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The free energy generated by photosystem I and photosystem II of green and blue-green algae

Arcelay, Angel Rene, Ph.D.
The Ohio State University, 1988
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UMI
THE FREE ENERGY GENERATED BY PHOTOSYSTEM I AND PHOTOSYSTEM II OF GREEN AND BLUE-GREEN ALGAE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Angel R. Arcelay, B.S., M.S.

The Ohio State University

1988

Dissertation Committee: Approved by
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DEPARTMENT OF BIOCHEMISTRY
ACKNOWLEDGEMENTS

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<td>A</td>
<td>absorbance</td>
</tr>
<tr>
<td>σ</td>
<td>absorption cross-section</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>θ</td>
<td>angle</td>
</tr>
<tr>
<td>c</td>
<td>speed of light</td>
</tr>
<tr>
<td>chl</td>
<td>chlorophyll</td>
</tr>
<tr>
<td>CH₂O</td>
<td>carbohydrates</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>cps</td>
<td>counts per second</td>
</tr>
<tr>
<td>DCMU</td>
<td>3-(3',4'-dichlorophenyl-1,1-dimethyl urea</td>
</tr>
<tr>
<td>DLE</td>
<td>delayed light emission</td>
</tr>
<tr>
<td>E₀'</td>
<td>standard electrode potential</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>Ephy</td>
<td>physiological potential</td>
</tr>
<tr>
<td>ev</td>
<td>electron volts</td>
</tr>
<tr>
<td>f</td>
<td>ratio of clear aperture to focal length</td>
</tr>
<tr>
<td>F(λ)</td>
<td>fluorescence at a wavelength</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>$\Delta G$</td>
<td>Gibb's free-energy</td>
</tr>
<tr>
<td>$\Delta G^0$</td>
<td>standard free-energy</td>
</tr>
<tr>
<td>GaAs</td>
<td>gallium arsenide</td>
</tr>
<tr>
<td>$h$</td>
<td>Planck's constant</td>
</tr>
<tr>
<td>$H_2O$</td>
<td>water</td>
</tr>
<tr>
<td>hrs</td>
<td>hours</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>$I_{bb}$</td>
<td>intensity of blackbody radiation</td>
</tr>
<tr>
<td>$I_{emi}$</td>
<td>intensity of emission</td>
</tr>
<tr>
<td>$I_{ext}$</td>
<td>intensity of exciting light</td>
</tr>
<tr>
<td>$k$</td>
<td>Boltzman's constant</td>
</tr>
<tr>
<td>$n$</td>
<td>index of refraction</td>
</tr>
<tr>
<td>$N$</td>
<td># molecules/cm$^3$</td>
</tr>
<tr>
<td>$N_2$</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NADP$^+$</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>ns</td>
<td>nanosecond</td>
</tr>
<tr>
<td>$O_2$</td>
<td>oxygen</td>
</tr>
<tr>
<td>O.D.</td>
<td>optical density, absorbance</td>
</tr>
<tr>
<td>$P$</td>
<td>pigment</td>
</tr>
<tr>
<td>$P_i$</td>
<td>phosphate</td>
</tr>
<tr>
<td>P-680</td>
<td>reaction center of photosystem II</td>
</tr>
<tr>
<td>P-700</td>
<td>reaction center of photosystem I</td>
</tr>
<tr>
<td>PMT</td>
<td>photomultiplier tube</td>
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\( \xi \)
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>psi</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>PS-I</td>
<td>photosystem I</td>
</tr>
<tr>
<td>PS-II</td>
<td>photosystem II</td>
</tr>
<tr>
<td>R</td>
<td>gas constant</td>
</tr>
<tr>
<td>R_{lum}</td>
<td>luminescence rate</td>
</tr>
<tr>
<td>R_{therm}</td>
<td>rate of thermal transitions</td>
</tr>
<tr>
<td>s, sec</td>
<td>second</td>
</tr>
<tr>
<td>T</td>
<td>absolute temperature</td>
</tr>
<tr>
<td>μs</td>
<td>microsecond</td>
</tr>
<tr>
<td>ν</td>
<td>frequency</td>
</tr>
<tr>
<td>V</td>
<td>volume</td>
</tr>
<tr>
<td>w</td>
<td>watt</td>
</tr>
<tr>
<td>φ</td>
<td>quantum yield</td>
</tr>
<tr>
<td>λ</td>
<td>wavelength</td>
</tr>
<tr>
<td>y(s)</td>
<td>sample signal in cps</td>
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Photosynthesis is the process by which radiant energy is converted into chemical energy stored in chemical bonds. The process is unique because no other process is known to be so efficient in trapping and converting energy in such a useful form. This process is the object of a large effort in research, and its understanding and usefulness are challenging tasks for future generations.

The largest group of organisms that perform the process over the surface of the Earth are the plants. Other organisms which perform the process are the eukaryotic algae and the prokaryotic bacteria, present mainly in aquatic systems.

The general equation for the process involves the use

\[ 2nH_2A + nCO_2 + hv \rightarrow n(CH_2O) + 2nA + nH_2O \]  

(1.1)

of reducing power provided by a hydrogen donor \( (H_2A) \), to reduce carbon dioxide with the production of a sugar compound and the liberation of oxidized donor \( (A) \).
The process is divided into two different sets of reactions: the light and the dark reactions. The light reactions are mediated by pigments which absorb and transfer the energy into an electron carrier system with a decreasing potential (Sauer, 1981).

\[ \text{H}_2\text{A} + h\nu + \text{ADP} + P_i + \text{NADP}^+ \rightarrow \text{NADPH} + \text{H}^+ + \text{ATP} + 2\text{A} \] (1.2)

These reactions are performed in membranous systems which contain the electron carriers and pigment protein complexes (Thornber, 1987). The organization of these electron carriers creates a potential (charge separation) that transfers hydrogen ions from one side of the membrane to the other. This vectorial hydrogen gradient is used to produce ATP. Electrons are transferred from water molecule(s) to \text{NADP}^+, through two photosystems.

When water is used as a hydrogen donor, one product of the light reactions is \( \text{O}_2 \) and the process called oxygenic photosynthesis. The oxygen liberated is a vital supply for the heterotrophic life on Earth. The sulphur bacteria and some cyanobacteria with one photosystem use hydrogen sulphide as donor instead of water, with the product being elemental sulphur and the process is named anoxygenic photosynthesis.
The dark reactions of photosynthesis are those where the ATP and reducing power (NADPH) are used for the fixation of CO₂. In this way the reactants of the light reactions are regenerated

$$\text{CO}_2 + \text{ATP} + \text{NADPH} + 3\text{H}^+ \longrightarrow \text{H}_2\text{O} + (\text{CH}_2\text{O}) + \text{ADP} + \text{P}_1 + \text{NADP}^+ \quad (1.3)$$

to complete a cycle. The enzymes of the dark process are localized in the non-membranous region of the photosynthetic system (stroma).

The radiant energy to be stored as chemical energy has to be transmitted through biochemical reactions in different environments to a final product such as a glucose molecule. Limitations on the storage of this energy can be explained in terms of two factors: thermodynamics and kinetics.

As explained by a Carnot engine and the Second Law of Thermodynamics, not all the energy used or supplied in such an engine can be obtained as useful work. In theory there is a maximum efficiency for a quantum converter. Ross and Hsiao (1977) established an efficiency limit of 31% for all quantum converters which resemble photosynthesis. The theory for photochemical energy conversion has been developed by Ross and others (see Bolton et al., 1981).
Real systems contain other losses which are not predicted by theory. Non-radiative transitions return photoproducts to the ground state and short circuits return electron back to low potentials. After all these constraints, the observed maximum efficiency should be less than 20% for photochemical systems (Bolton et al., 1981).

In plants because of such processes as photorespiration and reactions when there is no photosynthesis (dark), the yield is decreased to 6% (Bolton and Hall, 1979).

STRUCTURAL ELEMENTS OF PHOTOSYNTHESIS IN CYANOBACTERIA

This explanation of the photosynthetic apparatus will establish similarities and differences between components of eukaryotes (plants, green algae) and those of prokaryotes (cyanobacteria).

A good summary of photosynthesis in cyanobacteria is chapter 8 of the book "The Biology of Cyanobacteria" (Carr and Whitton, 1982).

Cyanobacteria are classified as prokaryotes. There are major differences between cyanobacteria and the chloroplasts of eukaryotic plants. One is the presence of phycobilisomes on the surface of the photosynthetic membranes of prokaryotes. This structure is shared with the eukaryotic red algae and has the same function as the chlorophyll b (chl b) light harvesting complex of chloroplasts. The second difference is the organization of photosynthetic
membranes. In cyanobacteria these membranes pervade the cytoplasm (Fig. 1.11) in varying arrangements and densities. In eukaryotes the photosynthetic membranes are packed within distinct structures called grana. Besides these two differences, there are great similarities in the details of photosynthetic electron transport and photophosphorylation mechanism between eukaryotes and cyanobacteria.

The photosynthetic apparatus of cyanobacteria is localized on intracytoplasmic membranes. The cytoplasmic membrane normally appears appressed to the cell wall as a triple-layered unit membrane (Fig. 1.12). In most species the thylakoids are arranged peripherally in three to six layers running parallel to the envelope layer. However, in some organisms such as Oscillatoria, the thylakoids are oriented perpendicular to the longitudinal cell wall. This radial thylakoid arrangement was demonstrated in Phormidium retzi and Oscillatoria (Golechi and Drews, 1982). The amount of thylakoid membranes per cell is variable due to growth conditions and the specific organism and can change from cell to cell within the same culture. The appearance of the thylakoids can change from parallel to convoluted.
Figure 1.1 Arrangement and distribution of thylakoid membranes.\textsuperscript{a}
\textsuperscript{a}Adapted from Carr and Witton, 1982, p.127.

1. Cross-section showing peripheral localization of thylakoid membranes. ICM are the intracytoplasmic membranes.

2. Section of a cyanobacterium showing the cell wall (CW), the cytoplasmic membranes (CM), and intracytoplasmic membranes (ICM). The arrow shows the appressed position of the thylakoid membranes.
In contrast to the thylakoids of higher plants, those of cyanobacteria form stacked regions only to a very limited extent.

Phycobilisomes are supramolecular pigment aggregates that serve as light harvesting antennae in red algae and in cyanobacteria (Glazer, 1985). These aggregates, composed primarily of phycobiliproteins, absorb over a wide range in the visible spectrum from 470 to 650 nm. There are three main classes of phycobiliproteins: phycoerythrin, phycocyanin and allophycocyanin. Phycocyanin and allophycocyanin are universally present in red algae and in cyanobacteria, while phycoerythrin is present in most red algae and in some cyanobacteria. Another class is the phycoerythrocyanin found in a few cyanobacteria.

MEMBERS OF THE ELECTRON TRANSPORT CHAIN

Figure 1.2 shows the scheme for the members of the electron transport sequence. Evidence obtained so far identifies these as the same as those found in higher plant chloroplasts. The figure does not indicate a membrane position of the components, but the sequence of electron transfer from water to NADP⁺. The vertical axis shows the standard redox potentials (Eₘ) and does not indicate the physiological potentials as affected by the concentration of species during electron transport (Blankenship and Prince, 1985).
Figure 1.2  Members of the electron transport chain

Fp  Ferredoxin-NADP⁺ oxidoreductase
Fd  Ferredoxin
FeS  Iron-Sulphur Center
A  Early acceptors
P700  Photosystem I reaction center
PC  Plastocyanin
FeSR  Rieske iron-sulphur protein
Cyt  Cytochrome
Q  Quinone
Ph  Pheophytin acceptor of PS-II
P680  Photosystem II reaction center
O.E.C.  Oxygen-evolving complex
The dashed line indicates cyclic electron flow around PS-I.
Figure 1.2
The molecules involved in the reductive process of the dark reactions are ATP and NADPH. The enzyme that gives electrons to NADP⁺ is a flavodoxin enzyme called ferredoxin-NADP⁺ oxidoreductase. Ferredoxin is a small, non-haem, iron-sulphur protein which receives electrons from PS-I and transfers them to various reductases. In cyanobacteria, ferredoxin can be replaced, under conditions of iron deficiency, by the flavoprotein flavodoxin.

In the reaction center of PS-I, one finds the participants of the light-driven electron-transfer reaction P-700 and its oxidant. P-700 is known from its light-induced change in absorbance at 700 nm, but has not been purified as a homogeneous chemical entity (Hipkins and Baker, 1986). The sequence of acceptors between P-700 and ferredoxin has been tentatively identified as:

P-700 → A0 → A1 → Fe-Sx → Fe-Sa → Fe-Sb → Fd.

The sequence includes two intermediary electron acceptors (A0, A1) and three iron-sulphur centers (Smith et al., 1987).

PS-I, the photosystem that stores less energy, receives electrons from a series of dark reactions which are supplied with electrons from PS-II. Figure 1.3 shows the distribution of photosystems in the thylakoid membranes. Plastocyanin or cytochrome C-553 supplies electrons to P-700 either directly or through a compound which has not yet been characterized.
Figure 1.3  Organization of some components of the process of photosynthesis in the thylakoid membranes. The diagram includes photosystem I complex (PS-I), photosystem II complex (PS-II), core complex of PS-II and light harvesting complex of PS-II (LHCP). Chloroplast lipids (L), coupling factors (CF₁), and coupling factor base complex (CF₀) are also included in the diagram.
The next participant in the chain of reductants, which brings electrons from PS-II to PS-I, is cytochrome f. Beyond cytochrome f there is less clear description of the biochemistry of electron transport. Cytochrome f is reduced by a non-haem iron-sulphur protein. Namba and Katoh (1984) found evidence to identify this iron-sulphur center as the Reiske iron-sulfur protein.

The plastoguinone pool is the next electron carrier in the sequence. This pool in cyanobacteria and higher plants seems to be identical with respect to function. The electron is first transferred to a primary acceptor (Qa), where it is stabilized, and then transferred to a second quinone (Qb) where it stays for a longer time. Finally a second electron is transferred to Qb, then Qb goes into the plastoguinone pool of the electron transport chain.

There are two major sites on the PS-II reaction center for electron movement. One is the reducing site, receiving electrons from the pigment P-680, a chlorophyll molecule in the excited state. The other is the oxidizing site taking electrons from the water molecule and releasing molecular oxygen and hydrogen ions. The primary acceptor of electrons from P-680 is identified as pheophytin.

Oxygen evolution measurements have demonstrated that the oxygen-evolving complex of cyanobacteria functions in a similar way to that of plants. There are 4 states for O_2 evolution and the electron donor system functions in the
same pattern (Kawamura and Fujita, 1983).

ANOXYGENIC PHOTOSYNTHESIS

Some species of cyanobacteria have been found to grow with either plant-type oxygenic photosynthesis or bacterial-type anoxygenic photosynthesis. Anoxygenic photosynthesis was clearly demonstrated using the strain Oscillatoria limnetica from the sulphide rich layers of the Solar Lake (near Elat, Israel). It was found (Cohen et al., 1975) that at low sulphide concentration (0.1-0.2 mM), CO$_2$ photoassimilation is inhibited several hours after light is supplied. Continuous exposure to light for about 2 hours even at high (3 mM) sulphide concentration causes regeneration of CO$_2$ photoassimilation. This CO$_2$ photoassimilation is insensitive to DCMU (3(3,4-dichlorophenyl)-1,1-dimethyl urea). DCMU inhibits photosystem II by blocking the electron transport reaction at the quinone level.

It is postulated therefore that anoxygenic photosynthesis is independent of PS-II and is driven by PS-I with hydrogen sulfide as an electron donor. In some photosynthetic systems such as Anacystis nidulans, Aphanotohece halophitica and isolated chloroplasts, sulphide (0.1-0.5 mM) acts in a site preceding PS-II (Oren et al., 1979) as an electron transport inhibitor. Oren et al. reported that the yield of variable fluorescence decreased
to the non-variable value in the presence of PS-II light, but was fully restored upon addition of DCMU.

Using far red light oxygenic photosynthesis was inhibited in sulphide but anoxygenic CO₂ photoassimilation was operative with PS-I (Cohen et al., 1975). Sybesma and collaborators (1987) found evidence to identify the electron donating site for PS-I at the plastoquinone level.

CHEMICAL POTENTIAL GENERATED BY PHOTOSYSTEMS

After light absorption a pigment is raised to the first singlet, $P^*$, excited state. The fate of the excited state is explained in a Jablonski diagram as shown in figure 1.3. It is from this excited state that energy can be transferred to obtain photoproducts, $P^+$, $A^-$, in oxidized and reduced state. There is an electron transfer with subsequent transfer of energy, which can be stored as chemical energy in a bond.

Luminescence is a result of back reactions of the energy storing system after illumination (Malkin, 1977). Luminescence from photosynthetic organisms have been used to elucidate mechanisms and components of the energy storing systems present in these organisms. Prompt fluorescence is emitted during illumination of a sample while delayed light is emitted after cessation of the actinic source. Some review articles such as Jursinic (1986); Govindjee and Jursinic, (1979); Lavorel (1975); and Amesz and van Gorkum
Figure 1.4  Diagram of the energy levels after absorption of light. The excited state decays by charge separation (photochemistry); fluorescence; phosphorescence and non-radiative decay.

\[
\begin{align*}
P & \quad \text{pigment in the ground state} \\
P^* & \quad \text{pigment in the excited state} \\
P_3^* & \quad \text{pigment in the triplet state} \\
P^+ & \quad \text{pigment in the oxidized state} \\
A^- & \quad \text{reduced acceptor}
\end{align*}
\]
Figure 1.4
(1979) discuss the similarities and differences between prompt and delayed light emission (DLE) fluorescence. Since luminescence is a characteristic of the photosynthetic machinery, its understanding reveals properties of photosynthetic organisms.

Luminescence is a phenomenon mainly associated with PS-II. Various authors report the exceptional delayed fluorescence arising from PS-I as reviewed by Jursinic (1986). Bertsch and collaborators (1967) reported that DLE from PS-I is at least 250 times less intense than that of PS-II. The luminescence from PS-II is far more intense than that from PS-I, normally obscuring it.

The free-energy difference between the ground and excited state of a photochemical system can be determined by the equation:

$$\Delta G = \text{constant} + kT \ln \text{(luminescence intensity)} \quad (1.4).$$

The thermodynamics used to derive the formula can be reviewed from Ross (1966, 1967). This formula was used for the first time by Ross and Calvin (1967) who calculated the ratio of the rates of the transitions of chl molecules to the excited state in both the light and dark. Assuming a value of 1.0 for the quantum yield with luminescence calculated from the Plank-Law, they reported the maximal free-energy difference as 1.16-1.19 eV for system I; 1.23 eV
for system II; and 0.79 eV for photosynthetic bacteria.

The emission properties of an excited electronic state are usually independent of how it was created. This is because the lifetime of most molecular excited states is sufficiently long that there is time for all the substates to reach equilibrium prior to emission.

Equilibration among an excited state is the usual observation, but incomplete equilibration is known for some aromatic molecules within the fluorescence lifetime (Ross, 1975). This is due to the lack of equilibration of the electronic states with the surrounding solvent. The lack of equilibration is observed as a change in emission spectrum or emission kinetics upon excitation at the long-wavelength absorption edge. A change in emission spectrum over time following illumination also demonstrates lack of equilibration (reviewed by Lakowicz, 1983). For the purpose of free-energy determination it will be assumed that equilibration is achieved. From this assumption it is inferred that the excited state has well-defined thermodynamic properties, such as free-energy. Therefore, the free-energy difference between ground and excited state must be independent of the path joining them.

The free-energy provided by the light-driven step of a photochemical process is equal to the free-energy of the non-radiative process
\[ P = P^*, \tag{1.5} \]

where \( P \) and \( P^* \) are the ground and excited states of the pigment. This free energy change is given by the Nernst equation as:

\[ \Delta G = \Delta G^o + kT \ln\left(\frac{[P^*]}{[P]}\right). \tag{1.6} \]

The radiative transition from \( P^* \) to \( P \) is accompanied by luminescence (see figure 1.5), with the intensity of this emission proportional to the concentration \([P^*]\). Thus, the free-energy of the transition varies as \( kT \ln(\text{luminescence intensity}) \).

Using a similar but more formal and general line of reasoning (Ross, 1967; Ross and Calvin, 1967; Ross, 1975; Marchiarullo and Ross, 1981) one can establish a relationship between the intensity of luminescence and the free-energy of the light-driven transition. This relationship requires only equilibration within the electronic states for its validity.

The general equation for the free-energy change is simply (Marchiarullo and Ross, 1981)

\[ \Delta G = kT \ln\left(\frac{R_{\text{lum}}}{R_{\text{therm}}}\right), \tag{1.7} \]
Figure 1.5  Mechanism for luminescence production. A metastable state is produced after light absorption, which after a time delay returns electrons back to the reaction centers with subsequent luminescence.
Excited state \rightarrow \text{Metastable} \rightarrow h\nu \rightarrow \text{Ground state}

\text{DELAYED FLUORESCENCE}

Figure 1.5
where $R_{\text{lum}}$ is the rate of luminescence emission, and $R_{\text{therm}}$ is the rate of transitions occurring, in either direction, due to absorption of thermal blackbody radiation,

$$R_{\text{therm}} = \int \sigma(\lambda) I_{\text{bb}}(\lambda) \, d(\lambda) \quad (1.8)$$

where $\sigma(\lambda)$ is the absorption cross-section at $\lambda$, and $I_{\text{bb}}(\lambda)$ is the intensity of radiation emitted by a blackbody at the ambient temperature. In the visible and near-visible, the radiation flux emitted by a blackbody of temperature $T$ is (Planck, 1959):

$$I_{\text{bb}}(\lambda) = \frac{8\pi n^2 c \lambda^{-4}}{\left[ \exp(hc/\lambda kT) - 1 \right]^{-1}} \quad (1.9)$$

If the sample does not absorb and emit light equally in all directions, $\sigma(\lambda)$ must be averaged over solid angle and polarization.

Accurate calculation of absolute $\sigma(\lambda)$ can be difficult or not possible, but $\Delta G$ can still be evaluated accurately using the following set of equations. First, we note that for any specimen in a particular instrument;

$$Y = \eta R_{\text{lum}} \quad (1.10)$$

where $Y$ is the luminescence signal recorded by the instrument, and $\eta$ is a measure of the sensitivity of the
Second, we note that for a photoluminescent specimen, one which luminesces as a direct consequence of illumination, the rate of luminescence may be written as

\[ R_{\text{lum}} = \phi_{\text{lum}} \int I_{\text{ext}}(\lambda) \, d(\lambda), \]  

(1.11)

where \( \phi_{\text{lum}} \) is the quantum yield of luminescence, and \( I_{\text{ext}} \) is the intensity of incident illumination. Combining Eqns (1.10) and (1.11), one may then in principle evaluate \( \eta \) for an instrument by using a reference specimen having a known quantum yield. However, this evaluation need not be done explicitly. Rather, Eqn (1.10) may be applied to both the sample (s) having unknown \( \Delta G \) and to the reference (r) having known quantum yield. Then, substituting in Eqn (1.8) for the sample and Eqn (1.11) for the reference, we find that

\[ \frac{R_{\text{lum}}}{R_{\text{therm}}} = \frac{\phi_{\text{lum}}(r) \int I_{\text{ext}} \sigma(r) \, d(\lambda)}{\phi_{\text{lum}}(r) \int I_{\text{bb}} \sigma(s) \, d(\lambda)} \]  

(1.12)

for the sample, which may then be used in Eqn (1.7) to find \( \Delta G \). If the sample and reference have the same geometry, any quantity proportional to \( \sigma(\lambda) \) may be used in its place. In optically-thin specimens, absorbance is proportional to cross-section, so that absorbance may be used in Eqn (12).
A material with high fluorescence quantum yield may be used as the reference, so there is no experimental difficulty in using a reference specimen which is optically thin at all wavelengths of interest. However, with a sample having very weak luminescence, one may need a high optical density in order to get an accurately measurable signal. Fortunately, the conflict between high optical density for good sensitivity and low optical density for accurate use of absorbance in Eqn (1.12) is not so great as it may at first appear, because most of the integral of Eqn (1.8) is at wavelengths at which the absorbance is relatively low.

Through the Kennard-Stepanov relationship (Stepanov, 1957; Ross, 1967) the integrand of Eqn. 1.8 is also the emission spectrum.

\[ \sigma(\lambda)I_{bb}(\lambda) = F(\lambda) \quad (1.13) \]

where \( F(\lambda) \) is emission in relative quanta per second per wavelength interval. By definition, this integrand is largest at the wavelength of the emission peak. The left side of Eqn 1.13 is evaluated from a short wavelength at which its value has become quite small out to the longest wavelength at which the absorbance can be measured. The right side of the equation is evaluated over the wavelength range in which emission can be measured. A fit of these two curves, weighted for the relative reliability of individual
points, determines the best scaling of the right side and the best estimate of the integrand. The resulting value of \( R_{\text{therm}} \) will have an error of about 20\% for a typical aromatic molecule, with a consequent error in \( \Delta G \) of 0.2 kT (Ross, 1975).

Equations 1.6-1.8 hold both in the presence and in the absence of external illumination. The intensity of emission during illumination or during the first few nanoseconds following illumination can be used to determine the \( \Delta G \) between the ground and excited state of pigments in the presence of actinic light. A microsecond or more after any actinic illumination has ceased, any detectable pigment luminescence must be from excited states which themselves have a much longer lifetime than the several nanoseconds of a typical singlet state, or which have been populated from long-lived states.

Any photochemical system with high energy conversion efficiency must emit luminescence from excited pigments which have been repopulated by reversal of the energy storage pathway (Ross and Calvin, 1967). At a time \( \Delta t \) after illumination, luminescence is due primarily to back reaction of energy-storage intermediates which have pseudo-first order reverse rate constants near \( 1/\Delta t \). The intensity of this luminescence is thus a fairly accurate measure of the \( \Delta G \) between chemical intermediates which are a "distance" \( \Delta t \) on either side of the light-driven step.
In a thermodynamically-ideal photochemical energy converter, all energy-storage intermediates derived from the excited state are in equilibrium with the excited state, so that the population of the excited state is held constant, with a consequent constant luminescence, until the concentration of these storage intermediates changes. However, previous measurements (Haug, 1972; Marchiarullo and Ross, 1981) of luminescence from PS-II have shown that the intensity of luminescence a few microseconds after illumination is about 200 times less than the intensity of the luminescence during illumination, showing that fairly early energy-storage intermediates are about $kT \ln 200 = 0.14$ eV out of equilibrium with the average free energy of the pigments responsible for fluorescence during illumination. Part of this drop occurs between the antenna pigments and the reaction center, and is due to the finite rate of energy transfer to the reaction center compared to the rate of fluorescence from the antenna. The rest of the drop appears to occur between the reaction center and intermediates which appear in nanoseconds, and may be due to a loss pathway such as the tunneling of a radical-pair intermediate back to the ground state.
WORK IN THIS DISSERTATION

The research reported here is in the use of prompt and delayed fluorescence from green algae and cyanobacteria to evaluate the free energy generated by photosystems I and II present in these photosynthetic microorganisms.

The amount and variability of these free energies will be determined using the intensity of luminescence emitted by each of the two photosystems. Luminescence from PS-II is far more intense than that from PS-I, normally obscuring PS-I luminescence. It is thus of special interest to study the intensity of luminescence generated by PS-I and compare its intensity to that of PS-II from prokaryotes and eukaryotes.

An integrating sphere is used to illuminate samples uniformly and collect luminescence efficiently. Equation 1.7 is used for the determination of the free energy generated by the photosystems. Actinic beam intensity is determined by chemical actinometry and luminescence yield is calibrated with the standard Cresyl Violet Perchlorate. The integrand in the denominator of eq. 1.13 used the Kennard-Stepanov relationship for the determination of the thermal transition density for each photosystem in the different samples.

The free energy generated by each photosystem can be determined if the intensity of luminescence from PS-I and PS-II can be distinguished and measured. For the reason that the two photosystems work in series within an integral
environment, systems are necessary where one photosystem is not functional in order to measure the other. Luminescence from wild-type *Scenedesmus obliquus* is assigned to PS-II. A mutant from *Scenedesmus* deficient in plastoquinone A will be used to study luminescence from PS-I. The intensity of delayed light emission from the wild-type *Scenedesmus obliquus* is used to refine a previous determination of the free-energy change in photosystem II. The weak luminescence from mutant #11 of *Scenedesmus* is used to place an upper limit of the free-energy change in eukaryotic PS-I.

The cyanobacterium *Anacystis nidulans* will be grown in a normal and calcium-depleted media to observed luminescence from PS-I and PS-II in prokaryotes. Cyanobacteria exhibit anoxygenic photosynthesis under sulfide treatment, with PS-II inactive and PS-I functional for these highly reducing conditions. The marine strain *Aphanothece halophitica* is treated with sulphide in an attempt to determine the amount of the free-energy generated by PS-I.

Luminescence from normal grown *Anacystis* is assumed to be due to PS-II and the free-energy determined as for *Scenedesmus*. The luminescence observed from *Anacystis* treated with a calcium-depleted media is used to test for any observable luminescence from PS-I. Luminescence from sulphide-treated *Aphanothece halophitica* is used to determine the free-energy change of PS-I in prokaryotes.
CHAPTER II
METHODS

GROWTH OF PHOTOSYNTHETIC MATERIAL

Normal and mutant cultures of the green algae *Scenedesmus obliquus* were purchased from The Culture Collection of Algae at the University of Texas. The wild-type (UTEX 393; known as Gaffron's D-3, from Emerson laboratory) was grown in a modified Kessler's medium (Table 2-1) as described by Bishop and Senger (1971) with continuous illumination provided by a bank of fluorescent lamps (General Electric, cool white) at an intensity of 1 W/m² at 26°C. Mutant #11 (UTEX 2016) produced by Bishop (1962) and deficient in plastoguinone A (Bishop, 1971) was grown heterotrophically in the dark with the Kessler's medium supplemented by 0.5% glucose and 0.025% yeast extract (Kaltwasser et al., 1969). Asynchronous cultures of the strains were kept with continuous light and constant shaking in a closed growing chamber where the temperature was regulated at 26°C, until used for experiments.

*Anacystis nidulans* (UTEX 625 and 1550; strain Tx20, PCC 6301) a cyanobacterium was grown in BG-11 (Table 2-1) as recommended by Stanier and collaborators (1971) using the
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Kessler</th>
<th>BG-11</th>
<th>ASN-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>0.809</td>
<td>-</td>
<td>-</td>
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<tr>
<td>NaCl</td>
<td>0.468</td>
<td>-</td>
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<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
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<tr>
<td>H₃BO₃</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>7.5x10⁻⁵</td>
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<td>-</td>
</tr>
<tr>
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<td>-</td>
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</tr>
<tr>
<td>Sodium Citrate</td>
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<td>-</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
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<td>0.75</td>
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<td>CaCl₂·2H₂O</td>
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<td>0.036</td>
<td>0.5</td>
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<td>MgCl₂·6H₂O</td>
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</tr>
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<tr>
<td>NaN₃</td>
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<td>0.75</td>
</tr>
<tr>
<td>K₂HPO₄·3H₂O</td>
<td>-</td>
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<td>0.02</td>
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<td>Citric Acid</td>
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<td>0.003</td>
</tr>
<tr>
<td>Ferric Ammonium</td>
<td>-</td>
<td>0.006</td>
<td>0.003</td>
</tr>
<tr>
<td>Citrate</td>
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<td>0.003</td>
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<tr>
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<tr>
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<td>0.02</td>
</tr>
<tr>
<td>Vit B₁₂</td>
<td>-</td>
<td>-</td>
<td>10 μg/ml</td>
</tr>
<tr>
<td>Trace Metal Mix A5</td>
<td>1 ml L⁻¹</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trace Metal Mix A5 + Co</td>
<td>1 ml L⁻¹</td>
<td>1 ml L⁻¹</td>
<td>1 ml L⁻¹</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1000 ml</td>
<td>1000 ml</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Trace metal mix A5 + Co contains (g/L): H₃BO₃, 2.86; MnCl₂·4H₂O, 1.81; ZnSO₄·7H₂O, 0.222; Na₂MoO₄·2H₂O, 0.390; CuSO₄·5H₂O, 0.079; Co(NO₃)₂, 0.0494.
same growing conditions as for the green algae *Scenedesmus*. This strain was purchased as an axenic culture from the University of Texas Culture Collection.

*Aphanothece halophitica* (ATCC 29534) a cyanobacterium purchased from the American Type Culture Collection was grown in the medium ASN-III (Table 2-1) as recommended by P. Pienta (Personal Communication) from ATCC. Description of the medium is published by the ATCC Media Handbook (1984). It uses the same micronutrients A-5 as BG-11. This strain has absolute requirements for vitamin B$_{12}$ which is added to the medium in a concentration of 10 µg/ml. The reason for the use of ASN-III for *Aphanothece* is that most cyanobacteria from marine sources (e.g. Solar Lake, Israel), cannot grow in BG-11 (Rippka, et al., 1979). Analysis of their nutrition showed that such cyanobacteria have elevated requirements for Na$^+$, Cl$^-$, Mg$^{+2}$, and Ca$^{+2}$. ASN-III medium has a similar sea water base and is supplemented with minerals of BG-11 at half strength.

Photosynthetic bacteria were grown with 1-2% dilution from an old medium. Fresh samples less than 48 hours old with low cell density were used for measurements principally those of DLE experiments. The samples used for delayed light emission (DLE) experiments were analyzed by the technique of flow cytometry. Results provided by the different profile of the light scattered from the cells showed that most of the cells were active photosynthetic
cells (90-95%), while a small number were in mitosis (2-3%) and some were old cells (1-2%). Doubling time for the eukaryotic cells was an average of 15 hours. Cells used for experiments were harvested in their logarithmic phase, pelleted, washed twice, and suspended in fresh medium with 0.5% methyl cellulose added to inhibit settling during luminescence measurements.

**Ca²⁺-DEPLETED MEDIUM**

*Anacystis nidulans* has the property of growing in a Ca²⁺-depleted medium with inactive PS-II (Brand et al., 1983; Becker and Brand, 1982). The procedure for this particular experiment was obtained from Dr. Jerry Brand (Personal Communication from the University of Texas, Austin). The composition of the depleted medium contained the following reagents in grams/L: MgSO₄·7H₂O, 0.25; K₂HPO₄, 0.05; KNO₃, 1.0; and CaNO₃·4H₂O, 0.025. The medium used a 1.0 ml per L of an Fe-EDTA solution made of 2.0 grams of the free-acid EDTA neutralized with 2N KOH until dissolves and 0.8 grams of Fe₂(SO₄)₃·7H₂O into a volume of 200 ml and pH adjusted to 7.5-8.0. The micronutrient solution contained the following chemicals in grams/L: MnCl₂·4H₂O, 2.86; ZnSO₄, 1.81; MoO₃, 0.0177; and CuSO₄·5H₂O, 0.079. One ml of the modified A-5 micronutrient solution was used per L of depleted-medium. Glycylglycine was added to the depleted medium as 1.5 grams/L and the pH of the medium adjusted to
8.25. The glassware used for experiments and to prepare solutions was rinsed with 1-1 v/v HNO$_3$, HCl solutions. It is important that all Ca$^{+2}$ and Na$^+$ be excluded from the depleted medium. This strain is grown at high light intensity under a bank of 12 fluorescent GRO-LUX lamps (Sylvania) at a temperature of 39°C +/- 2°C with and without Ca$^{+2}$.

CO$_2$ at 1% (v/v air) was supplied for the cells grown at high light intensities. Cells from Anacystis grown at high light intensity under a bank of 12 fluorescent lamps at 39°C, showed a shorter doubling time (8 hrs) than those grown in the incubating chamber (12 hrs) because of the high light and CO$_2$ effect.

The samples were pelleted in a Sorval GLC-1 centrifuge at 2000 RPM for 10 min, rinsed twice with Ca$^{+2}$-depleted medium and resuspended in a dilute suspension at 3-5 µg/ml chl. Samples are run in at least two batches for measurements. No oxygen evolution is observed from Ca$^{+2}$-depleted samples after 24 hours of treatment and the growth is slow (16 hr doubling time) when compared with control cells (Becker and Brand, 1982).

ANOXYGENIC MEDIUM

The cyanobacterium Aphanothece halophitica is capable of shifting between the anoxygenic bacterial-type photosynthesis and the oxygenic plant-type photosynthesis.
(Rippka et al., 1979). Cells of *Aphanothece* were treated in a sulphide O₂-free environment under the following conditions.

The sulphide inhibition of PS-II is carried out in a sealed-serum flask with a CO₂/N₂ atmosphere. The medium for the treatment has a high salt content (~3%) and cannot be boiled or autoclaved without causing precipitation, because of the product of solubility of the salts. Water for the medium is boiled and added to a 1 L flask where salts, micronutrients and vitamin B₁₂ were present. The solution is taken to pH 7.3 and adjusted to 1 L. N₂ is bubbled through the solution for an hour. This procedure will keep the solution with the lowest possible oxygen content. Any O₂ left will be oxidized by the sulphide added.

The solution described above was filter sterilized and N₂ was bubbled thru the solution for an hour. The serum flasks used for the PS-II inhibition were filled with the N₂/CO₂ mixture at 20 psi and autoclaved. After the ASN-III medium is oxygen-free and the flasks autoclaved, these materials are transferred to a closed anoxygenic-chamber with a N₂/CO₂ atmosphere where 50 ml of the medium were added to each flask and the flasks sealed. Flasks were removed from the chamber and degassed with a pump. A mixture of CO₂/N₂ at 25 psi is added to each flask to maintain internal pressure and inhibit leaks.
Once the serum flasks were filled with the ASN-III medium and sealed, CO₂ and sulphide were added to the flasks. The Na₂S solution was prepared in a boiled double distilled water solution adjusted to pH 12 with NaOH. Four grams of Na₂S.9H₂O were added to 50 ml of the basic water solution, the flask sealed in the N₂-anoxygenic chamber and autoclaved. A sodium carbonate solution is prepared with 3.763 g of the material diluted to 50 ml under the same conditions as the sulphide solution except that the double distilled water was not basic. A sample is back-titrated to pH=7.3 with an aliquot of 5 M NaOH, which is added to the rest of the flasks.

Sodium sulphide was added to flasks with a syringe, with .225 ml added from a 0.33 M solution in sulphide which produced a 1.5 mM sulphide solution for Aphanothece experiments. Sodium carbonate (0.5 ml) was added with another syringe to a concentration of 7.1 mM for the experiments. Samples of oxygenic grown Aphanothece were pelleted and washed twice with the ASN-III medium which contained sulphide and sodium carbonate. A control which lacks sulphide ran simultaneously. Both control and sulphide-treated Aphanothece were kept in the growing chamber for at least 24 hours before luminescence measurements. Cells used for experiments were washed twice with sulphide and sodium carbonate medium prior to experiments. Samples were suspended in 1 ml of medium and
transferred to the serum flask for treatment. Before luminescence measurements, cells are washed and suspended in medium with 0.1% methyl cellulose added to inhibit settling.

**ABSORPTION MEASUREMENTS**

Absorption measurements were run in a Cary 118 spectrophotometer or corrected for this instrument's sensitivity when obtained from other spectrophotometer. The absorption spectra was corrected for light scattering as described below.

Absorption measurements are constrained by two major effects: the sieve and the light scattering effect. The sieve effect occurs when the concentration of absorbing molecules in the sample is uneven, usually due to packaging, such as that of chlorophylls in chloroplasts. The scattering effect is a result of the size of the molecule and this effect varies with the wavelength of absorption.

Correction for light scattering can be explained using theory developed by Latimer and Eubanks (1962). In this method two absorption measurements are obtained at two different solid angles of measurements $\Theta_1$ and $\Theta_2$. $\Sigma \Theta_2$ is an absorption obtained from the scatterer compartment next to the PMT at the Cary and $\Sigma \Theta_1$ is obtained at the normal position at the center of the sample compartment far from the PMT. The absorption free of scattering [$\Sigma s$] from the cell suspension is given by the formula: 
where $A$ is a constant characteristic of the optical system and independent of wavelength. The value of $A$ may be found by the equation:

$$A = \left[\frac{\Sigma_{02} - A(\Sigma_{01} - \Sigma_{02})}{\Sigma_{01} - \Sigma_{02}}\right]_{\lambda}$$ \hspace{1cm} (2-2)

where $\lambda$ is a wavelength at which there is no absorption. With $A$, $\Sigma_{01}$ and $\Sigma_{02}$ the absorbance free of scattering, $\Sigma_S$, can be obtained using equation (2-1).

In our measurements $\lambda$ was 800 nm, a wavelength where there is no absorption for our cell suspension. Our method corrects for scattering effects, but not for the sieve effect.

**Chlorophyll determination**

The extraction of pigments from intact algae is a difficult task. For live cells of green algae or cyanobacteria methanol is recommended as a successful solvent (Hipkins and Baker, 1986). For our chl determination an aliquot of 5.00 ml is accurately pipetted from the cell suspension. The sample was transferred to a test tube and centrifuged for 10 min at 2000 RPM. The supernatant was decanted and the cell's pellet was resuspended in approx. 4 ml of methanol. The sample was
passed several times through a pasteur pipette to ensure homogeneous mixing and centrifuged for 5 min at 2000 RPM in a Sorval desk-top centrifuge. The supernatant was collected and diluted accurately to 5.00 ml with methanol in a volumetric flask. The absorbance of the solution was measured immediately at 650 and 665 nm. Since no dilution effect was involved in the test, substitution of absorbances in the following formula (Hipkins and Baker, 1986) yielded the total chl amount in μg/ml.

\[
\text{Total chl} = 25.8 \ A_{650} + 4.0 \ A_{665}
\]  

(2-3)

**Fluorescence spectra**

Fluorescence spectra were obtained using an SLM AMINCO SPF-500C spectrofluorometer. The instrument was automated with a Commodore C-64 microcomputer for data collection. The excitation source was a 250 watt xenon arc lamp. The excitation monochromator uses a 1200 lines/mm holographic grating with maximum efficiency at 300 nm. The emission monochromator uses a 1200 lines/mm grating blazed at 500 nm.

The reference PMT monitors a small sample of the excitation radiation produced by the xenon lamp and transmitted by the excitation monochromator. The reference PMT is a Hamamatsu R777 with a wide range and flat response. The signal PMT, a Hamamatsu R928P, has a higher sensitivity to collect fluorescence from the samples.
The samples were run in a ratio mode, in which the fluorescence readings were adjusted for transient lamp fluctuations and for the differential excitation intensity at different wavelengths. The microcomputer stored and transferred the data for analysis of spectral components present.

**FILTER FLUORIMETER**

Delayed light measurements were obtained from a custom-made filter fluorimeter built to measure very low yield signals, such as those arising from PS-I. Figure 2.1 shows a layout for the instrument. The instrument was mounted on a metal base to which modular parts can be added or removed as needed. The modular design of the instrument, nonetheless, provided light-tight measurements. The fluorimeter was L-shaped, suitable for prompt and delayed fluorescence measurements. Three important sections of the instrument will be explained individually: excitation/intensity arm; sample compartment; and detection arm.

**Excitation/intensity arm**

This is the part of the fluorimeter used to give appropriate actinic light of required wavelength and intensity. The light source was a 100-watt tungsten-halogen lamp with 2.3 x 4.2 mm filament. The lamp had an Oriel 6385 housing with an f/0.85 pyrex aspheric condensing
Figure 2.1  Layout of filter fluorimeter. The figure shows a block diagram of the custom-made instrument used for delayed luminescence measurements. Explanation for each part and its use can be found in the text.
Figure 2.1

**LAYOUT OF FILTER FLUORIMETER**
lens (Oriel 6327), powered by a 12-volt A.C. power supply (Oriel 6329). The actinic beam was passed through a 3.2 cm water filter before wavelength selection with color filters. A collimating system inside of a plastic tube was made of a metal tubing of 14.5 x 2.1 cm, blackened in the inside and outside, and used to reduce the light scattering effect from the bright source. The most used actinic beam had a peak at 564 nm with a half-bandwidth of 24 nm. This actinic beam was obtained using Corning 3-67, 4-96, and 4-97 colored-glass filters in combination with a Ditric 580 nm short-pass interference filter. Figure 2.2 shows the wavelength selection by the filter combination (maximum 564 nm) and the half-bandwidth obtained of 22-24 nm. For actinic beams of different wavelengths, interference filters of the appropriate wavelengths were used.

Light intensity was regulated by two neutral density wedges (Kodak). The combination of the wedges provided a decrease in intensity from 0 to 4 optical density units. These optical units are log units, so the unit has an adjustment of intensity from 1 to 10,000 times less light for the experiments. Timing of illumination was controlled with a Uniblitz shutter (Vincent, Ass.; #26L0A0T5). The delay time for opening after the current was applied was 1.0 msec, with a minimum dwell time of 0.5 msec and 0.9 msec for closure, according to specifications from the manufacturer. Before reaching the sample, light was
Figure 2.2  Actinic light bandwidth
Corning 3-67, 4-96 and 4-97 colored-glass filters with a
Ditric 580 nm short-pass interference filter were used to
obtain the actinic beam. The half-bandwidth is 22-24 nm.
collimated by a lens with adjustable position to ensure brighter and homogeneous light distribution onto the integrating sphere.

**Sample compartment**

The sample compartment was a square box made of black plastic with copper tubing at the bottom for temperature control. An integrating sphere was the most important component of the sample compartment. The primary purpose of the sphere was to average out the exciting light hitting the samples so that cells are illuminated uniformly by multiple passes of light through the samples. By the same mechanism the luminescence emitted by the samples can be observed and analyzed.

An integrating sphere is a globe with highly reflective surface in the inside. Demas and Crosby (1971) discussed the uses of integrating spheres and situations where they are most useful. For our instrument a small sphere was hollowed out of a block of Teflon. This material was selected because of uniformly high reflectivity of all visible wavelengths and washable. The sphere had three apertures: one at the top, 10 mm in diameter to hold the sample tube; and two apertures of 6 mm each at right angles facing the center of the excitation and emission paths. The sphere was made into two halves. The inside diameter of the sphere was 25 mm. One ml samples were held inside the
sphere as a cylinder of 8 X 23 mm through the top aperture. The sample test tube was made from a 10 mm quartz tubing at the glass shop of The Ohio State University Chemistry Department. The quartz material was found to be less luminescent than any other material from tubes commercially made; therefore, the tubes for the experiments were made from quartz tubing.

**Detection arm**

Luminescence leaving the sphere was collected by a lens with adjustable position to collimate emitted light for maximum yield into the PMT. Before reaching any filter for wavelength selection, luminescence from samples was passed through a Corning 2-60 (37% transmittance at 619-628 nm) filter to eliminate residual luminescence from optical components of the fluorimeter. When prompt fluorescence measurements or high yield signals were obtained, neutral density filters were used to protect the photomultiplier tube (PMT) from damage due to high yield of luminescence. Additional interference or cutoff filters were used when emission of specific wavelengths was needed. Time of luminescence exposure for the PMT was controlled by a Uniblitz shutter with the same specifications as that used for the excitation side.
Luminescence was collimated by a lens into the photocathode with an area 10 x 10 mm\(^2\) of the PMT. A GaAs PMT tube (Hamamatsu R943-02) suitable for photon counting was used. This PMT has a quantum efficiency of over 10% out to a \(\lambda\) of 800 nm. This PMT was thermoelectrically-cooled by a housing with pulse amplifier and discriminator built into it (Pacific Photometrics 3470/AD6). The power supply was adjusted to 1700 volts with the discriminator at 100 mv. The output from the PMT was fed to a Commodore C-64 minicomputer, by an electronic box made at the Visual Research Center Lab., for data analysis and transfer.

**CALIBRATION**

Measurements of relative excitation intensity in the filter fluorimeter were obtained with a photodiode/Keithley electrometer combination. The current generated by the photodiode (Hamamatsu S1226-5BQ, Serial #A); attached to the side of the integrating sphere, was measured by the Keithley electrometer. Spectral calibration of the photodiode was performed by former graduate student Mark Tirpack (Tirpack, 1986) from the Visual Research Center Laboratories. The graph for the relative spectral response versus wavelength is shown in Figure 2.3. This system gave relative measurements which were made absolute using chemical actinometry. It is the object of the photodiode/Keithley system to obtain reliable measurements with an alternate
Figure 2.3  Spectral response of the photodiode. Relative spectral response of the incident beam was obtained from a photodiode. Spectral calibration of the photodiode was performed at the Visual Research Center Laboratory by Mark Tirpack. The response was measured with a Keithley Electrometer as amperes per incident photons.
Figure 2.3
and stable system which is faster and easier to handle.

ACTINOMETRY

Chemical actinometry provided the most suitable method for determination of the intensity of the exciting light. Two results are accomplished with the method: (1) the number of photons absorbed for a specimen of absorbance $A_j$ are obtained; (2) the intensity of light from one direction which would give the same number of absorbed photons per second for each absorbing molecule. The actinometer solution used was the potassium Reinecke's salt following the recommendations described by Wegner and Adamson (1966).

To find the intensity, $I_o$, of monochromatic beam within the reaction cell the reactant used was potassium Reinecke's salt which absorbs light at the wavelength of interest (see equation 2.5). The quantum yield, $\Phi$, for the photochemical reaction for some product B must be known accurately for the experiments conditions. The temperature, concentration and other known conditions for the reactant photochemistry are adjusted before the experiment. Then the reactant is exposed to the light beam for $t$ seconds. The fraction of light absorbed by the reactant is determined from known experimental values: $1-(I/I_o) = 1-10^{-\varepsilon[A]l}$, where $\varepsilon$ is the molar extinction coefficient, $[A]$ the concentration of $A$ and $l$ the pathlength of the reaction sample. The
number of molecules of product B formed (see equation 2.4), $N_b$, in the time $t$ are determined; and from these the light intensity of the exciting beam, $I_o$, can be calculated from the formula:

$$I_o = \frac{N_b}{[(Q_b)(1-10^{-8[A]_1})t]} \text{photons/sec.} \quad (2.4)$$

$Q_b$ is the quantum yield for the production of molecule B, a known quantity for our experiments. The reactions below show the photochemical aquation of Reinecke's salt with the production of thiocyanate. The thiocyanate produced is measured spectrophotometrically and the effective intensity determined for the experiments.

$$KCr(NH_3)_2(NCS)_4 + h\nu \longrightarrow KCr(NH_3)_2(NCS)_3H_2O + NCS \quad (2.5)$$

$$NCS^- + Fe^{+3} \longrightarrow FeNCS^{+2} \text{ (yellow)} \quad (2.6)$$

**Preparation of Reinecke's salt solutions**

Reinecke's salt (Ammonium tetrathiocyanodiammonochromate) is commercially available as the ammonium salt. Photochemistry cannot be done with this salt because it is unstable in water solution; therefore, the potassium salt
is recommended for measurements. For conversion of the ammonium Reinecke's salt into the potassium salt, 0.2 grams of the ammonium salt are dissolved in 10 ml of warm water (40-50°C). An excess (0.2 grams) of solid potassium nitrate are added, then the solution is cooled and filtered in a Buchner funnel using Whatman paper #1. The product is recrystallized using 5 ml of warm water containing a few (3%) percent added potassium nitrate, filtered, washed with cold water and dried in a desiccator. Operations are carried in dim (red) light to obtain a minimum blank from the potassium salt.

Solutions of the Reinecke's salt of sufficient concentration to partially absorb (see Table 2-2) incident radiation were prepared. When 1.0 mg of the recrystallized salt are dissolved to 10 ml in 0.1 N H₂SO₄, the absorption of this solution at 565 nm is 0.2 absorbance units with the Cary 118 spectrophotometer. Fresh solutions were prepared for each experiment and the samples were exposed for at least an hour to the actinic beam because of the low extinction coefficient of the salt solution.

Photons absorbed by the samples

The extent of thermal or photochemical aquation of Reinecke's salt was determined by analysis for free thiocyanate ion. A 1.0 ml aliquot of the Reinecke's salt solution to be analyzed is mixed accurately to a volume of
Table 2-2
Extinction coefficient\textsuperscript{a} of Reinecke's salt\textsuperscript{b}

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Extinction Coefficient</th>
<th>Quantum Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>303</td>
<td>$1.01 \times 10^4$</td>
<td>-</td>
</tr>
<tr>
<td>520</td>
<td>106.5</td>
<td>0.286</td>
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<tr>
<td>545</td>
<td>90.5</td>
<td>0.282</td>
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<tr>
<td>585</td>
<td>43.8</td>
<td>0.270</td>
</tr>
<tr>
<td>600</td>
<td>29.0</td>
<td>0.276</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Solutions in 0.1 N H\textsubscript{2}SO\textsubscript{4}

\textsuperscript{b} Data obtained from Wegner and Adamson, 1966
5.00 ml of a daily prepared solution consisting of 0.1 M Ferric Nitrate in 0.5 M Perchloric Acid. The resulting iron-thiocyanate complex has an absorption coefficient of 4.30 x 10^3/M cm at 450 nm. The optical density at this wavelength gives the iron-thiocyanate ion concentration directly. A parallel control was made in the dark at the reaction instrument which is subtracted from the photochemical reaction, so that the thiocyanate released from photochemistry is determined by differential spectrophotometry. The concentration of photoreleased thiocyanate was obtained by dividing the corrected absorbance for the iron-thiocyanate complex by the extinction coefficient. This concentration times the volume of irradiated solution (1 ml), divided by the quantum yield and corrected for the volume of 1 ml in a L gave the molarity of photons absorbed for the irradiation time. This molarity of photons absorbed is multiplied by Avogadro's number (6.02 x 10^{23}), divided by the absorbance of the sample (.198), and divided by the irradiation time in seconds to obtain the number of photons absorbed per second per absorbance unit.

For a sample which ran an average time of 67 minutes the absorbance at 450 nm produced from the iron-thiocyanate complex was 0.099 and the control had an absorbance of 0.00. The reading of the photodiode/Keithley system was .101 x 10^{-7} amp. The photons absorbed by the sample are obtained
as explained in the paragraph above and substituted as follows:

\[ \frac{0.099 \times (6.02 \times 10^{23})}{4.3 \times 10^3} \left[ \frac{1000 \times (0.283) \times (0.198) \times (67) \times (60)}{1000 \times (0.283) \times (0.198) \times (67) \times (60)} \right] = 6.18 \times 10^{13} \text{ photons absorbed/sec per absorbance} \]

An average value of \(6.18 \times 10^{13}\) absorbed photons/sec per absorbance (standard deviation of 5.1% in 3 determinations) was obtained for the light intensity of the actinic beam producing a reading of \(0.101 \times 10^{-7}\) amp from the photodiode.

**Effective intensity**

Chemical actinometry provided the absorbed photons/sec in a sample of volume \(V\) and absorbance \(A\) (for 1 cm.) In order to obtain the second objective from actinometry one must ask: "What is the equivalent illumination intensity, \(I_o\), for directional radiation?" The absorbed photons in a sample obtained from actinometry are related to the intensity of the actinic beam by the following formula:

\[ I_o = \frac{(R_a \ln10)}{V} \quad (2-7) \]

where \(I_o\) are the photons/cm\(^2\) sec reaching the sample or illumination intensity for directional radiation; \(R_a\) is the absorbed photons/sec from actinometry in a sample of volume
V.  Ra was an average of $6.18 \times 10^{13}$ photons/sec, the volume of the sample was 1.00 cm$^3$ which gives by substitution an average of $1.42 \times 10^{14}$ photons/cm$^2$ sec from the source. These intensities are used to scale the abscissa (actinic beam) for the free-energy graphs.

Calibration of Detection Efficiency

The standard used in our measurements was Cresyl Violet Perchlorate with a quantum yield of 0.54. The absorption spectrum of cresyl violet perchlorate is shown in figure 2.4. A solution of 0.01 absorbance units at 564 nm was excited with a weak beam producing a photodiode reading of $0.11 \times 10^{-10}$ amp ($1.42 \times 10^{11}$ photons/cm$^2$ sec). The correction used for the different intensities for measurements with actinometry and Cresyl Violet are included in the next equation:

$$Io^R = \frac{Ra \times i(R)}{i(A)} \quad (2-8)$$

where Ra is the absorbed photons/sec from actinometry measurements ($6.18 \times 10^{13}$ photons/sec per absorbance); $i(R)$ is the current measured from the Cresyl Violet experiment ($0.11 \times 10^{-10}$ amp.); and $i(A)$ is the current from the actinometry experiment ($0.101 \times 10^{-7}$ amp.; $1.42 \times 10^{14}$ photons/cm$^2$ sec). The value for Io$^R$ calculated from this
Figure 2.4  Absorption spectrum of cresyl violet perchlorate in methanol.
Figure 2.4
The efficiency of the system was determined after the emission from the standard was calibrated for the system. At the low excitation rate described above, the emission from Cresyl Violet Perchlorate was 5,600 counts/sec. This signal was attenuated by a neutral density filter which decreased it by a factor of 8.163 and the 2-60 cutoff filter which decreased it by a factor of 1.933. Using the absorption of this sample at 564 nm as 0.01 and a quantum yield of 0.54, the detected absorption events for a standard having unit absorbance was calculated as \(1.64 \times 10^7\) count/sec \([(5,600 \times 8.163 \times 1.933)/(0.54 \times 0.01)]\). The amount of photons actually absorbed by the sample corrected for the actinometry and standard experiment was found to be \(6.73 \times 10^{10}\) photons/sec. When \(1.64 \times 10^7\) is divided by \(6.73 \times 10^{10}\), the efficiency of the system was obtained as \(2.43 \times 10^{-4}\) counts/photons. This efficiency is used to calculate the absolute emission of the samples from the raw emission as counts per sec.
Figure 2.5  Fraction of light passed by the Corning 2-60 filter. A solution of cresyl violet perchlorate with absorbance of 0.033 at 550 nm was used. The excitation wavelength was 550 nm. Both monochromators had a bandpass of 2.5 nm.
Figure 2.5
FREE-ENERGY DETERMINATION

The free-energy generated by the light-driven step was calculated using equation 1-7, as explained in the introduction:

\[ \Delta G = RT \ln \left[ \frac{R_{\text{lum}}}{R_{\text{therm}}} \right]. \] (1-7)

The gas constant, \( R \), is used with units of eV per Kelvin; \( 8.62 \times 10^{-5} \) eV/K. The absolute temperature for the experiments was 298K, room temperature.

The ratio of the emission rate to the excitation rate is defined as:

\[ \frac{\xi_{\text{lum}}}{\xi_{\text{therm}}} = \frac{R_{\text{lum}}}{R_{\text{therm}}} \] (2.9)

where \( \xi_{\text{therm}} \) is defined by:

\[ \xi_{\text{therm}} = \int I_{\text{bb}}(\lambda) \sigma(s) \, d(\lambda) \] (2.10)

and \( I_{\text{bb}} \) is defined by equation 1.9 and \( \sigma(s) \) is normalized to 1.0 at 680 nm as shown in Table 3.2

\[ \xi_{\text{lum}} = \frac{\gamma(s)}{\eta} A(s) \] (2.11).
\( \gamma(s) \) is the counts/sec from the experiment, \( \eta \) is the detection efficiency as computed on pages 53-60 and \( A(s) \) is the actual photosystem absorbance at 680 nm.

To determine the \( \xi_{\text{therm}} \), the black-body radiation between 651 and 693 nm for PS-I and between 651 and 684 nm for PS-II was calculated using equation (1.9). This \( I_{\text{bb}} \) was multiplied by the action spectrum for the corresponding wavelengths with the result being the thermal transition density. The fluorescence spectrum is scaled to this transition density (see equation 1.13 on page 25) and the scaling factor averaged for the wavelength interval used for the \( I_{\text{bb}} \) determination. The averaged scaling factor is multiplied by the fluorescence spectra starting at 696 nm for PS-I and 687 nm for PS-II to a final wavelength of 750 nm. This data is shown in Tables 3-2 and 3-3. The action data points were interpolated for each 3 nm from published graphs. The computer program used is listed in the appendix and was written in BASIC language which ran with an IBM PC microcomputer.

After summation of the emission spectra from absorption and fluorescence measurements, the \( \xi_{\text{therm}} \) value was obtained by multiplication of a factor to correct for the different units (nm) and the point collection at 3 nm intervals. The equation used for calculations is:

\[
\int I_{\text{bb}} (\lambda) \sigma(\lambda) \, d(\lambda) \approx \Sigma I_{\text{bb}} (\lambda) \sigma(\lambda) \Delta \lambda \quad (2.12)
\]
where $\Delta \lambda = 3\text{nm} = 3 \times 10^{-7} \text{cm}$. With the data for Scenedesmus, the value of the $E_{\text{therm}}$ for PS-I is $1.09 \times 10^{-7} \text{s}^{-1} \text{per cm}^2$ and the value for PS-II is $2.99 \times 10^{-8} \text{s}^{-1} \text{per cm}^2$.

Substitution in equation 2.9 for the ratio of the emission rate to the excitation rate and using equations 2.10 and 2.11.

$$\frac{E_{\text{lum}}}{E_{\text{therm}}} = \frac{\gamma(s)/\eta A(s)}{\int I_{bb}(\lambda) \sigma(s) d(\lambda)}$$

When the data from the experiments is substituted into the previous equation:

$$\frac{E_{\text{lum}}}{E_{\text{therm}}} = \frac{24,489}{2.43 \times 10^{-4}} (0.126)[2.99 \times 10^{-8}]$$

$$\Delta G = RT \ln (2.67 \times 10^{16})$$

$$= 0.97 \text{ eV}$$

where 24,489 is the counts/sec from the sample corrected for the 1.0 o.d. filter; $2.43 \times 10^{-4}$ is the efficiency; 0.126 is the PS-II absorbance at 680 nm (63% of a total absorbance of 0.2); $2.99 \times 10^{-8}$ is the thermal transition rate. The $\Delta G$ of 0.97 eV is for time delay of 20-250 msec.
after illumination.

DATA COLLECTION

The light source and PMT power supply were stabilized for one hour prior to experiments. During this hour, the samples were suspended in methyl-cellulose medium and their optical density adjusted. The conditions of the dark level counts of the PMT were tested before the experiments for the optimum level of 5-15 counts/sec. The Keithley/photodiode system was used to adjust the light intensity required for each particular experiment.

The program used to run the experiments for DLE measurements was written by graduate student Bilal Ezzeddine, and is shown in the appendix. The program first requests any data to be taken from a disk as: "DISK DATA? <y/n>". The input given is no, "N", whenever a new data set will be collected. After the data question, the program will ask for an "EXPERIMENT TITLE?:". The title is given with sample identification and information about the wavelength and intensity of exciting light and the emission band was described with the date of the run. The various parameters entered into the program are shown in Tables 2.3 and 2.4 for reference and sample, respectively. These parameters are all given in seconds with prompts from the computer as shown next:
After the time parameters the number of points (bins) and cycles are given for each experiment:

NUMBER OF BINS AVAILABLE? 250
REFERENCE: # OF CYCLES? 40
# OF REF. ADAPTATION CYCLES? 5
SIGNAL : # OF CYCLES? ≥ 100
# OF SIG ADAPTATION CYCLES? 60

The samples were subjected to alternating periods of 6.0 sec of illumination and 4.0 sec of darkness. Samples were adapted for this light regime for 10 min before the start of data collection, which then continued for several hundreds cycles for each sample. The emission shutter was opened just after the excitation shutter was closed, and was then closed for a 1.0 sec measurement of dark counting rate prior to the next period of illumination. Data collection began 7 msec after the excitation shutter was half-closed, to reduce the spurious signal (Schmidt, 1987) due to luminescence from the glass parts of the instrument, which was observable for an additional 50 msec following
excitation at high light intensities. A control for background counting rate was observed with a blank sample containing growing medium with methyl-cellulose at the integrating sphere prior to each run of data collection; this is called the reference.

Action spectra for excitation were obtained using interference filters having bandwidths of 9-12 nm for half-maximum transmittance. The period of dark adaptation and the timing of the sequence of light/dark/background of each cycle was the same as that of the previous experiments (see Tables 2-3 and 2-4). For each filter, the luminescence was observed at three or more different excitation intensities. Interpolation on a log-log plot of emission intensity versus excitation intensity, shown in figure 2.6, was used to identify the excitation intensity producing the emission intensity chosen as the criterion for equal action. The action spectrum was obtained from the reciprocal of the intensity chosen as the criterion for equal action for each wavelength.

The relative shapes of the emission spectra were compared by measuring the DLE intensity from samples using only the Corning 2-60 filter, and with the addition of Schott colored glass filters having cutoff wavelengths of 695 and 715 nm.
### Table 2-3
Parameters for the reference

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<th>Sec</th>
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<td>3</td>
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### Table 2-4
Parameters for the sample

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<td>2</td>
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<td>0</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>.01</td>
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</table>
DATA ANALYSIS

The raw decay of DLE is difficult to analyze (Schmidt and Senger, 1987a). For this reason the average intensities during certain periods of time were analyzed and plotted. A computer program written by Robert T. Ross was used to analyze intensity for some time average of the DLE curve. This program was written in Fortran and ran at the IRCC of The Ohio State University. The program is listed in the appendix. The data files are prepared with the name that the program will read. A line is inserted at the beginning of the program with the number of the data files to be analyzed. The program will print first the name of the data file as given when the experiment ran. Then, it will print the number of data bins, usually 250. Then, the time range per bin as 0.01 sec/bin; the number of reference cycles with the dark average per sec; and the number of signal cycles with the dark average per sec are given. The data points as collected for the reference and the signal are shown next. After the data is listed, the statistical analysis for the data is shown. The reference and signal background is given first with the standard deviation. The signal corrected for the reference with the log of the signal intensity is given with the range or error for the signal. This $\log_{10} (\text{signal})$ and the range are used to plot the luminescence curves (see figures 3-1 and 3-2).
Figure 2.6  Log luminescence versus log excitation intensity for different wavelengths illuminating samples of Scenedesmus. The top 4 decays belong to the wild-type while the bottom 4 decays belong to the mutant samples. The horizontal lines (A — > and B — >) indicate an emission intensity used as criterion for identification of an excitation intensity at the abscissa of the plot. The reciprocal of the excitation intensity gave the action for each wavelength as shown on figure 3-7.
Figure 2.6
Another program used to analyze data was FA1DLE. The program is listed in the appendix and is written in Fortran, running with the same instructions as the DLEFIT program. The program gave the same data analysis as the DLEFIT program, and divided the data in cells or different groups which were analyzed for the time range of data collection. It also printed the log (signal) and the signal intensity with the standard deviation for the cell width.
CHAPTER III
RESULTS AND DISCUSSION

LUMINESCENCE FROM SCENEDESMUS

The luminescence studied from the samples was of wavelengths greater than 650 nm, since the Corning 2-60 filter used in all the experiments would pass light of those wavelengths. Figures 3.1 and 3.2 show raw DLE data obtained from samples of wild-type Scenedesmus cells. The strong signal from figure 3.1 was due to the higher light intensity (.11 x 10^{-7} amp.) used from the actinic beam when compared to the incident intensity (.11 x 10^{-10} amp.) of the low yield signal from Fig. 3.2, as incident intensity reported from Keithley/photodiode system. The first data point for each curve was obtained 7 msec after the emission shutter was closed and the curve represents a decay to 2.5 sec after the shutter was closed. The top part of the figures shows the raw signal as obtained without any processing of the data. The decay at the center is the signal with the background subtracted. The behavior of the reference control can be seen at the bottom part of the figures.
Figure 3.1  Strong luminescence signal from wild-type *Scenedesmus*. Output as obtained at the screen of the Commodore C-64 minicomputer after a sample run. The raw decay with background is shown first. The middle decay is corrected for dark level of the PMT, and the reference level is observed at the bottom.
Figure 3.1
Figure 3.2  Weak yield luminescence signal from wild-type *Scenedesmus*. The legend is the same as that from Fig. 3.1.
Figure 3.2
Previous luminescence measurements made in this laboratory (Marchiarullo, 1983) used an absorbance of 1.0 units because the original instrument had a low sensitivity. A new instrument was made with f/0.7 optics which reduced the amount of photosynthetic material (absorbance) required for reliable signals.

Most of the experiments for DLE were performed with an optical density (absorbance) of 0.2 units measured in the scatterer compartment of the Cary 118 and corrected for light scattering at 800 nm. This optical density was selected after experiments were obtained where signals could be processed for data analysis at low light intensities. These results are explained on figure 3.3. Experiments such as action spectra, where signals of high yield were needed, used an absorbance of no more than 0.3.

The dependence of the prompt and delayed fluorescence signal on the absorbance of the sample in the integrating sphere was observed using a suspension of wild Scenedesmus cells. Samples were excited with an intensity of 2.06 x $10^{12}$ photons/cm$^2$ sec. The absorbance of the samples was corrected for the light scattering effect by subtraction of residual absorption at 800 nm, and diluted when the absorbance was too high for accurate measurements. Data for the DLE curve of each absorbance from figure 3.3 was obtained from the intensity of the corrected signal at 10 msec after the shutter was closed. The prompt fluorescence
Figure 3.3  Dependence of luminescence intensity on sample absorbance. Samples used an excitation intensity of $2.06 \times 10^{12}$ photons/cm$^2$ sec of 564 nm light. Prompt fluorescence measurements used a 1.0 O.D. filter in the emission side. Sample is wild-type Scenedesmus.
Figure 3.3
gave a steady (flat) response; consequently, the average of the signal during the 2.00 sec of the measurement was used for the graph. The results are displayed in figure 3.3.

The kinetics of DLE from wild-type *Scenedesmus* with time is shown on figure 3.4. The sample absorbance is 0.2 units. The figure was obtained from the intensity of luminescence for different times after the shutter was closed, at four different intensities of excitation, each different from the other by a factor of 10. The data was analyzed by the FA1DLE program, being divided into sets and computed for that time range with the error for the intensity of the data points used during the time analysis. The number of cells used in FA1DLE was 20 for each luminescence data set analyzed. Two different data sets were collected for each light intensity, one for up to 2.5 sec and the other for up to 25 sec after illumination. Due to the high yield of luminescence at shorter times, the errors are not significant. More noise was observed at longer times because there was less signal with consequent larger error bars. The error bars in the figure are the standard deviation calculated from expected counting errors using Poisson's distribution with FA1DLE. The data was plotted as obtained from the output of the FA1DLE program. The software used to make the graph is SIGMAPLOT. The data was fed in columns which are the axis of the plot. One of the columns was defined as error column and the input
Figure 3.4  Time course of delayed luminescence for wild-type Scenedesmus. The exciting light was 564 nm with intensities starting at $3.69 \times 10^{14}$ photons/cm$^2$ sec and decreasing in intensity by a factor of 10. The emission side had a neutral density filter of 1.0 O.D.
Figure 3-4

LOG INTENSITY (counts/sec)

LOG TIME (sec)
obtained from FA1DLE.

The vertical axis of figure 3.4 was raw counts/sec as obtained from the intensity of the data analyzed by FA1DLE.

Figure 3.4 shows faster decay at short times for the higher light intensity curves. The results from the two higher light intensity experiments demonstrated similar decay kinetics. For the two top curves, the DLE intensity decays faster to about 10 sec, then the decay is less rapid. At $3.69 \times 10^{12}$ photons/cm$^2$ sec (third curve) the decay is less rapid, and the slope of the time curve does not change drastically throughout the curve. At the lowest intensity the response is almost flat; the high error for each point was due to the use of 1.0 optical neutral density filter at the emission side. The results from the time course of DLE are in agreement with previous measurements (Marchiarullo, 1983).

Figure 3.5 shows the intensity of delayed luminescence as a function of the exciting light intensity. This axis is corrected for absorbance and counting efficiency. The two top curves show the behavior of the wild-type at two different average delay times after the shutter was closed. The two lower curves show the corresponding behavior of the mutant. The two top curves for each pair were obtained from the average of the decay from 20 to 250 msec (bin or point 5 to 20) after the shutter was closed of each DLE curve as represented in figures 3.1 and 3.2. The two lower for each
pair curves was the average of 1.5 to 2.5 sec (bin or point 150 to 250) after closure of the shutter.

The two curves in Figure 3.5 for a particular culture diverge at high light intensity because of the more rapid rate of decay of relative luminescence intensity under these conditions. The two wild-type curves have greater separation than the two mutant curves, showing that the wild-type has larger rate of decay in the one-second time region.

The logarithmic curves showed linear behavior before saturation or a change in the slope of the curve. The behavior at low intensities does not obey the I^2 law as predicted. This behavior may be due to the use of continuous actinic source and not pulses which were found necessary (McCauley and Ruby, 1981) to observe square law behavior under certain conditions of actinic intensities.
Figure 3.5  Intensity of delayed luminescence from *Scenedesmus* as a function of exciting light intensity. Both axes are calibrated for absorption cross-section in dilute solution at 564 nm. Upper curve of each pair is the average intensity 20 to 250 msec after illumination; lower curves of each pair is for 1.5 to 2.5 s after illumination.
Figure 3.5

LOG INTENSITY OF LUMINESCENCE (photons/cm² sec)

WILD

MUTANT #11

LOG INTENSITY OF EXCITING LIGHT (photons/cm² sec)

FREE ENERGY (eV)

0.95
0.89
0.83
0.77

Figure 3.5
The compensation point is the light intensity at which the rate of CO₂ fixation from photosynthesis is equal to the rate of CO₂ consumption by respiration (Rabinowitch, 1951). This compensation point for *Scenedesmus* was found to be at an intensity of 0.3 W/m² or $8 \times 10^{13}$ photons/cm² sec. For simple enzyme kinetics, a log-log plot of velocity versus substrate concentration will have a slope of 1/2 when half of the catalytic sites are occupied and the velocity is half of the maximum. For the 20-250-ms curve of the wild-type, the log-log plot of Fig. 3.5 has a slope of 1/2 at an excitation intensity of $8 \times 10^{13}$ photons/cm² sec, which is equivalent to 0.3 W/m² [230 lux]. Both curves for the mutant have a slope of 1/2 at an intensity of $7 \times 10^{13}$ photons/cm² sec, and the 1.5-2.5 second curve for the wild-type has this slope at an intensity of $1 \times 10^{13}$ photons/cm² sec.

The results from the mutant are in agreement with those of Bertsch and collaborators (1967). They found that emission from the mutant was 250 times less intense than that from the wild-type between 0.75 and 4.2 sec with saturating light intensities. I found a ratio of 310 times at intensities higher than the compensation point. The differences between this result and that of Bertsch and collaborators are small and may be due to the fact that Bertsch used absorbance 10 times higher with the cells grown heterotrophically in the dark.
The luminescence from wild Scenedesmus was used to refine a previous determination of the free-energy generated by PS-II of eukaryotes. The weak luminescence from mutant #11 of Scenedesmus was used to place a limit on the free-energy generated by PS-I.

The vertical scale on the right side from Fig. 3.5 shows the ΔG for the light-driven step, calculated from eq. 1-7 with substitution into equation 2-13 as explained on pages 63-65. The free-energy was determined assuming that wild-type Scenedesmus emission is entirely from PS-II, that 63% (Melis et al., 1987) of the absorbance at 680 nm is due to PS-II, and that the action and emission spectra are as described in Marchiarullo and Ross (1985) and Melis, et al. (1987).

Figure 3.6 compares the thermal transition density calculated for the wild-type and mutant Scenedesmus. PS-I thermal transition density is 3.6 times higher than that of PS-II, which was due to the longer wavelength action of PS-I as shown on Tables 3-1 and 3-2. The area under the curve for the thermal transition densities are used as the denominator of eq. 2-13 for the free-energy determination.

At an excitation intensity of 0.3 W/m², the intensity of emission from the wild strain 10 ms after excitation corresponds to a ΔG of 0.99 eV. This value is in agreement with a previous determination (Marchiarullo and Ross, 1981). The intensity of emission from the mutant is 310 times less
than that from the wild-type. Were this emission due entirely to PS-II, it would correspond to a $\Delta G$ of 0.83 eV.; were it is due entirely to PS-I, it would correspond to a $\Delta G$ of 0.81 eV.

Action spectra were obtained as explained on page 68. Figure 2.6 shows the log-log plot of emission intensity versus excitation intensity used to determine the action. The top four decays from the figure belong to the wild-type while the bottom decays belong to the mutant. The horizontal line across the decays indicates the emission intensity which would be the criterion for the action. At the intercept of the decays with the line, an excitation intensity was obtained. The reciprocal of the excitation intensity for each wavelength resulted in the corresponding action.
Figure 3.6 Thermal transition density graph for PS-I and PS-II.
Figure 3.6
Table 3-1
Data for $R_{\text{therm}}$ calculation for PS-I

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<tr>
<th>WAVELENGTH (nm)</th>
<th>ACTION SPECTRUM</th>
<th>FLUORESCENCE SPECTRUM</th>
<th>THERMAL TRANSITION</th>
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$R_{\text{therm}} = 1.09E^{-7} \text{ photons/cm}^2 \text{ sec}$
### Table 3-2

Data for $R_{\text{therm}}$ calculation for PS-II

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$R_{\text{therm}} = 2.99E^{-8} \ \text{photons/cm}^2\ \text{sec}$
Fig. 3.7 compares the long-wavelength action spectra of mutant and wild samples. The action spectrum of the mutant resembles that of PS-II and the difference shown is close to the experimental error. Since little if any of the luminescence from mutant shows PS-I characteristics, the contribution from PS-I to mutant luminescence should be determined.

In order to obtain the contribution of PS-I to mutant luminescence, the emission characteristics of the mutant samples were studied under several conditions. Table 3-3 shows the relative emission in different wavelength bands from wild and mutant samples following excitation at different wavelengths. The estimate of the emission from PS-I was based on the assumptions (1) that all of the delayed emission from the wild sample is from PS-II and (2) that between 30 and 50% of the prompt emission from the wild sample is from PS-I.

The two empirical values of wild delayed and prompt were used in the following formula to obtain the estimated PS-I relative emission.

\[ F_{PS-I} = \frac{[F_{prompt} - F_{dle}(1-x)]}{x} \]  \hspace{1cm} (3-1)

where \( F_{PS-I} \) is the estimated PS-I luminescence from green algae samples excited with 564 nm light. \( F_{prompt} \) is the prompt fluorescence as obtained in the second row and \( F_{dle} \)
Figure 3.7  Action spectra for mutant and wild-type *Scenedesmus*. Action spectra normalized to 680 nm. The average for three different times from the decay curves (10 msec, 500 msec and 2.0 sec) was used for each point in the graph.
Figure 3-7

A C T I O N

1.000

0.100

0.010

675 680 685 690 695 700 705 710

WAVELENGTH (nm)

WILD

MUTANT # 11
is the DLE yield as obtained from the first row in the table. The variable $x$ is the known number of PS-I reaction centers to estimate its contribution to the luminescence.

The two numbers in the third column for each band of emission represent a high (50%) and low (30%) value of PS-I reaction centers (Melis et al., 1987).

Factor analysis provided a method for estimating the amount of PS-I luminescence from the mutant samples. The data described as mutant delayed in Table 3-3 was placed in a three-way array (Lee, 1988). The array consisted of three excitation wavelengths (670, 688 and 708 nm) by three emission bands (emission longer than 650 nm, longer than 695 nm and longer than 715 nm), by ten time delays since illumination. The luminescence for the ten time delays was measured in 10 periods of equal width in log(time).

This array was subjected to PARAFAC3 analysis (Lee, 1988). The two-component results showed one of its components to be very similar to the one-component result, and a weaker component equal to 20% of it. It was not clear that the weaker component was real, therefore the estimated PS-I is less than 20% of the total emission with excitation at any of the three wavelengths.
Table 3-3
Relative emission from wild and mutant #11 with different wavelength bands

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<tr>
<td>564</td>
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Excitation at 708 nm favors PS-I over PS-II by a factor of 10 (Kelly and Sauer, 1965; Reid, 1972) when compared to excitation at wavelengths shorter than 680 nm. The two estimates from above will place the emission from PS-I to be less than 2% of the total emission from 10 ms to 2.5 s caused by excitation at wavelengths shorter than 680 nm. Very rapid decay kinetics will decrease the detectability of PS-I emission no more than a factor of 2 or 3. Accordingly, it is inferred that emission from PS-I is less than 5% of the 10 msec emission in the mutant samples.

What is the actual source of emission? Among several possibilities it could be assigned to: (1) contaminant wild-type; (2) residual activity of mutated PS-II; (3) a second kind of PS-II; (4) PS-II; (5) some source other than the energy storage pathway of a photosystem. It was found that the emission from the mutant and wild strains have different kinetics, which argues against the mutant emission being due to contamination from wild type. On the other hand, the mutant and wild strain have similar excitation spectra, emission spectra and saturation curves. It could be speculated that mutant emission is from a second kind of photosystem II, such as the non-B type which has been discovered lately (Jursinic, 1987 and Schreiber, 1984).
The action spectrum for mutant luminescence is at shorter wavelengths than that of PS-I and emission is at longer wavelength than that of PS-II. This evidence points to a photosystem with emission at longer wavelengths than PS-II which could be a second kind of PS-II as discussed earlier.

A mutant sample was incubated in an anoxygenic environment with hydrogen under three atmospheres of pressure. The luminescence intensity of hydrogen-treated samples was of the same yield as those samples grown heterotrophically in the dark and used for experiments. Hydrogen is known to inhibit PS-II (Lavorel, 1969).

If PS-I comprises 5% of this emission, its $\Delta G$ would be $0.81 \text{ eV} + kT \ln(0.05)$, or 0.73 eV. This becomes the provisional upper limit on the free-energy generated by PS-I from eukaryotes.
Discussion

The decay curves of DLE have a complex behavior. The complex behavior is due to the different species formed after excitation of reaction centers (Mathis and Paillotin, 1981). These different species will rereduce oxidized reaction centers (Jursinic, 1986) to give the observed complex behavior of luminescence.

At higher light intensities the pools of oxidized donors and reduced acceptors are larger, so they decrease more rapidly when excitation is removed. DLE is a phenomenon predominantly originating from PS-II (Govindjee and Jursinic, 1979). Reaction centers and antenna pigments are involved in the luminescence process (Schmidt and Senger, 1987) as in from the original DLE spectra such as Azzi (1966).

The metastable chemical states whose back reaction leads to DLE consists of several different components, causing the time-decay kinetics to be complex. DLE after a short time delay is due to species formed rapidly after light absorption (Jursinic, 1986). The quinone pool and oxidized reaction centers are responsible for DLE in microseconds after the actinic light is turned off. The slower components of DLE are associated with recombination of the $S_2$ and $S_3$ states of the oxygen-evolving complex. The cytochromes and other components such as NADPH will release electrons back to the reaction centers at longer times with
the result of the long term DLE.

The free energy determined by this method is the driving force from the photosystems available for chemistry after light absorption, as explained in the introduction (see page 25). On the same page it is reasoned that the photosystem with shorter wavelength action, PS-II, would store more energy than the one with longer wavelength action, PS-I. The reason for the low energetics of PS-I is still unknown. As a consequence of the low energetics, a low luminescence yield characterizes PS-I.

Various other methods can be used to estimate this driving force which moves electrons in the photosynthetic chain. The midpoint redox potentials are used to position (Blankenship and Prince, 1985) members of the electron transport chain in a diagram known as the Z scheme. For that diagram the energy input from light is described by the standard potential of the primary donor and acceptor involved in the photochemical reaction.

The redox properties of the donor and acceptor sides of PS-I (Cramer and Crofts, 1982) are better known than those of PS-II (Avron, 1981). However, little is known about the working potentials of these donors and acceptors (Allen and Holmes, 1986), which can differ from the standard potentials by 0.10 volts or more.

Other methods have been used to estimate the energetics of electron transport. Seely (1978) used polarographic data
to evaluate ground-to-excited-state electrochemical potentials of chlorophyll and other compounds. The potentials presented are not physiological potentials.

With a stochastic modeling of light-energy conversion Ross and collaborators (1976) determined that primary donor and primary acceptor half-cells must act at very nearly the same potentials. The forward reactions need to be many times the net rate, therefore, limits are imposed in the concentration of the species involved. These limits work by adjusting the species concentration to minimize free-energy losses.

The free energies reported here may appear to be inconsistent with the known properties of PS-I and PS-II. In particular, the primary donor of PS-I has a midpoint potential of +0.45 V, and early acceptors have midpoint potentials between -0.5 and -0.7 V, suggesting a free energy change of 1.0 V or more. This may seem to be in conflict with our finding that the actual free energy change in PS-I is 0.7 volts or less.

The answer to this apparent conflict lies in the large difference between midpoint potentials and actual working potentials in efficient photochemical systems (Allen and Holmes, 1986). We recall that

\[ E_{\text{phy}} = E^0 - 0.059/n \log([\text{red}]/[\text{oxd}]) \]  

(3-2)
where $E^\circ$ is the standard electrode potential, [red] and [oxd] are the concentrations of the reduced and oxidized forms of the electron carrier, and $n$ is the number of electrons transferred.

Most of the donor P700 should be in the reduced state, ready for photochemistry. If the ratio $[P700]/[P700^+]$ is between 100 and 1000 at light intensities just sufficient to drive net photosynthesis, then the corresponding potential of the primary donor is between +0.27 and +0.33 V. The secondary donor plastocyanin has a midpoint potential of +0.37 V (Sanderson, et al., 1986).

The acceptor side of PS-I has several very transient components which must be mostly in the oxidized state, ready to accept an electron from the excited P700. Being highly oxidized at low light intensities, these components will have working potentials which are much less negative than their midpoints. These early acceptors are then followed by ferredoxin ($E^\circ'=-0.42$) and NADP ($E^\circ'=-0.33$). The NADP pool is 50% reduced in illuminated chloroplasts during CO₂ fixation (Krause and Heber, 1976).

With this reasoning, we suggest that PS-I under weak illumination operates between +0.3 and -0.4 volts, for a free energy change of 0.7 eV, in agreement with the results obtained from our luminescence measurements.
Table 3-4

Table of redox potentials of components of the electron transport chain

<table>
<thead>
<tr>
<th>Component</th>
<th>$Em$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P680 complex</td>
<td>$\sim1,000^a$</td>
</tr>
<tr>
<td>Pheophitin</td>
<td>$-500^a$</td>
</tr>
<tr>
<td>Quinones</td>
<td>0-100$^a$</td>
</tr>
<tr>
<td>Plastoquinone</td>
<td>$-$</td>
</tr>
<tr>
<td>FeS (PS-II)</td>
<td>$+290^b$</td>
</tr>
<tr>
<td>Cytochrome b557</td>
<td>$+60^b$</td>
</tr>
<tr>
<td>Plastocyanin</td>
<td>$+370^c$</td>
</tr>
<tr>
<td>P700</td>
<td>430-530$^b$</td>
</tr>
<tr>
<td>Fe-S (PS-I)</td>
<td>$-360^d$</td>
</tr>
<tr>
<td>Ferredoxin</td>
<td>-430</td>
</tr>
</tbody>
</table>

$^a$ Blankenship and Prince, 1985
$^b$ Avron, 1981
$^c$ Sanderson, et al., 1986
$^d$ Cramer and Crofts, 1981
LUMINESCENCE FROM *ANACYSTIS*

The decay of luminescence from samples of *Anacystis* has a similar complex behavior to that from *Scenedesmus* (examples in figures 3.1 and 3.2). The absorbance of the samples for *Anacystis* was the same as those from *Scenedesmus* at 680 nm. As we discussed in the Introduction, DLE is a phenomenon mainly associated with PS-II. The evidence obtained so far describes the members of the photosynthetic machinery in cyanobacteria as being the same as those in eukaryotes (Kawamura and Fujita, 1983). Action and emission spectra for both organisms seemed very similar (Arnold and Thompson, 1955) with emission predominantly from chlorophylls. Thus, the origin of DLE from *Anacystis* should be from the same pool as that from eukaryotes, which is discussed on pages 103-106. Nevertheless, the intensity of luminescence from samples of *Anacystis* was of lower yield and had different time-kinetics than those of *Scenedesmus* under the same experimental conditions; see figure 3.8.

In cyanobacteria the number of PS-II reaction centers is equal to those of PS-I (Vierling and Albereto, 1980). On the contrary, for eukaryotes the number of PS-II units is 1.6 times that of PS-I (Melis et al., 1987). It is not certain how the different number of reaction centers for both samples would affect the yield and kinetics of DLE.
Figure 3.8  Time course of delayed luminescence from wild-type Scenedesmus and normally-grown Anacystis. Absorbance of the samples was 0.3 optical units at 680 nm. The intensity of exciting light was $3.69 \times 10^{14}$ photons/cm$^2$ sec of 564 nm light. Samples with a 100 sec cycle were excited for 60 sec with 40 sec in dark for which 10 were used to monitor PMT dark level and 25 sec for luminescence measurements. Samples had 5 cycles of adaptation time and 10 cycles of experiment. The Scenedesmus sample had a 1.0 od neutral density filter in the emission side. For Scenedesmus the vertical scale is for raw counts if the neutral density filter had not been present. The Anacystis data was collected on March 22, 1988. The luminescence intensity emitted by samples of Anacystis from this figure is 13 times larger than the intensity of Anacystis samples used for free-energy measurements as shown in figure 3.10. The explanation for this discrepancy is unknown.
Figure 3.8
The time decay of samples from Anacystis has a different kinetics from those of Scenedesmus as shown in figure 3.8. The decay at short times from an exciting intensity of $3.69 \times 10^{14}$ photons/cm$^2$ sec is less rapid for Anacystis. As observed from the figure the emission of normally-grown Anacystis samples was about 10 times less than those of wild Scenedesmus.

The time course of DLE from Anacystis is shown on figure 3.9. At high light intensities of $3.06 \times 10^{13}$ and $10^{14}$ photons/cm$^2$ sec, the decay diverges less than the decays at the two lowest exciting light intensities and a change in slope is observed at the one second time region. The third and fourth curves showed little change in slope at most of the times. At an intensity of $3.69 \times 10^{12}$ photons/cm$^2$ sec the yield is relatively less when compared to the two upper curves. At the lowest exciting intensity ($3.69 \times 10^{11}$ photons/cm$^2$ sec) greater separation from the upper curves is observed. At longer times, the decays from the three higher light intensities converge into a common yield, but the DLE intensity for the lowest exciting light does not show such a convergence.

Figure 3.10 shows the behavior of luminescence as a function of exciting light intensity for samples of Anacystis. The two top curves represent the behavior of the normally-grown Anacystis while the two bottom curves are for the calcium-depleted. The top curve is the average of the
Figure 3.9  Time course of delayed luminescence from normally-grown *Anacystis*. Exciting light as described on figure 3.4. Experiments for the two top curves used a cycle time which is 10 times longer than for the two bottom curves; the short time cycle is explained on pages 66-67. The long term-DLE (top figures) used 5 cycles of adaptation and 10 cycles of data collection. The two bottom curves used cycles explained on page 67. This data was collected on March 22, 1988 on a sample having an absorbance of 0.3 optical units.
luminescence from 25 to 250 msec after cessation of actinic light. The bottom curve represent the average of 1.5 to 2.5 sec. The saturation point for the normally-grown *Anacystis* is at a different intensity than that of wild *Scenedesmus*. For the 25-250 msec *Anacystis* decay this point is at about 5 x 10^{12} photons/cm^2 sec. This intensity is 16 times less than that for green algae. At the lowest exciting intensities, the 25-250 msec curve has a limiting slope of 1.5. The emission from normally-grown *Anacystis* diverges at high light intensities due to more rapid decay of DLE.

The calcium-depleted curve shows 6 times less emission than normally-grown *Anacystis* at the saturation point for the 25-250 msec curve. No saturation is observed for the slow and fast curve of the depleted *Anacystis*. The linear behavior of the curves could be due to PS-II activity. Therefore, the presence of PS-II luminescence masks that of any PS-I, and this system could not be not used to determine PS-I energetics.

The vertical scale of figure 3.10 was calibrated assuming that luminescence from normally-grown *Anacystis* is from PS-II and that 50% (Vierling and Alberte, 1980) of the reaction centers belong to this photosystem. The procedures for the free-energy determination were as for *Scenedesmus*. Table 3-5 shows the data used for the thermal transition density determination. Action spectrum were obtained from
Figure 3.10  Intensity of delayed luminescence from normally-grown Anacystis as a function of exciting light intensity. The legend as from figure 3.4. The normally-grown Anacystis had an absorbance of 0.3 optical units and was collected on July 27, 1987. The calcium-depleted specimen had an absorbance of 0.3 optical units and was collected on July 06, 1987.
Figure 3.10

LOG INTENSITY OF LUMINESCEENCE
(photons/cm² sec)

LOG INTENSITY OF EXCITING LIGHT (photons/cm² sec)

FREE ENERGY (eV)
Wang and collaborators (1977) and fluorescence spectrum from Azzi (1966). The data was substituted into equation 2.13 for scaling of the figure. At 0.3 W/m², the compensation point for green algae and Anacystis (Rabinowitch, 1951), the intensity of luminescence from the normally-grown Anacystis samples provided a ΔG of 0.88 electron volts. This value is 0.11 electron volts less than that of PS-II from green algae.

Figure 3.11 compares the action spectra from samples of mutant Scenedesmus and depleted Anacystis. The action overlaps to a wavelength of 700 nm which express emission from PS-II. In addition, the light curves of figure 3.10 showed that emission from Ca⁺²-depleted samples do not present PS-I behavior. Therefore, a different strain will be used to measure the free-energy generated by PS-I.

Discussion

The less pronounced decay at short times for Anacystis could be due to the less number of PS-II reaction centers when compared to Scenedesmus.

In addition the pigments for light harvesting are different for both organisms. With the presence of phycobilisomes and the absence of chl b, cyanobacteria use chl a for reaction centers I and II. The effect of the different pigments in the DLE intensity and kinetics cannot be determined at this point.
Table 3-5
Data for Rtherm calculation for *Anacystis* PS-II

<table>
<thead>
<tr>
<th>WAVELENGTH (nm)</th>
<th>ACTION SPECTRUM</th>
<th>FLUORESCENCE SPECTRUM</th>
<th>THERMAL TRANSITION</th>
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<td>0.79</td>
<td>8.49E-5</td>
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<td>0.101</td>
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</tr>
<tr>
<td>751</td>
<td>-</td>
<td>0.107</td>
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</tr>
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</table>
Figure 3.11 Action spectra for mutant #11 from Scenedesmus and Ca$^{2+}$-depleted Anacystis. Action was normalized to 680 nm.
Figure 3.11

- ○ ○ Mutant #11
- △ △ Anacystis Ca$^{+2}$-depleted
LUMINESCENCE FROM APHANOTHECE

It has been a major objective of the work presented here to find the intensity of luminescence from samples where signals from PS-I and PS-II could be distinguished. This could lead to the determination of the free-energy generated by each photosystem separately during the photoact.

Because PS-II emission from prokaryotes is of lower yield, then the ΔG for the light-driven step is smaller by 0.11 eV compared to eukaryotes. Consequently, to compensate for this lack, the PS-I from prokaryotes may have a higher energetics than that of eukaryotes.

Among the cyanobacteria strains which perform anoxygenic photosynthesis, Aphanothece halophitica was selected for our experiments. In anoxygenic photosynthesis operating with PS-I, cyanobacteria have the advantage of surviving in sulphide-rich habitats. Since PS-II is known to be inhibited at low sulphide concentration (0.1-0.2 mM), cyanobacterial CO₂ photoassimilation insensitive to DCMU must be driven by PS-I with sulphide as an electron donor (Padan and Cohen, 1982).

Aphanothece removes two electrons from hydrogen sulphide to produce sulphur with subsequent extracellular sulphur deposition. The range of sulphide utilization at pH=6.8 by Aphanothece is 0.1-0.15 mM. The predominant ionic form of hydrogen sulphide is HS⁻. The first dissociation
constant (pKₐ₁) for hydrogen sulphide is near pH = 7.0. Above this pH, HS⁻ works with a reducing potential in the range of -0.2 to -0.3 V (Huxtable, 1986). The second dissociation constant for hydrogen sulphide is above pH=13. The typical pH of an Aphanothece environment is 7.3. The reaction in which sulphide is used as an electron donor differs from the photosynthetic water reaction in that oxygen is not evolved:

\[
2\text{H}_2\text{S} + \text{CO}_2 + \text{hv} \rightarrow 2\text{S}^0 + \text{H}_2\text{O} + (\text{CH}_2\text{O})
\]

Since sulphide is oxidized by oxygen, the sulphide concentration is influenced by oxygenic photosynthesis activity and oxygen diffusion. Therefore, an absolute anoxygenic environment is used for Aphanothece during sulphide treatment. Any small amount of oxygen in the reaction flask is oxidized by excess sulphide.

Due to the lower luminescence yield of the samples an absorbance of 0.3 was used for the measurements. This is 50% higher than for the other experiments but in the linear range of luminescence response at the integrating sphere (see figure 3.3).

Emission obtained from different wavelength bands are shown on Table 3-6 and showed that Aphanothece samples treated with sulphide behaved as predicted for PS-I emission characteristics (see Table 3-3). The emission
Table 3-6

Emission with different wavelength bands from *Aphanothece*

<table>
<thead>
<tr>
<th>Excitation (nm)</th>
<th>Emission cutoff filter (nm)</th>
<th>Oxygenic</th>
<th>Anoxygenic</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>650</td>
<td>695</td>
<td>715</td>
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beyond 715 nm is 30% of that from 650 nm, obtained from the 2-60 filter, which should be predominantly from PS-I. These results are different from those of samples from mutant *Scenedesmus* and calcium-depleted *Anacystis* which showed 10% emission beyond 715 nm. It will be assumed that emission from sulphide-treated *Aphanothece* is due to PS-I for free-energy determinations.

Is luminescence from anoxygenic *Aphanothece* due to PS-I? Previous experiments have demonstrated that PS-II is very sensitive to treatment with sulphide (Oren et al., 1979). No oxygen evolution or variable fluorescence are observed from treated samples, which assure that the oxygen-evolving complex is inhibited. Thus if PS-II is not functional with anoxygenic conditions, PS-I photochemistry provides the energetics for cells to obtain products from photosynthesis (Oren et al., 1979).

That luminescence observed from the samples is due to chlorophylls may be inferred from the emission distribution in Table 3-6.

The evidence obtained so far describes PS-I as the source of luminescence from samples of sulphide-treated *Aphanothece*. This assignment provides a tentative basis from determining the ΔG of this photoact in prokaryotes.

The pigments in *Aphanothece* are phycocyanin, allophycocyanin and chlorophyll which are the same as those in *Anacystis*. Cultures of *Anacystis* and *Aphanothece* had
Table 3-7

Data for Rtherm calculation for prokaryotic PS-I

<table>
<thead>
<tr>
<th>WAVELENGTH (nm)</th>
<th>ACTION SPECTRUM</th>
<th>FLUORESCENCE SPECTRUM</th>
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<td>0.96</td>
<td>0.98</td>
<td>1.31E-2</td>
</tr>
<tr>
<td>685</td>
<td>0.91</td>
<td>0.87</td>
<td>1.67E-2</td>
</tr>
<tr>
<td>688</td>
<td>0.78</td>
<td>0.75</td>
<td>1.91E-2</td>
</tr>
<tr>
<td>691</td>
<td>-</td>
<td>0.60</td>
<td>2.15E-2</td>
</tr>
<tr>
<td>694</td>
<td>-</td>
<td>0.38</td>
<td>5.52E-3</td>
</tr>
<tr>
<td>697</td>
<td>-</td>
<td>0.32</td>
<td>4.65E-3</td>
</tr>
<tr>
<td>700</td>
<td>-</td>
<td>0.26</td>
<td>3.78E-3</td>
</tr>
<tr>
<td>703</td>
<td>-</td>
<td>0.21</td>
<td>3.05E-3</td>
</tr>
<tr>
<td>706</td>
<td>-</td>
<td>0.19</td>
<td>2.75E-3</td>
</tr>
<tr>
<td>709</td>
<td>-</td>
<td>0.14</td>
<td>2.03E-3</td>
</tr>
<tr>
<td>712</td>
<td>-</td>
<td>0.13</td>
<td>1.89E-3</td>
</tr>
<tr>
<td>715</td>
<td>-</td>
<td>0.13</td>
<td>1.89E-3</td>
</tr>
<tr>
<td>718</td>
<td>-</td>
<td>0.12</td>
<td>1.74E-3</td>
</tr>
<tr>
<td>721</td>
<td>-</td>
<td>0.11</td>
<td>1.60E-3</td>
</tr>
<tr>
<td>724</td>
<td>-</td>
<td>0.10</td>
<td>1.45E-3</td>
</tr>
<tr>
<td>727</td>
<td>-</td>
<td>0.094</td>
<td>1.37E-3</td>
</tr>
<tr>
<td>730</td>
<td>-</td>
<td>0.089</td>
<td>1.29E-3</td>
</tr>
<tr>
<td>733</td>
<td>-</td>
<td>0.087</td>
<td>1.26E-3</td>
</tr>
<tr>
<td>736</td>
<td>-</td>
<td>0.083</td>
<td>1.21E-3</td>
</tr>
<tr>
<td>739</td>
<td>-</td>
<td>0.079</td>
<td>1.15E-3</td>
</tr>
<tr>
<td>742</td>
<td>-</td>
<td>0.075</td>
<td>1.09E-3</td>
</tr>
<tr>
<td>745</td>
<td>-</td>
<td>0.038</td>
<td>1.04E-3</td>
</tr>
<tr>
<td>748</td>
<td>-</td>
<td>0.068</td>
<td>9.87E-4</td>
</tr>
<tr>
<td>751</td>
<td>-</td>
<td>0.064</td>
<td>9.29E-4</td>
</tr>
</tbody>
</table>

\[ R_{\text{therm}} = 4.33 \times 10^{-8} \text{ photons/cm}^2 \text{ sec} \]
matching absorption spectra taken in our laboratory. The action spectra of PS-I and PS-II used for the free-energy determination are those of Anacystis taken from Wang and collaborators (1977). The DLE spectrum for PS-II energetics is from Anacystis obtained by Azzi (1966). For PS-I energetics the fluorescence spectrum is from 3-component decomposition of the prompt fluorescence spectrum of Anacystis of Lee (1988). This is the best data available for these organisms. Table 3-7 shows the data used to calculate the PS-II $R_{\text{therm}}$. Action spectrum data was used to a wavelength of 688 nm to obtain the scaling factor which produced the thermal transition to a wavelength of 751 nm. The value obtained for cyanobacteria PS-II $R_{\text{therm}}$ was $8.05 \times 10^{-9}$. When substituted in equation 2.13 for $\Delta G$ determination with 50% of reaction centers assigned to PS-II and all luminescence from oxygenic Aphanothece due to PS-II, the $\Delta G$ obtained from the yield is 0.90 electron volts. This $\Delta G$ was obtained with 470 cps/2.43 x $10^{-4}$ (0.15) $8.05 \times 10^{-9}$, where 470 cps is the signal at 10 msec after illumination, $2.43 \times 10^{-4}$ is the efficiency, 0.15 is 50% of 0.3 absorbance and $8.05 \times 10^{-9}$ is the thermal transition rate. In the same manner, the $\Delta G$ obtained for PS-I was 0.79 electron volts.

The PS-I free-energy is higher than that of eukaryotes. The reason may be that PS-II of prokaryotes provides less energy, which has to be compensated by PS-I to obtain stable products from photosynthesis.
Figure 3.12  Intensity of delayed luminescence as a function of exciting light intensity for oxygenic and anoxygenic Aphanothece. The two top curves are oxygenic Aphanothece and the bottom curve is anoxygenic. Figure legend is as from figure 3.5. The anoxygenic curve is the average of 20 to 250 msec after illumination.
Figure 3.12
Luminescence from the back reaction of the photosynthetic energy-storing systems is observed for hours after illumination. The intensity of luminescence is several thousands times less than the input. For this reason a very sensitive instrument with optics of f/0.7 was made to measure signals of low yield from the photosynthetic samples. An integrating sphere was used to illuminate samples uniformly and to collect luminescence efficiently. The integrating sphere provided uniform excitation in the samples, helping with the problems of high optical densities. The highly reflective surface of the Teflon sphere also provided for very sensitive detection of weak luminescence.

The integrating sphere allowed the use of samples with low absorbances. The absorbance used for the samples was of 0.2 units corrected for light scattering at the long wavelength edge.

The intensity of luminescence emitted by the energy-storing systems present in green and blue-green algae was used to determine the free-energy generated by photosystem I.
and photosystem II of these organisms. The luminescence from wild-type *Scenedesmus obliquus* was used to re-evaluate the free-energy generated by photosystem II of eukaryotes. Assuming that all luminescence from wild-type is due to PS-II and 63% of reaction centers are PS-II, the $\Delta G$ for the light-driven step is 0.99 eV at an excitation intensity of 0.3 W/m$^2$ of 564 nm light. This value was obtained at 10 msec after cessation of actinic light. This energetics was found at an excitation intensity close to the point where there is no gas (CO$_2$) exchange, the compensation point, for green algae.

The weak luminescence arising from mutant #11 of *Scenedesmus obliquus* was used to place an upper limit on the free-energy generated by photosystem I of eukaryotes. The emission is 310 times less than that of the wild-type at the compensation point. The luminescence from mutant samples did not show PS-I characteristics. Consequently, emission at different wavelengths from the samples was analyzed by the technique of three-mode principal component analysis. It was found that the emission from PS-I is less than 2% of the total emission from 10 msec to 2.5 s caused by excitation at wavelengths shorter than 680 nm. Very rapid decay kinetics would decrease the detectability of PS-I emission, but no more than a factor or 2 or 3. Thus, it is concluded that emission from PS-I is less than 5% of the 10 msec emission of mutant samples at any wavelength. If PS-I
comprises 5% of the emission then its ΔG should be 0.73 eV. This is an upper limit of the free-energy generated by PS-I of eukaryotes. For this calculation, thermal equilibrium within the excited state is assumed, which is questionable for PS-I but the best available estimate of the efficiency of this light-energy conversion process.

The luminescence from the cyanobacterium Anacystis nidulans was studied in a normal and in a calcium-depleted medium to determine the free energy generated by photosystem I and photosystem II of prokaryotes. The free energy generated from PS-II of normally-grown Anacystis was found to be 0.88 electron volts at the compensation point and 10 msec after cessation of illumination. This ΔG is 0.11 eV less than that of wild-type Scenedesmus and the saturation point (slope of 1/2 for log-log luminescence curve) is at an intensity 6 times smaller. The luminescence yield from normally-grown samples of Anacystis is 170 times less than that of wild-type Scenedesmus at 20-250 msec after illumination.

No saturation of luminescence was observed from the calcium-treated samples of Anacystis and the DLE intensity was of higher yield than that predicted for PS-I. Therefore, the cyanobacterium Aphanothece halophitica was treated with sulphide in another attempt to determine the energetics from PS-I in a functional system. The weak luminescence from anoxygenic samples of Aphanothece showed
PS-I characteristics. The intensity of the sulphide-treated samples is 200 times less than that of oxygenic samples. The intensity of luminescence at 10 msec after cessation of illumination provided a $\Delta G$ of 0.79 electron volts. This $\Delta G$ is 0.06 eV greater than the upper limit determined for eukaryotic PS-I.

The combined energetics for both photosystems from green algae and cyanobacteria results in 1.7 eV. That this addition gives the same result for both organisms tends to corroborate the values obtained for the two different organisms.

The potential difference necessary to move electrons from water to NADP$^+$ is 1.14 V. Under conditions of physiological ATP production, an estimated additional 0.49 V are required for ATP formation. Thus, 1.63 V is the estimate of the total potential difference used to obtain stable products in photosynthesis. The combined energetics reported here for PS-I and PS-II provides just enough energy for the stabilization and production of chemical compounds in the process.
LIST OF REFERENCES


APPENDIX

This appendix contains several computer programs used to collect and analyzed luminescence data. The programs used for data collection and analysis of components are included in C. H. Lee dissertation. The program DLE written by B. Ezzeddine in BASIC ran a microcomputer Commodore C-64 for data collection and is displayed on pages 141-145. The programs DLEFIT and FA1DLE written by R. T. Ross were used for data analysis and are displayed on pages 146-157. The program used to calculate free-energy values is displayed on pages 158-159.
141

PRINT"WELCOME TO DFL"
PEEK(2)=25 THEN 3
POKE 255,116;POKE 56,116;POKE 64,0;POKE 64,116;CLR
REM ***** SUBROUTINE READ VARIABLE LIST & INIT USER PORT *****
PA=5676;PB=5677;AB=5678;BB=5679;FF=5680;HI=5681;LO=1;F=199;LP=195
POKE AB,65;POKE PA,HP;POKE BB,0;RETURN
REM ***** SUBROUTINE FOR BYTE OUTPUT *****
POKE BB,FF;POKE PB,AD;POKE PA,LP;POKE PA,HP;RETURN
REM ***** SUBROUTINE FOR CLEARING FLAG2 *****
PRINT("FD")
REM **** SUBROUTINE FOR WAITING ON FLAG2 *****
NEXT I
BS=STRS(B):BS=MIDS(BS,3,LEN(BS))
BS="0"+BS:11=11-1:
IF BS="." OR BS="E" THEN 312
BS=BS+"."314 PRINT#2,BS316 RETURN
IF B=0 THEN B=.001318
11=0
B=B*10:11=11+1:IF B<.01 THEN 320
BS=STRS(B):BS=MIDS(BS,3,LEN(BS))
BS="0"+BS:11=11-1:
IF BS="." OR BS="E" THEN 324
BS="."+BS327 GOTO 312
600 REM ***** SET UP TEST *****
REM ** A()=TEMP. B()=PLOT. C()=ORIG DARK. DO=ORIG DATA.**
REM **603 IS=21:DIMA(256).B(256),C(256),D(256),E(256):GOSUB 2321:GOSUB2311
604 PRINT"ENTER EXPERIMENT TITLE:";TS:PAUSE
605 GETAS:IF AS<>"Y" THEN 608
606 IF AS<"N" THEN G0T0 608
607 PRINT"ENTER BACKG. DATA COLLECTION TIME";D1:PRINT"CYCLE TIME";TT:PRINT".*
611 IF D1+PP+D2<TT THEN 6
NB=250:INPUT "NUMBER OF BINS AVAILABLE";NB:
IF NB>250 THEN 616
617 IF NB=0 OR NB>250 THEN 616
618 IF NB=1
620 TR=0:INPUT "REFERENCE";A:PRINT"# OF CYCLES";T:PRINT"TAB(26);TR:PRINT",";TAB(26):TR
621 AK=0:INPUT ",# OF REF. ADAPTATION CYCLES";AK:PRINT",";TAB(29):AK
622 TS=1:INPUT "# SIGNAL ";# OF CYCLES";TS:PRINT"";TAB(26):TS
623 IF TS=1 THEN 422
624 NA=0:INPUT ",# OF SIG. ADAPTATION CYCLES";NA:PRINT"";TAB(29):NA:AS=NA+TT
625 FOR X=0 TO 255:RA=X:RB=X:RC=X:RD=X:RE=X:NEXT X:CA=0:KA=0:SC=0:SD=0
626 REM ***** REFERENCE RUN *****
629 TR=0:INPUT "THEN RK=1:ET=TR;NR=A":GOTO450
630 REM ***** SIGNAL RUN *****
632 RK=0:ET=TS:NR=NA
650 PRINT"REPLACE SAMPLE":
651 IF RK=1 THEN PRINT"REFERENCE";
654 IF RK=0 THEN PRINT"SIGNAL";
659 PRINT"IN BOID";
662 PRINT"& PRESS 'F'";
665 GETA:IFA<="Y" THEN 658
669 PRINT"A",TAB(2):"LUMINOMETER PARAMETERS ARE ";ST

141
142

662 IF RK=0 AND TR<>0 THEN 740
700 REM ***** COMPUTE PARAMETERS ****
723 W1=0:W2=0:W1<>N THEN W1=NB
733 MT=W1:GOSUB200:W1=MT:AI=DD
740 REM ***** LIST LUMIDMETER PARA **
751 PRINT TAB(14):IF RK=1 THEN PRINT"(REFERENCE)" ELSE PRINT"(SIGNAL)"
744 PRINT"CYCLE TIME =":TT:"SEC"
746 PRINT"EXCITATION TIME =":PP:"SEC"
725 REM ****** ADAPTATION MODE ="AK"
749 PRINT"PERIOD BINS SEC/BIN SEC"
743 PRINT"TRIGGER CYCLE TIME =":TT:"SEC"
747 GETA$:IF A$="" THEN 740
772 R=NM
748 PRINT"EXPERIMENT TYPE =":NE:"CYCLES.BB":"SEC"
750 PRINT"BBERIMENT =":NE:"CYCLES.BB":"SEC"
774 I$="";CHANGE:RUN "L":PRINT G:QUIT:--------:"
788 IF IN="" THEN 790
752 PRINT"DATA COLLECTION MODE =":AK
782 PRINT TAB(136);"RUNNING"
755 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
812 GOSUB2321
814 GETA$:IF A$="" THEN 814
820 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
821 REM ***** ADAPTATION LOOP ****
822 IF R=0 THEN 830
823 T$="000000"
824 IF R>0 THEN 830
825 GOSUB2311:T$="000000"
826 PRINT"DATA COLLECTION MODE =":AK
827 GOSUB2311:T$="000000"
828 IF R>0 THEN 830
829 IF R>0 THEN 830
830 REM ****** IS=21 ******
871 REM ****** ADAPTATION LOOP *****
872 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
874 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
876 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
878 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
880 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
882 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
884 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
886 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
888 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
890 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
892 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
894 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
896 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
898 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
900 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
904 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
908 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
912 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
916 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
920 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
924 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
928 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
932 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
936 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
940 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
944 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
948 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
952 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
956 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
960 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
964 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
968 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
972 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
976 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
980 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
984 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
988 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
992 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
996 PRINT"INSTRUMENT IS READY.";PRINT"PRESS ANY KEY TO BEGIN"
1000 IF RK=0 THEN 1100
1005 REM ****** READING DATA *****
1010 PRINT"READING DATA!!!!"
DLEFIT

// JOB,
// REGION=4096K,TIME=1
// JOBPARM L=6G00,DISKIO=8000,V=E
// EXEC PLOTV,PSIZE=30,
// PARM,FORT="NODECK,NOLIST,OPT(0),LANGLVL(77),NCDP,SRCDLG*",
// PARM,LKED="LET,LIST*",
// TIME=60=1
// SYSIN DD *
C

************

PLOTGRAM DLEFIT

EDITION OF 03 MAR FEB 87

INSTRUCTIONS FOR PREPARATION OF DATA

1. TYPE: USE DATASU UNNUM
   (WHERE "DATASU" IS DATA-SET-NAME FROM C-6M)

2. TYPE: X LUCASE
   FOLLOW INSTRUCTIONS, USING "1" FOR FIRST LINE, "L" FOR LAST

3. TYPE: INSERT 0,5
   INSERT THE NUMBER OF DATA FILES

4. TYPE: SAVE DLEDAT UNNUM

5. TYPE: USE DLEFIT CLEAR

VARIABLES IN COMMON

*****

NFEFCY NUMBER OF REFERENCE CYCLES
NS1GCY NUMBER OF SIGNAL CYCLES
BG5SIG BACKGROUND COUNTS/SEC FOR SIGNAL DATA
BG5REF BACKGROUND COUNTS/SEC FOR SIGNAL DATA
NDATA NUMBER OF DATA BINS
TEIN TIME PER BIN (SEC/CYCLE)
REF REFERENCE DATA (COUNTS/SEC, TRUNCATED)
SIG RAW SIGNAL DATA (COUNTS/SEC, TRUNCATED)
REF1 REFERENCE INTENSITY (BACKGROUND CORRECTED)
REF2 REFERENCE INTENSITY (REF1 SMOOTHED)
SIG1 SIGNAL INTENSITY (BACKGROUND CORRECTED)
SIG2 SIGNAL INTENSITY (BKG & REF CORRECTED)
EREF1 STANDARD DEVIATION OF REF1(I)
EPREF2 STANDARD DEVIATION OF REF2(I)
ERSIG1 STANDARD DEVIATION OF SIG1(I)
ERSIG2 STANDARD DEVIATION OF SIG2(I)

IMPLICIT REAL*8 (A-H,P-Z)

PRINT HEADER
WRITE (6,100)

100 FORMAT(* DLEFIT, EDITION OF 03 MAR 87*)

INITIALIZE PLOT ROUTINE

CALL PLTS1(0,0,0)

READ (2,*) NSET
DO 900 I=1,NSET
C
READ DATA
C
CALL RDDATA
C
CALL CALC
C
IF(NPLT.EQ.0) GOTO 200
C
CALL PLTXM(NPLT)
C
200 CALL PLOT (0.,0.,+999)
C
900 CONTINUE
C
END
C

SUBROUTINE RDDATA
C
READ A FILE DATA FILE
C
REVISED 26 FEB 87 BY R. T. ROSS
C
C
IMPLICIT REAL*8 (A-H,P-Z)
CHARACTER*68 TITLE
COMMON /TITL/ TITLE
COMMON /DAT1/ NDATA,TBIN,NREFCY,NSIGCY,BKGPEF,BKGSIG
COMMON /DAT2/ IREF(250),ISIG(250)
COMMON /DAT3/ REF1(250),REF2(250),SIG1(250),SIG2(250)
COMMON /DATA/ ERREF1(250),ERREF2(250),ERSIG1(250),ERSIG2(250)
C
I=2
IH=6
C
C
READ HEADER INFORMATION
C
READ(IR,100) TITLE
FORMAT(A)
100 READ(IR,*) TBKG
READ(IR,*) TBASE1
READ(IR,*) TBASE2
READ(IR,*) TBASE3
READ(IR,*) FNBIN
READ(IR,*) FNREFC
READ(IR,*) FNSIGC
READ(IR,*) BKGREF
READ(IR,*) BKGSIG
READ(IR,*) REFCY
READ(IR,*) SIGMAX
READ(IR,*) TOTTIM
READ(IR,*) FNDATA
READ(IR,*) TBIN
C
NREFCY = FNREFC
NSIGCY = FNSIGC
NCATA = FNCDATA

C READ REFERENCE DATA (COUNTS/SEC, TRUNCATED TO INTEGERS)
READ (IR,*) (IREF(I), I=1,NDATA)

C READ SIGNAL DATA (COUNTS/SEC, TRUNCATED TO INTEGERS)
READ (IR,*) (ISIG(I), I=1,NDATA)

C WRITE (1W,200) TITLE
200 FORMAT (// TITLE = ',A68)
WRITE (1W,210) NDATA,TBIN,NREFCY,BKGREF,NSIGCY,BKGSIG
210 FORMAT (/I8,* DATA BINS*/
    ,F8.3,* SECONDS/BIN*/
    ,F8.3,* REFERENCE CYCLES, BACKGROUND=*,F7.3,*/SEC*/
    ,F8.3,* SIGNAL CYCLES, BACKGROUND=*,F7.3,*/SEC*/) C WRITE (1W,220)
220 FORMAT (' REFERENCE DATA')
WRITE (1W,230) ( IREF<  I  ) ,  1  =  1 ,NDATA)
230 FORMAT (1018)
C WRITE (1W,240)
240 FORMAT (' SIGNAL DATA')
WRITE (1W,250) ( ISIG(I), I=1,NLATA)

RETURN
END

C +++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
C SUBROUTINE CALC
C C CORRECT INPUT DATA AND COMPUTE EXPECTED ERRORS
C REVISED 03 MAR 87 BY R. T. ROSS
C IMPLICIT REAL*8 (A-H,P-Z)
COMMON /DAT1/ NDATA,TBIN,NREFCY,NSIGCY,BKGREF, BKGSIG
COMMON /DAT2/ IREF(250),ISIG(250)
COMMON /DAT3/ REF1(250),REF2(250),SIG1(250),SIG2(250)
COMMON /DAT4/ ERREF1(250),ERREF2(250),ERSIG1(250),ERSIG2(250)

C FOLLOWING SHOULD BE READ IN, BUT IS NOW SUPPLIED HERE
TBKG=1.C0
NFIRST=5
NLAST=250
NUSE = NLAST-NFIRST+1

C COMPUTE ERROR IN BACKGROUND
ERRFBK=DSQRT(BKGREF/TBKG*NREFCY))
EPSGBK=DSQRT(BKGSIG/TBKG*NSIGCY))

C COMPUTE SUMMARY STATISTICS
DO 60 I=NFIRST,NLAST
   SUMREF=SUMREF+IREFIIJ+0.5D0
   SUMSIG=SUMSIG+1SIG<IJ+0.5D0
60 CONTINUE
   R£FAVG=SUHREF/NUS£ - BKGREF
   SIGAVG=SUMSIG/NUSE - BKGSIG
   REFERR=CSQRT((SUMREF/INREFCY*TB1/M1>NUSE**2)  + ERRFKK**2)
   SIGERR=CSQRT(SUKSIG/(NSIGCY*T8IN*NUSE**2) + FRSC;iK*#2)
   IF (REFAVG.GT.3.D0*REFERR) THEN
      SG2AVG=SIGAVG-REFAVG
      SG2ERR=DSQRT(SIGERR**2 + REFERR**2)
   ELSE
      SG2AVG=SIGAVG
      SG2ERR = S IGERR
   ENDIF
   HCLD=SG2AVG
   IF (HOLD.LT.1.0-10) HOLD=1.0-10
   YMI=DLG10(HOLD)
   HCLD=SG2AVG-2.00*SG2ERR
   IF (HOLD.LT.1.0-10) HOLD=1.0-10
   YLO=DLG10(HOLD)
   HCLDF=SG2AVG+2.00*SG2ERR
   IF (HOLD.LT.1.0-10) HOLD=1.0-10
   YHI=DLG10(HOLD)
   C
   WRITE (6,70) NFIRST,NLAST,BKGREF,ERRFK,ER,KGFI,ERSGPK,
   REFAVG,REFERR,SG2AVG,SG2ERR,
   YMI,YLO
70   FORMAT (// SUMMARY STATISTICS FOR BINS I4, I4 thru I4, //
   REFERENCE LEVEL=*,F8.3,*, STD.DEV. =*,F7.3/
   SIGNAL LEVEL = *,F8.3,*, STD.DEV. =*,F7.3/
   SIGNAL - REF = *,F8.3,*, STD.DEV. =*,F7.3/
   LOG10(SIGNAL) =*,F7.2,* RANGE=*,F7.2,* TO*,F7.2//)
   C
   C COMPUTE BACKGROUND-CORRECTED REFERENCE INTENSITY, REF(I)
   DC 160 I=1,NDATA
   REF=REF(I)+0.5D0
   REF(I) = REF - BKREF
   ERREF1(I) = DSQRT((REF/TE1N+BKREF/TPK)/NREFCY)
   160 CONTINUE
   C
   C COMPUTE AVERAGE STATISTICS FOR REFERENCE CHANNEL
   C
   DO 200 I=1,NDATA
   SUM=SUM+REF(I)/ERREF1(I)**2
200 CONTINUE
   C
   C COMPUTE SMOOTHED REFERENCE SIGNAL, REF2(I)
   C PRESENTLY A DUMMY SEGMENT, NO SMOOTHING
   C
   DO 250 I=1,NDATA
   REF2(I)=REF1(I)
ERREF2(I) = ERREF1(I)

250 CONTINUE

C COMPUTE BACKGROUND-CORRECTED SIGNAL INTENSITY, SIG1(I)

DO 300 I = 1, NDATA
   FSIG = ISIG(I) + 0.500
   SIG1(I) = FSIG - BKGSIG
   ERSIG1(I) = DSQRT((FSIG/TEIN + BKGSIG/TEIN)/NSIGCY)
300 CONTINUE

C COMPUTE REFERENCE-CORRECTED SIGNAL INTENSITY, SIG2(I)

DO 400 I = 1, NDATA
   SIG2(I) = SIG1(I) - REF2(I)
   ERSIG2(I) = DSQRT(ERSIG1(I)² + EREF2(I)²)
400 CONTINUE

RETURN
END
PROGRAM FA1DLE

EDITION OF 12 OCT 87

INSTRUCTIONS FOR PREPARATION OF DATA
1. TYPE: USE DATASN UNNUM
   (WHERE "DATASN" IS DATA-SET-NAME FROM C-64)
2. TYPE: X LUCASE
   FOLLOW INSTRUCTIONS, USING "1" FOR FIRST LINE, "L" FOR LAST
3. TYPE: INSERT 0.5
   INSERT THE NUMBER OF DATA FILES
4. TYPE: SAVE OLEDAT UNNUM
5. TYPE: USE FA1DLE CLEAR
   RUN

VARIABLES IN COMMON
/****/

NREFCY NUMBER OF REFERENCE CYCLES
NSIGCY NUMBER OF SIGNAL CYCLES
BKGSIG BACKGROUND COUNTS/SEC FOR SIGNAL DATA
BKGREF BACKGROUND COUNTS/SEC FOR REFERENCE DATA
NOATA NUMBER OF DATA BINS
T8 IN TIME PER BIN (SEC/CYCLE)
IREF REFERENCE DATA (COUNTS/SEC, TRUNCATED)
ISIG RAW SIGNAL DATA (COUNTS/SEC, TRUNCATED)
REF1 REFERENCE INTENSITY (BACKGROUND CORRECTED)
REF2 REFERENCE INTENSITY (REF1 SMOOTHED)
SIG1 SIGNAL INTENSITY (BACKGROUND CORRECTED)
SIG2 SIGNAL INTENSITY (BKG C REF CORRECTED)
ERREF1 STANDARD DEVIATION OF REF1(I)
ERREF2 STANDARD DEVIATION OF REF2(I)
ERSIG1 STANDARD DEVIATION OF SIG1(I)
ERSIG2 STANDARD DEVIATION OF SIG2(I)

IMPLICIT REAL*8 (A-H,P-Z)

PRINT HEADER
WRITE (6,100)
100 FORMAT(' FA1DLE, EDITION OF 12 OCT 87')

INITIALIZE PLOT ROUTINE

CALL PLOTS(0,0,0)

READ (2,*), NSET
DO 900 I=1,NSET
    ISET=I
C
    READ DATA
    CALL RDDATA
C
    CALL CALC
    CALL CELL
    CALL FAPREP(ISET, ISET)
C
    IF(NPLT.EQ.0) GOTO 200
C
    CALL PLTXMINPLT
    CALL PLOT (0., 0., +999)
C
    900 CONTINUE
C
    CALL WRFILE
    END
C
+++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

SUBROUTINE RDDATA
C
READS A DLE DATA FILE
C
REVISED 26 FEB 87 BY R. T. ROSS
8 OCT 87: COMMON /DAT5/ ADDED
C
IMPLICIT REAL*8 (A-H,P-Z)
CHARACTER*68 TITLE
COMMON /TITL/ TITLE
COMMON /DAT1/ NDATA, TBIN, NREFCY, NSICCY, BKGREF, BKGSIG
COMMON /DAT2/ IREF(250), ISIG(250)
COMMON /DAT3/ REF1(250), REF2(250), SIG1(250), SIG2(250)
COMMON /DAT4/ ERREF1(250), ERREF2(250), ERSIC1(250), ERSIC2(250)
COMMON /DAT5/ TLAG, NFIRST, NLAST, NCELL
C
IF=2
IH=6
C
READ HEADER INFORMATION
C
READ(1R,100) TITLE
100 FORMAT(A)
READ(1R,9) TKG
READ(1R,9) TBASE1
READ(1R,9) TBASE2
READ(1R,9) TBASE3
READ(1R,9) FWHM
READ(1R,9) FNREFC
READ(1R,9) FNSIGC
READ(1R,9) BKGREF
READ(1R,9) BKGSIG
READ(1R,9) REFMAX
NCCELL = 1C

NUSE = NLAST - NFIRST + 1

COMPUTE ERROR IN BACKGROUND

ERRFBK = DSQRT((BKGREF / (TBKG * NREFCY)))
ERSGBK = DSQRT((BKGSG / (TBKG * NSIGCY)))

COMPUTE SUMMARY STATISTICS

SUMREF = C.DO
SUMSIG = 0.DO
DO 60 1 = NFIRST, NLAST
   SUMREF = SUMREF + IREF(I + 0.5DO)
   SUMSIG = SUMSIG + ISIG(I + 0.5DO)
60 CONTINUE

REFAVG = SUMREF / NUSE - BKGREF
SIGAVG = SUMSIG / NUSE - BKGSIG
REFFER = CSQRT(SUMREF / (NREFCY * TBIN * NUSE ** 0.5DO) + EREFBK ** 0.5DO)
SIGERR = CSQRT(SUMSIG / (NSIGCY * TBIN * NUSE ** 0.5DO) + ERSGBK ** 0.5DO)
IF (REFAVG > 3.0 * REFFER) THEN
   SG2AVG = SIGAVG
   SG2ERR = SIGERR
ELSE
   SG2AVG = SIGAVG
   SG2ERR = SIGERR
END IF

HOLD = SG2AVG
IF (HOLD < 1.D-10) HOLD = 1.D-10
YMID = LOG10(HOLD)
HOLD = SG2AVG - 2.0 * SG2ERR
IF (HOLD < 1.D-10) HOLD = 1.D-10
YLO = LOG10(HOLD)
HOLD = SG2AVG + 2.0 * SG2ERR
IF (HOLD < 1.D-10) HOLD = 1.D-10
YHI = LOG10(HOLD)

WRITE (6, 70) NFIRST, NLAST, BKGREF, ERRFBK, BKGSIG, ERSGBK,
   REFAVG, REFFER, SIGAVG, SIGERR,
   SG2AVG, SG2ERR, YMID, YLO, YHI
70 FORMAT (//' SUMMARY STATISTICS FOR BINS, 14, ' THRU', 14, '''
   ' REF BACKGROUND = ', F8.3, ', STD.DEV. = ', F7.3/
   ' SIG BACKGROUND = ', F8.3, ', STD.DEV. = ', F7.3/
   ' REFERENCE LEVEL = ', F8.3, ', STD.DEV. = ', F7.3/
   ' SIGNAL LEVEL = ', F8.3, ', STD.DEV. = ', F7.3/
   ' SIGNAL = ', F8.3, ', STD.DEV. = ', F7.3/
   ' SIGNAL = ', F8.3, ', STD.DEV. = ', F7.3/
   LOG10 (SIGNAL) = ', F7.2, ', RANGE = ', F7.2, ', TC = ', F7.2,
   //'

COMPUTE BACKGROUND-CORRECTED REFERENCE INTENSITY, REF(1)

DO 100 I = 1, NDATA
   FREF = IREF(I) + 0.5DO
   REF(I) = FREF - BKGREF
   ERRF(I) = DSQRT((FREF / TBIN + BKGREF / TBKG) / NREFCY)
100 CONTINUE

COMPUTE AVERAGE STATISTICS FOR REFERENCE CHANNEL
READ(IR,*) SIGMAX
READ(IR,*) TOTTIM
READ(IR,*) FNDATA
READ(IR,*) TBIN
C
NREFCY = FNREFC
NSIGCY = FNSIGC
NCATA = FNCDATA
C
READ REFERENCE DATA (COUNTS/SEC, TRUNCATED TO INTEGERS)
READ (IR,*) (IREF(I), I=1,NDATA)
C
READ SIGNAL DATA (COUNTS/SEC, TRUNCATED TO INTEGERS)
READ (IR,*) (ISIG(I), I=1,NDATA)
C
WRITE (IW,200) TITLE
200 FORMAT (//, TITLE = ' ,A68)
WRITE (IW,210) NDATA,TBIN,NREFCY,BKGREF,NSIGCY,BKGSIG
210 FORMAT (4I4,* DATA BINS*/
* F8.3,* SECONDS/BIN*/
* IS,* REFERENCE CYCLES, BACKGROUND=* ,F7.3,* /SEC*/
* IS,* SIGNAL CYCLES, BACKGROUND=* ,F7.3,* /SEC*/)
C
WRITE (IW,220)
220 FORMAT (* REFERENCE DATA*)
WRITE (IW,230) (IREF(I), I=1,NDATA)
230 FORMAT (1018)
WRITE (IW,240) (* SIGNAL DATA*)
WRITE (IW,250) (ISIG(I), I=1,NDATA)
RETURN
END
C
C******FOLLOWING SHOULD BE READ IN, BUT ARE NOW SUPPLIED HERE******
TBKG=1.C0
NFIRST =5
NLAST=250
TLAG=0.007D0
PRESENTLY A DUMMY SEGMENT

DC 200 I=1,NDATA
SUM=SUM+(REF1(I)/ERREF1(I)**2)
200 CONTINUE

C
C COMPUTE SMOOTHED REFERENCE SIGNAL, REF2(I)
C PRESENTLY A DUMMY SEGMENT, NO SMOOTHING
C
DC 250 I=1,NDATA
REF2(I)=REF1(I)
ERREF2(I)=ERREF1(I)
250 CONTINUE

C
C COMPUTE BACKGROUND-CORRECTED SIGNAL INTENSITY, SIG1(I)
C
DC 300 I=1,NDATA
FSIG = ISIG(I) + 0.5D0
SIG1(I) = FSIG - BKGSIG
ERSIG1(I) = DSQRT((FSIG/TBIN + BKGSIG/TEKG)/NSIGCY)
300 CONTINUE

C
C COMPUTE REFERENCE-CORRECTED SIGNAL INTENSITY, SIG2(I)
C
DC 400 I=1,NDATA
SIG2(I) = SIG1(I) - REF2(I)
ERSIG2(I) = DSQRT(ERSIG1(I)**2 + ERREF2(I)**2)
400 CONTINUE

C
RETURN
END

+++++++++++++++++++++++SUBROUTINE CELL++++++++++++++++++++++++

SUBROUTINE CELL

PUTS SIGNAL DATA INTO CELLS OF EQUAL WIDTH IN LOG(TIME)

REVISION OF 12 OCT 87

INPUT
NFIRST  INDEX OF FIRST BIN TO BE USED
NLAST   INDEX OF LAST BIN TO BE USED
TBIN    TIME PER BIN (SEC/CYCLE)
TLAG    TIME AT BEGINNING OF FIRST BIN
SIG2(I) SIGNAL INTENSITY IN BIN (I)
ERSIG2(I) STANDARD DEVIATION OF SIG2(I)
NCELL   NUMBER OF OUTPUT CELLS

OUTPUT
Y(J)   AVERAGE SIGNAL INTENSITY IN CELL J
S(J)   STANDARD DEVIATION OF Y(J)
IO(J)  INDEX OF FIRST BIN IN CELL J
TO(J)  TIME AT START OF CELL J
AVLOGT(J) AVERAGE LOG10(TIME) OF CELL J

IMPLICIT REAL*8 (A-H,P-Z)
COMMON /DAT1/ NOATA,TBIN,NREFCY,NSIGCY,BKGREF,BKGSIG
COMMON /DAT2/ IREF(250),ISIG(250)
COMMON /DAT3/ REF1(250),REF2(250),SIG1(250),SIG2(250)
COMMON /DAT4/ ERREF1(250), ERREF2(250), ERSIG1(250), ERSIG2(250)
COMMON /DAT5/ TLAG, NFIRST, NLAST, NCELL
COMMON /DATE/ IO(250), TO(250), AVLOGT(250), Y(250), S(250)

C

IO(1) = NFIRST
TO(1) = TLAG + TBIN*(NFIRST-1)
C TEND = TIME AT END OF DATA COLLECTION
TEND = TLAG + TBIN*NLAST
C
C COMPUTE DIVISION OF TIME AXIS INTO CELLS
C
DO 400 ICEL=1,NCELL-1
  NLEFT = NUMBER OF CELLS REMAINING
  NLEFT = NCELL - ICEL + 1
C DLNT = INCREMENT IN LOG(TIME) FOR EQUAL SPACING
  DLNT = DLOG(TEND/TO(ICELL))/NLEFT
  TCALC = CORRESPONDING TIME AT END OF CELL
  TULONGTCALL = TO(ICELL)*EXP(DLNT)
C ICALC = BIN NUMBER IN WHICH TIME=TCALC, LAST BIN OF CELL
  ICALC = ((TCALC-TOICELL)/TBIN) + 1
  IO(ICELL+1) = ICALC + 1
  TO(ICELL+1) = TLAG + ICALC*TBIN
  AVLOGT(ICELL) = (DLOG10(TO(ICELL)) + DLOG10(TO(ICELL+1)))/2.0
400 CONTINUE
C
IO(NCELL+1) = NLAST+1
AVLOGT(NCELL) = (DLOG10(TO(NCELL)) + DLOG10(TEND))/2.0
C
C COMPUTE Y AND S FOR THE CELLS
C
DO 600 ICEL=1,NCELL
  I1=IO(ICELL)
  I2=IC(ICELL+1)
  SUMY=0.D0
  SUMS=0.D0
  DO 500 IBIN=I1, I2
    SUMY=SUMY+SIG2(IBIN)
    SUMS=SUMS+ERSIG2(IBIN)**2
500 CONTINUE
  Y(ICELL)=SUMY/(I2-I1+1)
  S(ICELL)=DSQRT(SUMS/(I2-I1+1))
600 CONTINUE
C
WRITE (6,710)
710 FORMAT ('CELL IO TO LOG(TIME) SIGNAL STD.DEV.*')
DO 730 J=1,NCELL
  WRITE (6,720) J, IO(J), TO(J), AVLOGT(J), Y(J), S(J)
720 FORMAT (I4,15,F9.4,F8.4,F12.5,F12.5)
730 CONTINUE
C
RETURN
END
C
SUBROUTINE FAPREP(NSET, ISET)
C
FORMATS DATA FOR BILINEAR ANALYSIS
C
REVISION OF 12 OCT 87 BY R. T. ROSS
C
IMPLICIT REAL*8 (A-H,P-Z)
CHARACTER*2 ROWNAM, COLNAM
CHARACTER*68 TITLE
COMMON /CTRL/ NGRP, NROW, NCOL, NF
COMMON /DATA/ DATAJK(50, 50, 6)
COMMON /TITL/ TITLE
COMMON /XVAL/ XROW(52), XCOL(52)
COMMON /STAT/ SIGMA(50, 50), IMISS(50, 50), VMAX
COMMON /PASS/ RPASS(12, 128), NROW, NCOLR, IPASS(7, 128), NROWI, NCOLI
COMMON /DAT1/ NDATA, TBIN, NREFCY, NSICCY, BKGPEF, BKGSIG
COMMON /DAT2/ IREF(250), ISIG(250)
COMMON /DAT3/ IREF(250), ISIG(250)
COMMON /DAT4/ IREF(250), ISIG(250)
COMMON /DAT5/ TITLE, NFIRST, NLAST, NCELL
COMMON /DAT6/ TITLE, NFIRST, NLAST, NCELL
C
NROW = NSET
NCOL = NCELL
IROW = ISET
XROW(IRCW) = IROW
DO 200 ICODE = 1, NCOL
   XCOL(ICODE) = AVGLOGT(ICOL)
   DATAJK(IRCW, ICODE) = Y(ICOL)
   SIGMA(IRCW, ICODE) = S(ICOL)
   IMISS(IRCW, ICODE) = 0
200   CONTINUE
C
NGRP = 1
NF = 2
C
RETURN
END
C
+++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
SUBROUTINE WRFILE
WRITES DISK FILE FOR USE BY FA2
VERSION OF 13 SEP 85 Copied from FA1SLM on 8 OCT 87
C
IMPLICIT REAL*8 (A-H,P-Z)
CHARACTER*2 ROWNAM, COLNAM
CHARACTER*68 TITLE
COMMON /CTRL/ NGRP, NROW, NCOL, NF
COMMON /DATA/ DATAJK(50, 50, 6)
COMMON /TITL/ TITLE
COMMON /XVAL/ XROW(52), XCOL(52)
COMMON /STAT/ SIGMA(50, 50), IMISS(50, 50), VMAX
COMMON /PASS/ RPASS(12, 128), NROW, NCOLR, IPASS(7, 128), NROWI, NCOLI
C
IMODEC = 0
C
RWNAM = 'CONDITION NUMBER'
COLNAM = 'LOG(TIME IN SECONDS)'
C
IDSK = 1
NROWI = 2
10 REM FREE-ENERGY 1990 VERSION march 1990
20 REM
30 REM THIS PROGRAM CONTAINS DATA FOR THE PS-II ENERGETICS FROM
40 REM ANACYSTIS NIDULANS
50 REM
60 REM PROGRAM USED TO EVALUATE THE PARAMETERS NEEDED TO OBTAIN A VALUE
70 REM FOR THE ENERGY AVAILABLE FROM PHOTOSYSTEMS TO PRODUCE WORK USED IN
80 REM ORGANISMS FOR METABOLISM BY THE FORMULA \( dG = kT \ln (R_{\text{lum}}/R_{\text{therm}}) \)
90 REM
100 REM THE PROGRAM WILL EVALUATE FIRST THE \( R_{\text{therm}} \) OR EXCITING ENERGY
110 REM USING BLACK-BODY EMISSION AND ACTION SPECTRA AS PARAMETERS BY THE
120 REM FORMULA \( R_{\text{therm}} = \sum (I_{bb}(\lambda) \times A_{abs}(\lambda))d(\lambda) \) WHERE \( I_{bb} \) IS THE BLACK-
130 REM BODY EMISSION INTENSITY FOR EACH WAVELENGTH OR \( \lambda \) AND \( A_{abs}(\lambda) \) IS
140 REM THE ABSORPTION AT EACH WAVELENGTH THE FORMULA FOR \( I_{bb} \)
150 REM
160 REM IS \( A * 3.14 * N^2 * c * \lambda^-4 * (\exp(h*c/\lambda*k*T) - 1)^-1 \) WHERE
170 REM
180 REM
190 REM \( N \) IS THE REFRACTIVE INDEX OF WATER = 1.333
200 REM \( h \) IS THE PLANCK'S CONSTANT = 6.6*10^-27 ERG SEC
210 REM \( c \) IS THE SPEED OF LIGHT = 3*10^10 CM SEC^-1
220 REM \( k \) IS THE BOLTZMAN'S CONSTANT PER MOLECULE = R/NO
230 REM \( T \) IS THE ABSOLUTE TEMPERATURE T = 0C+273=25+273=298K
240 REM
250 REM
260 REM
270 REM THE INPUT FOR THE FIRST PART OF THE PROGRAM IS THE WAVELENGTH AND
280 REM THE ABSORPTION PER WAVELENGTH. THE WAVELENGTH IS GIVEN AS "NM"
290 REM WHICH IS CONVERTED TO "CM" TO BE USED IN THE FORMULAS BECAUSE OF
300 REM "CGS" UNITS. THE VARIABLE A DETERMINES THE PRE-EXPONENTIAL PORTION
310 REM AND THE VARIABLE L THE EXPONENTIAL PORTION OF THE INTEGRATED RADIATION
320 REM THE VARIABLE G OBTAINS THE COMBINED BLACK-BODY RADIATION AND ABSORBANCE
330 REM THE WAVELENGTH IS INCREASED BY 3 "NM" AND THE ACCUMULATOR INTEGRATES
340 REM THE EXCITING RADIATION FOR THE FIRST PART OF THE PROGRAM
350 REM
360 REM
370 REM AS="wavelength" :BS="integrated emission"
380 LPRINT AS,BS
390 LPRINT LAMBDA=625 :ACUM=0 :DIM G(45),FLUO(45),MULT(45)
400 FOR I=1 TO 19
410 E=LAMBDA*10^-7) :F=E*(-4) :READ DAT
420 B=(4.814*10^-3)/E :A=(1.3391*10^-12)*F
430 H=EXP(B)*L*(H-1)^(-1) :G(I)=G(I)+B*A*H
440 LAMBDA=LAMBDA + 3 :ACUM = ACUM + G(I)
450 IF LAMBDA<682 THEN 480
470 NEXT I
480 WAVE = 625 :CFLUO=0 :SMULT=0 :SRAD=0
490 BEEP
500 FOR M=1 TO 43 :READ FLUO(M) :NEXT M
510 FOR N=1 TO 19
520 MULT(N)=G(N)/FLUO(N) :SMULT=SMULT+MULT(N)
530 WAVE=WAVE+3 : CFUO=CFUO+FLUO(N) : IF WAVE > 682 THEN 550
540 NEXT N
550 SRAD=SMULT/N :PRINT SRAD,N
560 FOR M=20 TO 43
570 CFUO=SRAD+FLUO(M) :LPRINT WAVE,CFUO : WAVE=WAVE+3
580 ACUM=ACUM+CFUO : IF WAVE>751 THEN 600
590 NEXT M
600 ACUM=ACUM+3*10^-7
610 LPRINT: LPRINT: LPRINT
620 LPRINT "Rtherm for ANACYSTIS PS-II = " ; ACUM
630 REM DATA AS EXCITATION SPECTRA READ BY DAT VARIABLE
640 DATA 1,1,92,77,72,68,63,59,52,48,43,38,35,32,315,3.28,28
650 DATA .23
660 REM END OF ACTION SPECTRA DATA
670 REM DATA AS FLUORESCENCE SPECTRA
680 DATA .026,.037,.053,.079,.101,.107,.134,.173,.206,.250,.312,.370,.413,.683
690 DATA .691,.778,.917,1,.998,.975,.927,.759,.635,.467,.408,.345,.294,.248,.226
700 DATA .214,.197,.186,.181,.182,.177,.166,.161,.149,.131,.113,.107
710 BEEP
720 END

wavelength integrated emission
625 3.105943E-05
628 4.402212E-05
631 5.720873E-05
634 6.740713E-05
637 8.843992E-05
640 1.168181E-04
643 1.508801E-04
646 1.963597E-04
649 2.397487E-04
652 3.056395E-04
655 3.76998E-04
658 4.573314E-04
661 5.765294E-04
664 7.193472E-04
667 9.635754E-04
670 1.245232E-03
673 1.57263E-03
676 1.970497E-03
679 2.345771E-03
682 2.807989E-03
685 1.217735E-03
688 9.70449E-04
691 8.341548E-04
694 6.134651E-04
697 5.359609E-04
700 4.532022E-04
703 3.862071E-04
706 3.257801E-04
709 2.955666E-04
712 2.811167E-04
715 2.58785E-04
718 2.443351E-04
721 2.37767E-04
724 2.390806E-04
727 2.390806E-04
730 2.325124E-04
733 2.259443E-04
736 2.180625E-04
739 2.114944E-04
742 1.957308E-04
745 1.720855E-04
748 1.484401E-04
751 1.405584E-04

Rtherm for ANACYSTIS PS-II = 6.453115E-09