PHOTOCHEMISTRY OF MASKED PYRENE-4,5-DIONE

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A Thesis
Submitted to the Graduate College of Bowling Green
State University in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

August 2013

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The photochemistry of 1,2-dipyridinium dihydrodioxin, 1,1-di(2-pyridyl) dihydrodioxin, 1,1-di(2-pyridyl) dihydrodioxin-Cu$^{2+}$, pyrene-4,5-dione, and their complexes with DNA were studied. Also, quantum yields of pyrene-4,5-dione release from 1,2-dipyridinium dihydrodioxin, 1,1-di(2-pyridyl) dihydrodioxin, and 1,1-di(2-pyridyl) dihydrodioxin-Cu$^{2+}$ were calculated to be $10^{-4}$, 0.1, and 0.3 respectively.

In case of 1,2-dipyridinium dihydrodioxin, it was found that DNA damage happens due to electron transfer from pyrene ring of 1,2-dipyridinium dihydrodioxin to DNA stack under excitation by UV-light. Evidence is given that release of pyrene-4,5-dione is not responsible for DNA cleavage, as it was believed before. After treatment of DNA with pyrene-4,5-dione under UV-light no decomposition of DNA was noticed. Pyrene-4,5-dione forms solvent separated radical-ion pair with Guanine of DNA after which both molecules go back to ground state.

Unfortunately, 1,1-di(2-pyridyl) dihydrodioxin and 1,1-di(2-pyridyl) dihydrodioxin-Cu$^{2+}$ do not dissolve in water and cannot be used as DNA-cutting agents. Although, photoexcitation of 1,1-di(2-pyridyl) dihydrodioxin-Cu$^{2+}$ gives better understanding of the mechanism of electron transfer from the pyrene-ring to an acceptor. 1,1-di(2-pyridyl) dihydrodioxin does not have a good electron acceptor in its structure therefore electron transfer does not occur.

Electron transfer from pyrene-ring of dihydrodioxin to electron acceptor, such as DNA molecule, water or Cu$^{2+}$, forms stable radical-cation species which has an absorption
band at 450 nm and can be recognized. 1,2-dipyridinium dihydrodioxin in water, 1,2-
dipyridinium dihydrodioxin with DNA, and 1,1-di(2-pyridyl) dihydrodioxin-Cu$^{2+}$ showed
in transient absorption spectra the presence of stable 450 nm band with a lifetime of more
than 1 ns.
In loving memory of my cat, Silva.
ACKNOWLEDGMENTS

I am exceptionally grateful to my advisor, Dr. R. Marshall Wilson for his help, patience, guidance, encouragement, kindness, and suggestions. His erudition, intelligence, and passion have been inspiring me throughout the whole time of my studying in BGSU. Without his countenance and support, I would not be able to accomplish this far.

I would also like to thank Dr. Alexander Tarnovsky for letting me to use the facilities in his laboratory. His advices and comments were very helpful as well as his “Photophysics” course.

I would like to acknowledge Dr. Peter Lu for allowing me to use equipment in his laboratory. I am as well thankful to my committee member, Dr. Mikhail Zamkov, for his questions and suggestions.

My big thanks I want to address to my husband/collaborator/best buddy/soul mate/critic/supporter/father of my son, Dr. Andrey Mereshchenko, for all his help and advices.

I owe special thanks to Dr. Alexei Shamaev for all his help in the research project and for being a great colleague.

I would like to acknowledge all my friends and colleagues, Evgeniya Butaeva aka Jennifer el Tano, Veniamin Ben Borin, Pavel Moroz, Anton Toha Alenko, Misha Rai, Suzana and Georgy Zanev, Heather Coruja Bergseth, Mami Jinshin Moon, Samuel Samchik Olaiya, Oluwatosin Owoseni, Zijian Z Wang, Yanka Lu, Bryn Wilke, Liubov Lifshits, and Janitha Walpita for their help and great company.

And of course I owe my biggest thanks to my family, my brother, mother, and son for their cheering and support.
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INTRODUCTION

Deoxyribonucleic acid, DNA, is a macromolecule which is responsible for storing, passing from generation to generation, and implementation of genetic information in the living organisms. DNA is stored in a cell nucleus for eukaryotes and bound to the cell membrane for prokaryotes. From a chemical point of view, DNA is a long polymer molecule consisting of nucleotides; each nucleotide has nucleobase, sugar, and phosphate group. In general, DNA molecules are composed of two helical chains connected by paired nucleobases. The nucleobases form hydrogen bonds between each other; however, pairing happens between specific bases. Typical nucleobases for DNA are Adenine, Guanine, Thymine, and Cytosine. According to the Watson Crick model Adenine pairs with Thymine (two hydrogen bonds) and Guanine pairs with Cytosine (three hydrogen bonds); every newly formed hydrogen bond is specific for each base-pair. The order in which nucleobases placed in DNA chain forms a unique sequence, which contains genomic information.

DNA is wrapped around nucleosomes and bound to proteins in cells, nucleosomes organized in higher order structure. The two polynucleotide chains in double helical DNA are anti-parallel forming two distinct grooves; the one with the larger width is called the major groove, the one with the smaller width, the minor groove. These anti-parallel chains form right-handed double helix. Polymeric DNA molecule has an extremely complicated structure. Nucleotides are covalently linked to each other forming polynucleotide chain. The backbone of each single strand is formed by interchanging desoxyribose sugar and phosphate groups; a nucleobase is attached to each sugar.
There are many DNA conformations found in the human body, whereas the major three are A-DNA, B-DNA, and Z-DNA. A- and B-DNA are right handed whereas Z-DNA is left handed. B-DNA is the most common conformation, it exists under normal conditions, but the A-form exists mostly in dehydrated samples. Z-DNA needs certain conditions to form, the structure is not favorable and no biological importance of it has been found.

There are many anticancer drugs known to be effective in different types of cancer, all of them affect cells in their own, special, way. Since there are not many biochemical differences between normal and abnormal cell, the search for the universal cure for all types of cancer is very difficult. Although modern medicine offer treatment for almost all types of cancer; efficiency of each anticancer drug is limited, and each of the drugs is specifically directed to eliminate certain types of cells.

In general, all anticancer drugs in one or another way affect the replication function of the cell. They all can be divided into four major groups: the first group eliminates the formation of nucleotides as major blocks for building the DNA molecule; second group stops the construction of DNA molecule from nucleotides; the third group blocks mitosis of the cell which prevent the further reproduction; and the fourth group intercalates into DNA molecule causing disruption and dysfunction.

The fourth group of cancer drugs works due to intercalation into DNA molecule between two base-pairs. Intercalating agents serve as a guest molecule and share a common characteristic, flat aromatic system that is able to insert between two aromatic nucleobases. The planar aromatic system of drug is stabilized by the \(\pi\)-electron systems of DNA base pair and it causes double helix to stretch, changing the length, and shifting other bases; thus, the normal structure is lost.
At the present time, there is a large number of known intercalators, and they all are derivatives of several basic molecules: phenanthroline, pyrene, anthraquinone, acridine, and anthracene. But the most effective DNA intercalators are the metallocomplexes of these aromatic molecules serving as the ligands.2-8

Design of artificial molecules which are able to bind, cleave, and damage the DNA molecule has been of interest to researchers for a few decades. Photoactivated DNA-cutting agents are the most interesting group since the activation of the cutting agent can be controlled. There are several classes of DNA photocleaving agents known, like metallocomplexes of organic molecules, riboflavins, some derivatives of quinones, nitrosubstituted aromatic molecules, naphthalimide analogs, and others.

Cleavage of DNA by irradiation of intercalated transition metal complexes of pyridine derivatives have been the focus of investigators for many decades. Complexes of platinum(II), ruthenium(II), rhodium(III), iridium(III), cobalt(III), zinc(II), osmium(II) and other metals with ligands are widely used in a variety of treatment therapies such as supplying iron for anemia, removal of toxic metals, treatment of pulmonary disease (Mn$^{2+}$, Ce$^{4+}$ and Mo$^{3+}$ bound to different types of polyoxiranes). These metals are very active in living systems due to their small radius, redox activity, stability, ability to modify structure of natural systems and the dynamics of some their processes, capacity to bind to O, N and S atoms of functional groups, and high charge density in their ionic form.

Stability of complexes of first row transition metals with ligands follows the sequence known as Irving-Williams order. The series are explained by ionic radius difference. Crystal Field Stabilization Energy also increases in that order. Complexes of these metals were found to be stable in that order, the most stable chelate is Zn$^{+2}$ and the least stable is Mn$^{+2}$. 
In the work of Lerman,\textsuperscript{1} intercalation of acridines in DNA was first inferred. Molecules of acridine derivatives were found to insert between two base pairs of DNA. In order to intercalate into DNA strand, the molecule should meet two main characteristics: the first, large π-system (π-π stacking between intercalator and base pairs) and, the second, positive charge (to facilitate electrostatic connection with negative charge on a phosphate backbone).\textsuperscript{9}

Complexes of these molecules with transition metals, metallointercalators, are special type of compounds that have a metal, usually a spectroscopically active transition metal, surrounded by two or more intercalators. First described aggregates of this type were platinum(II) complexes which contained planar aromatic heterocycles as a ligand.\textsuperscript{10-12} The main benefit of these complexes is the ability to target specific sites of DNA; different ligands have different shapes to match between certain base pairs in the DNA stack.

The spatial relationships between bidentant ligands and metal in center form a plane, and relative orientation of these planes to each other generate two possible structures of the complex, either ∆(right-handed propeller) or Λ(left-handed propeller), schematic representations of both are shown in Figure 1. These two forms stick in DNA with different efficiency. Right handed groove (A- or B-DNA) discriminates the Λ-form and prefers to bind with ∆-form while Z-DNA binds with both forms.\textsuperscript{13}
Zn(phen)$_3^{2+}$ complex intercalates into right-handed DNA, the circular dichroism spectrum showed that one Δ-enantiomer binds better then another one. It was shown that the complex was not damaged and DNA cleavage did not happen. Binding of Δ-isomer over the Λ-isomer into right-handed DNA was noticed and the explanation was given. The Δ-enantiomer of Zn(phen)$_3^{2+}$ complex intercalates into double-helix between base-pairs parallel to them and the other two phenanthrolines coincide together with the DNA groove (Figure 2). Whereas the Λ-isomer’s ligand stuck between base-pairs with two left-handed ligands left outside, the phosphate backbone groups and these ligands run against each other, forming steric inconvenience. Oxygen atoms on the phosphate groups are too far away from hydrogen atoms on a phenanthroline to form the hydrogen bonds between them, whereas in case of Δ-isomer this distance is shorter.
A-isomer complex of cobalt with 4,7-diphenyl-1,10-phenanthroline (DiP) is described in the work of J. K. Barton and others\textsuperscript{15}. A photocleavage of left-handed DNA was investigated by Mapping procedure described in literature\textsuperscript{16}; the A-isomer of Co(DiP)\textsubscript{3}\textsuperscript{3+} intercalates into left-handed DNA following photoactivation (irradiation at $\lambda=315$ nm). Using EcoRI enzyme the molecule linearizes, then nuclease S1 which cleaves DNA at sites opposite to the sites splitted by Co(DiP)\textsubscript{3}\textsuperscript{3+} and electrophoresis on 1% agarose gels one can determine the size of formed fragments. Scheme of this procedure is shown on Figure 3.
Fig. 3. Addition of Λ-isomer of Co(DiP)$_3^{3+}$ to supercoiled left-handed DNA and irradiation at wavelength 315 nm lead to formation of circular form; following addition of linearization enzyme and cutting agent help to recognize the single-stranded cleavage location of Co(DiP)$_3^{3+}$.

These studies showed that Λ- Co(DiP)$_3^{3+}$ complex cleaves specific sites of DNA in regions where geometry of DNA differs from the whole molecule conformation. Δ- Co(DiP)$_3^{3+}$ was found to bind B-form and Z-form of DNA, but it shows a no site-specific cleavage.

Structural features of metallocomplexes are also in a great interest of researchers. Structure of ligands in a complex can vary depending on a prospective that one wants to achieve. The most investigated ligands in this field are 1,10-phenanthroline, 2,2′-bipyridyl and their derivatives. X-ray studies of their complexes with Cu(II) showed that monomer complex perform a distorted octahedron around Cu(II).$^{17}$ The structure manages 4 short-length bonds between nitrogen and copper (Figure 4).
Fig. 4. The coordination of atoms of 1,10-phenanthroline around Cu(II); part of each ligand is shown (two nitrogen atoms with connected carbon atoms).\textsuperscript{17}

Δ- and Λ-forms of Tris(tetramethylphenanthroline)ruthenium(II) also showed discrimination towards B-form of DNA, and both showed good binding affinity to A-form helices.\textsuperscript{18} Guanine and Cytosine containing polynucleotides are also indicated preference in binding with Tris(tetramethylphenanthroline)ruthenium(II) rather than Adenine and Thymine containing polynucleotides.

There are many reports on complexes of metals with non-planar ligands are also capable of intercalating into the double helix of DNA.\textsuperscript{20-22} Ru(II) complexes with two flat ligands (2,2'-bipyridine) and one non-flat ligand 2-(2-chlorophenyl)imidazo[4,5-f]1,10-phenanthroline(CIP) or 2-(2-nitrophenyl)imidazo[4,5-f]1,10-phenanthroline(NIP) were investigated.\textsuperscript{22} Crystallographic data showed existence of two enantiomers, Δ and Λ-forms. It also showed that in the mixture of two forms, the non-flat ligands adjusted asymmetrically together by simultaneous penetration. The distance between ligands is so small that \(\pi-\pi\) stacking interactions
appear between them. Titration of both compounds with DNA did not show any shift in absorption spectra, but resulted in noticeable decay of the lowest-frequency absorption band.

All these metallointercalators have two things in common: oxidative mechanism of cleavage and, the most aggravating, an absence of site specific cleavage; they bind to DNA and cleave it at random sites. Due to arising need of site-specific tools to recognize and damage DNA molecules, the effort have been done towards the creation of tools which catalyzes reaction of DNA hydrolysis and provides site-specific DNA damage.

One of these approaches is using oligonucleotides which are able to bind to specific sites of a double-helix DNA forming a triplex and labeled with DNA-cleaving agent which is capable of cleaving the double-helix at the targeted site. But the main disadvantage of these complexes is that they cleave DNA hydrolytically, which requires addition of a hydroxide anion source.

In this work we report phototactivated quinone releasing agents, 1,2-DPyDHD and 1,1-di(2-pyridyl)DHD, as possible DNA cleavage agents. The formation of the complexes of aforesaid molecules with DNA, the quinone release quantum yield of 1,2-DPyDHD and 1,1-di(2-pyridyl)DHD molecules, and ultrafast photochemistry of pyrene-4,5-dione, 1,2-DPyDHD, and 1,1-di(2-pyridyl)DHD and their complexes with DNA are reported here.

References


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CHAPTER I. MATERIALS AND METHODS

Synthesis of compounds

Pyrene-4,5-dione, 1,1-di(2-pyridyl)DHD, and 1,2- DPyDHD were synthesized and purified in our laboratory by Alexei Shamaev. The cupric salt of 1,1-di(2-pyridyl)DHD was prepared by mixing 1,1-di(2-pyridyl)DHD with equimolar amount of Cu(ClO₄)₂ in acetonitrile, with following recrystallization from chloroform. Herring Sperm DNA was purchased from Sigma Aldrich. Potassium ferrioxalate for quantum yield measurements was prepared as previously described.¹

Ultrafast transient absorption spectroscopy

The ultrafast transient absorption spectrometer used in this experiment is based on a Ti:Sapphire regenerative amplifier and that produces a train of 800 nm pulses with 1 kHz repetition rate (E= 900 μJ/pulse). The beam splitter divides the output beam into two beams with equal intensities. One of them is sent to the TOPAS-C (Light Conversion Lt.) optical parametrical amplifier, which generates 350, 310, and 325 nm excitation pulses that go through the sample and are used as a pump light. The energy of excitation beam was 3 μJ per pulse at the sample position. The second beam goes through the delay line and produces white light continuum by focusing onto the 3 mm CaF₂ window. After CaF₂ window, this beam divides into two parallel beams: reference and probe. Both beams go to the monochromator, and then to the
dual-diode array detector, but only the probe beam gets through the sample. For this experiment 0.2 mm thick flow cell was used. The data were collected at room temperature (22 °C).

Quantum yield measurements

For irradiation of samples, HeCd laser dual continuous wavelength (325/442 nm) from Kimmon Koha was used in this experiment. The average beam power before sample was 22 mW. Samples were irradiated in 5 cm quartz quvette (Scientific Cell Cony). Absorbance spectra were taken using the Agilent 8453 UV-visible Spectroscopy System in 1 cm quartz quvette. Potassium ferrioxalate (K₃[Fe(C₂O₄)₃]) was used as an actinometer.²,³ The decomposition quantum yield was determined by the decay of the absorption spectra of the studied compounds. All measurements were provided at the temperature of 22°C.

References

CHAPTER II. PHOTOCHEMISTRY OF PYRENE-4,5-DIONE

Quinones are the family of aromatic hydrocarbons which have two carbonyl groups in their structure. There are two major classes – ortho-(carbonyl groups in ortho position) and para-quinones (carbonyl groups in para position). Because of their ability to be reduced to phenols, quinones participate in biological oxidations: as electron acceptors in electron transport through membrane; as precursors for synthesis of biologically important proteins. The medical use of quinines is mostly concentrated in the field of anticancer drug design.1-3

Most anti-tumor drugs activate molecular oxygen present in a cell in order to form reactive oxygen species, including singlet oxygen or hydroxyl-radical. Some quinones have long-lived triplet states which can be quenched by molecular oxygen, formed reactive oxygen species that damage a DNA molecule.4-6

Ortho-quinones are highly reactive compounds which make it difficult to use them as DNA-cutting agents directly, but masking them in photosensitive compounds solves this problem.7,8 Protection of ortho-quinones with olefins leads to formation of dihydrodioxins9 and release of a quinone can be done under UV or Visible-light. These compounds demonstrated effective DNA photo-cleavage. It is believed that released ortho-quinones are responsible for damaging DNA. In order to test whether the ortho-quinones do or do not damage DNA.

Transient absorption spectroscopy was performed for pyrene-4,5-dione and its mixture with Herring sperm DNA.

Ultrafast transient absorption (ΔA) spectra of pyrene-4,5-dione and complex of Herring Sperm-DNA with pyrene-4,5-dione solutions in 1:1 methanol:water mixture were measured upon 310 nm excitation. The transient absorption bands centered at 380, 550, and 680 nm are
formed within first 0.3 ps. Between 0.3 and 10 ps, simultaneous rise of 380 and 550 nm transient absorption bands is accompanied with decay of the 680 nm band with time a constant around 3 ps. Between 10 ps and 1 ns time delays, the $\Delta A$ spectrum completely decays without any reshaping with time constant in about 120 picoseconds.

**Fig. 1.** Transient absorption spectra of pyrene-4,5-dione in cacodylic buffer-methanol mixture(1:1), $\lambda_{irr}=310$ nm.
Fig. 2. Transient absorption spectra of pyrene-4,5-dione/DNA in cacodylic buffer-methanol mixture(1:1), \( \lambda_{irr}=310 \) nm.

At 0.3 ps delay time, \( \Delta A \) spectrum of the complex of Herring Sperm-DNA and pyrene-4,5-dione consist of four bands peaking at 380, 480, 550, and 680 nm and a shoulder at 420 nm at 0.3 ps. At short delay times, from 0.3 to 10 ps, the 380 nm centered band grows with time constant around 2 picoseconds. It was noticed that the 480 and 550 nm bands decay simultaneously with a time constant of 21 ps, which can indicate that 480 and 550 nm bands corresponds to the same species. Similarly to 480 and 580 nm bands, the shoulder at 420 nm and peak at 680 nm have the same lifetime around 4 ps. At long delay times, from 10 ps to 1 ns, all
four peaks and the shoulder at 420 nm decay with time constant 110±10 ps keeping constant spectral shape.

$\Delta A$ spectra of pyrene-4,5-dione with DNA and without have many similarities, both have peaks at 380, 550, 680 nm and shoulder at 420 nm with similar kinetics at longer timescale. The main differences is spectral evolution at short delay times and presence the band centered at 480 nm in pyrene-4,5-dione/DNA $\Delta A$ spectra. The peak at 700 nm was assigned as singlet excited state for both systems, whereas signals at 380 and 550 nm were assigned as first triplet state of pyrene-4,5-dion. The rapid decay of triplet state in both systems is a result of a triplet state quenching by the solvent, methanol, and formation of solvent separated ion pair with guanine of DNA molecule. ²⁰

In the $\Delta A$ spectra of pyrene-4,5-dione with DNA, the band at 550 nm is overlapping with another band which does not appear in pyrene-4,5-dione spectra itself. Since this new band is already in the $\Delta A$ spectrum and is decaying by the time of 0.3 ps, we do not see the rise of 550 nm band that we noticed in pyrene-4,5-dione $\Delta A$ spectra.

The pyrene-4,5-dion/DNA $\Delta A$ spectra displayed the new band at 480 nm which decays with time constant of around 16 and 100 ps. This band could be assigned as semiquinone radical formed after hydrogen abstraction or solvent separated radical-ion pair formed between pyrene-4,5-dione in the first triplet state with Guanine. Because the value of hydrogen abstraction rate is diffusion controlled and has magnitude of $10^{-7}$ it is unlikely to assigned the 480 nm band to semiquinone radical species. Therefore, we assigned this peak as solvent separated radical-ion pair (SSRIP), emission signal of SSRIP in highly polar solvents was showed to be at 480 nm. ¹⁰-¹⁴ The differential spectrum of pyrene-4,5-dione and its complex with DNA at 10 ps showed a broad band with center at 600 nm and a narrow peak at 480 nm which
assigned as SSRIP. The broad band at 600 nm is due to electron localized between solvated DNA bases.\textsuperscript{15}

\textbf{Fig. 3.} Jablonsky diagram for pyrene-4,5-dione/DNA excited at $\lambda=310$ nm. Singlet excited state ($^1Q^*$) of pyrene-4,5-dione($Q$) intersystem crosses to form triplet state($^3Q^*$). Then it follows either quenching by solvent (MeOH) or formation of solvent separated radical ion pair ($Q^-(S)G^+$) with guanine.

The character of singlet and triplet states of pyrene-4,5-dione were shown to be dependent on polarity of a solvent.\textsuperscript{16,17} In polar media first triplet state has dominant $\pi\pi^*$ character, while in nonpolar it has $n\pi^*$ character.\textsuperscript{18} The ketones with natural lowest triplet $\pi\pi^*$ are known to be less reactive than those with $n\pi^*$, as a result hydrogen abstraction with further formation of semiquinone radicals are less possible for pyrene-4,5-dione in mixture of methanol
and water. And none of the $\Delta A$ spectra showed the presence of hydrogen abstracted species. The singlet excited state of pyrene-4,5-dione has dominant character n\pi*, which is in agreement with the fact that larger splitting for the two lowest n\pi* states causes considerable lower energy (visible region) of the lowest singlet excited state (680 nm).\textsuperscript{19} Also the singlet-triplet splitting, where first triplet state has character \pi\pi* and first singlet excited state has character n\pi*, is predicted to be 330-200 nm.\textsuperscript{20} We found it to be \textasciitilde 200 nm for pyrene-4,5-dione with and without DNA which is within the range.

Pyrene-4,5-dione does not damage the DNA, the steady state spectra of DNA/quinone mixture before and after transient spectroscopy experiment did not show significant changes. Even though the formation of solvent separated radical-ion pair of pyrene-4,5-dione with Guanine was noticed (Figure 2), the damage of Guanine did not happen. Therefore we can propose that pyrene-4,5-dione relived from dihydrodioxin molecule is not responsible for DMA damage.

References


Currently, “caged molecules” are of significant interest to both chemists and biologists. In order to control chemical reactions in biological systems, “caged” molecules must be designed in a way that one would be able to supervise the structure, reactivity, biological activity and light sensitivity of these compounds. Biological environment adds complexity to the management of chemical processes. Applications of these kinds of molecules include design of molecular devices, optical sensors, drug delivery systems, etc. “Caged” molecules that are used in processes that occur in biochemical environment should meet these qualifications: protection should work throughout biological treatment without losing a masking group; the masking group must be stable to hydrolysis; photo-release of biologically active agent should be controlled and efficient; molecules must be soluble in water buffers; photochemistry should be initiated by wavelengths >300 nm; the photoproducts of masking group must be biologically inactive; and the products of photo-release should not absorb the light of the same wavelength as the activating irradiation. Endonucleolytic DNA photo-cleavage by light-activated “caged” molecules is an interesting process that might be controlled.

An example of a light-activatable molecule is the ene-diyne compound (Scheme 1) bearing a large planar pyrene group, that intercalates into DNA between two base pairs. Irradiation of ene-diyne in the presence of DNA plasmid leads to disruption of the DNA. ¹
Scheme 1. Example of ene-diyne with pyrene in its structure

Another type of “caged” molecules with a pyrene group are pyrene dihydrodioxins (PDHDs). PDHDs can photochemically release ortho-quinones (Scheme 2), which are known to be good DNA cleaving agents. The ortho-quinone family is a big class of DNA-cutting agents. Ortho-quinone reactivity is significant which makes their delivery to a targeted cell difficult, but masking the ortho-quinone with photosensitive masking group makes their delivery to a cell much more likely.

Scheme 2. Ortho-quinone release from DHD

An example of the pyrene-4,5-dione release mechanism from one of the PDHDs is shown in Scheme 3. This PDHD releases pyrene-4,5-dione under UV and Visible light through the
formation of pyrene radical-cation. Further exposure to oxygen results in creation of an olefin and a ketone.

Scheme 3. Pyrene-4,5-dione release from one of the PDHDs.

Targeting a specific site of the DNA to cleave is a challenging task. The main difficulty is that the radical-cation, “holes”, formed at the target site can migrate significant distances, which leads to cutting DNA at random sites. PDHD randomly binds to DNA strands and photochemically releases quinone, which in turn produces holes that can migrate within the DNA strands. Another problem is that quinone release is that it is more effective in the absence of DNA than in its presence. This means that DNA quenches quinone release. Synthesis of the monopyridinium dihydrodioxins (PyDHD) shown in Scheme 4 can solve this problem due to the
intramolecular electron traps which can compete with DNA stack in accepting the released electrons.

![Scheme 4. Mechanism of pyrene-4,5-dione release from monopyridinium DHD](image)

Thus quinone release is enhanced, by eliminating or reducing electron transfer with the DNA base pair stack. In previous work it was shown that the pyrene ring of a PDHD intercalates into double-stranded DNA and after its photorelease, the pyrene-quinone remains intercalated between the DNA base-pairs. In the work of E. Mack, the nanosecond spectroscopy of PyDHD in acetonitrile was studied and the results summarized in Scheme 3. Transient spectroscopy of PyDHD in acetonitrile showed an absorption peak centered at 450 nm which was assigned as PyDHD pyrene radical-cation (Figure 1).
Fig. 1. Transient absorption spectra of radical-cation produced by irradiating of PyDHD with 350 nm light in acetonitrile.²

Ultrafast time-resolved spectra of the 1,2-dipyridinium dihydrodioxin 1,2-DPyDHD (Figure 2) in water buffer and in acetonitrile (Figure 4) at pump/probe wavelengths 255/340-770 nm and between 0 and 1200 ps time delays were measured. Two absorption bands with the maxima at 450 and 530 nm appear within the instrumental time response of 250 fs in water (Figure 2). Then, the 450 nm band, when analyzed with a triple exponential equation, affords three components with time constant of τ=1.3, τ=6.3 and τ=115 ps. The 530 nm band re-shapes, and by 6 ps is completely transformed into a broad absorption between 480 and >615 nm. This broad signal remains at least into the μs time domain. The 480 centered peak that appears at longer timescale (from 10 ps to 1 ns) in transient absorption spectra of 1,2-DPyDHD in acetonitrile is assigned to an exciplex between the pyrene ring of 1,2-DPyDHD and acetonitrile (Figure 4).⁹,¹⁰
Fig. 2. Transient absorption spectra of 1,2-DPyDHD in water at the pump wavelength 350 nm at different time delays.

In 1,2-DPyDHD solution with added DNA (1,2-DPyDHD/DNA base pair), the transient absorption spectra (Figure 3) has many similarities to that of 1,2-DPyDHD without DNA, two absorption bands of 455 and 535 nm appear within the excitation pulse, 0.3 ps, and have the very similar behavior within the first 10 ps. The 535 nm band transforms to the broad absorption signal at ca. 600 nm within 6 ps. In 1,2-DPyDHD solution with DNA, the 455 nm band decays much more rapidly in the presence of DNA, ca. 10 ps, than in the absence of DNA, >1000 ps.
In this PyDHD research, it was noticed the pyrene radical-cation species with absorption maximum at ca. 450-455 nm was very long lived, \( \tau = 6.3 \, \mu s \) (Figure 1). In our ultrafast data, we also observed the 450 nm transient absorption band for the pyrene radical-cation of the 1,2-DPyDHD in acetonitrile and water formed within the excitation pulse (0.3 ps). Because transient spectra of 1,2-DPyDHD in acetonitrile and water are similar at short times, we also assigned the 450 nm band to the pyrene radical-cation.
After analysis of transient absorption spectra of 1,2-DPyDHD in water (Figure 2), we proposed, that the second transient absorption band with a maximum at 530 nm corresponds to the pyridine radical fragment. Moreover, we noticed that up to 3 ps, the 450 nm band (radical-cation) and 530 nm band (pyridine radical) decay simultaneously. Therefore, at the short time before 3 ps direct charge recombination process is observed. Then, the 530 nm band transforms into a broad absorption signal in the range 500-610 nm. It is known that solvated electron in water has a broad absorption in a red part of visible range. Due to the fact that water is good
electron acceptor, we assumed that the re-shaping of 530 nm band into the broad absorption signal corresponds to the electron transfer from the pyridine radical to the bulk water on the timescale of several picoseconds. Thus, at the long times we assigned transient absorption signals to the radical-cation and the water solvated electron. Simultaneous decay these two signals is explained as a transfer of the water solvated electron to the pyridine radical-cation.

Change in surrounding of 1,2-DPyDHD in presence of DNA shifts the absorption bands 5 nanometers to the red for the pyrene radical-cation and pyridine radical bands relatively to the water solution without DNA, 455 and 535 nm, respectively (Figure 3). Because in this case, 1,2-DPyDHD molecule is in a complex with DNA, in a timescale of several picoseconds electrons from the pyridine radical are transferred to the DNA molecule instead of water. Thus, in this case the broad transient absorption signal at long times is due to absorption of DNA solvated electrons. At the long times, we observed much faster decay of the 455 nm pyrene radical-cation band for 1,2-DPyDHD with DNA than without. It was proposed that an electron in the negatively charged DNA molecule (DNA solvated electron) bonded to the 1,2-DPyDHD molecule is much more localized than the electron solvated by bulk water. This causes faster transfer of solvated electron from the DNA molecule to the radical-cation resulted and a shorter lifetime of pyrene radical-cation absorption band.

We propose the following mechanism for photolysis of 1,2-DPyDHD (Scheme 5). Excitation of 1,2-DPyDHD at 350 nm wavelength leads to rapid formation of the pyrene radical-cation and radical on pyridine within 1 ps with absorption bands centered at 450 nm and 530 nm, respectively. These species have two pathways of decay: the major pathway of electron transfer from pyridine radicals to water producing solvated electrons and to pyrene radical-cation which annihilates within 100 ps to reform the 1,2-DPyDHD molecule, and the minor pathway, which is
release of ortho-quinone by the mechanism outlined in Scheme 6.

**Scheme 5.** Mechanism of electron transfer from 1,2-DPyDHD to water molecule
In order to prove binding between 1,2-DPyDHD and DNA, nuclear magnetic resonance (NMR) spectroscopy was investigated. Two-dimensional NMR spectroscopy was chosen as a reliable tool for the evaluation of intercalation of the pyrene in the DNA base pair stack. Therefore COSY in 99.96% D$_2$O was performed at room temperature.

Two-dimensional $^1$H-spectra for 1,2-DPyDHD, 1,2-DPyDHD\DNA -4:1 and 1,2-DPyDHD\DNA-1:1 were acquired on the Bruker AM500 MHz spectrometer. Since D$_2$O was used as a solvent in all solutions, suppression of the water signal was needed. DFGPPH19 COSY experiment was used in order to improve suppression of the water signal.$^4$ All solutions were prepared at room temperature in 4 mm NMR-tubes.

Two-dimensional nuclear magnetic resonance spectroscopy (2D NMR) is a method in which the spectrum intensity is plotted verses two frequency axes. Each peak has a position specified by these two frequencies. The simplest type of 2D NMR is a correlation spectroscopy.
(COSY). COSY spectrum can provide information about proton-proton coupling in molecule within three chemical bonds.

2D NMR spectra give more information about structure of big molecules than 1D NMR. 1D spectrum of complicated molecules is too difficult for interpretation, because most of the signals overlap strongly. Introduction of additional frequency dimension results in simplified spectra and extracting additional structure information.

Fig. 5. $^1$H-COSY NMR(500 MHz, D$_2$O) 1,2-DPyDHD

Addition of DNA changes 1,2-DPyDHD NMR spectrum. Signals of the pyrene protons of 1,2-DPyDHD which are between 6 ppm and 9 ppm decrease, and signal at $\delta = 6.4$ that corresponds to the protons on carbon next to the ring-oxygen disappears completely. The pyrene
signals disappear because all 1,2-DPyDHD molecules are intercalated in many different sites in
the DNA molecules.

The original DNA NMR spectrum also undergoes significant changes. Addition of 1,2-
DPyDHD in 1:1 ratio leads to considerable decrease of various signals.

Scheme 7. π-π stacking between pyrene ring of 1,2-DPyDHD and base pairs of DNA

What we see in NMR spectra is a result of 1,2-DPyDHD intercalating between two DNA
base pairs. This complex stabilized electronically by π-π stacking and dipole-dipole interactions
between positively charged nitrogen in the pyridinium ring and negatively charged phosphate
group.5 Protons on the pyrene ring sandwiched tightly between the heterocyclic DNA π-systems
of the bases - are in a highly shielding environment, and many different DNA binding sites are
occupied by the 1,2-DPyDHDs. The factors cause reducing of some of the DNA signals and
disappearance of Pyrene proton signals6. This provides evidence for intercalating of 1,2-
DPyDHD into DNA. But it was impossible to extract information about structural features.

Also quantum yield of quinone release was measured by comparative method with
irradiation at 442 nm (Table 1). In order to calculate quantum yield measurements two solutions
of 1,2-DPyDHD were made: 1. 1,2-DPyDHD in presence of oxygen; 2. 1,2-DPyDHD in absence
of oxygen.
Both solutions were irradiated for three different times, after each irradiation, the steady state spectra were determined. Change in absorption spectra was determined by decrease of absorption band at 343 nm.

Table 1. Quantum yields of 1,2-DPyDHD.

<table>
<thead>
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<th>$\Phi$</th>
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<tbody>
<tr>
<td>1,2-DPyDHD in presence of oxygen</td>
<td>$1.703 \times 10^{-4}$</td>
</tr>
<tr>
<td>1,2-DPyDHD in absence of oxygen</td>
<td>$1.968 \times 10^{-4}$</td>
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It was impossible to perform quantum yield studies with presence of DNA because of unusual behavior of DNA-1,2-DPyDHD complex. Absorbance of the complex after irradiation increases in the area between 358 nm and 220 nm. This could be a sign of formation of new products that have larger extinction coefficients than starting materials.

References


CHAPTER IV. PHOTOCHEMISTRY OF 1,1-DI(2-PYRIDYL)DHD / 1,1-DI(2-PYRIDYL)DHD-Cu$^{2+}$

Another member of DHDs family, 1,1-di(2-pyridyl)dihydrodioxin was prepared by reacting pyrene-4,5-dione with 2,2-ethenylidenebispyridine. The compound is insoluble in water, and therefore, cannot be used in DNA binding and damaging studies. Nevertheless, it demonstrated a great affinity to form metallocomplex with Cu$^{2+}$. two nitrogen atoms offer unshared electron pair to Cu$^{2+}$ and form bidentant complex.

\[
\text{Scheme 1. Formation of the cupric salt of 1,1-di(2-pyridyl)DHD}
\]

Quantum yields of pyrene-4,5-dione release from 1,1-di(2-pyridyl)DHD with and without Cu$^{2+}$ in acetonitrile were measured (Table 1). Irradiation wavelength used was 325 nm. In order to calculate quantum yield measurements, two solutions of 1,1-di(2-pyridyl)DHD with and
without Cu\(^{2+}\) were made: 1- 1,1-di(2-pyridyl)DHD C= 1.3 mM; 2 – 1,1-di(2-pyridyl)DHD, C= 1.6 mM.

Both solutions were irradiated for three different times, after each irradiation, the steady state spectra were determined. Change in absorption spectra was determined by decrease of absorption band at 343 nm.

**Table 1.** Quantum yields of 1,1-di(2-pyridyl)DHD and its cupric salt

<table>
<thead>
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<th>(\lambda) irradiation, nm</th>
<th>(\Phi)</th>
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<tbody>
<tr>
<td>325</td>
<td>1,1-di(2-pyridyl)DHD-Cu(^{2+})</td>
</tr>
<tr>
<td>325</td>
<td>1,1-di(2-pyridyl)DHD</td>
</tr>
<tr>
<td>442</td>
<td>1,1-di(2-pyridyl)DHD-Cu(^{2+})</td>
</tr>
<tr>
<td>442</td>
<td>1,1-di(2-pyridyl)DHD</td>
</tr>
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</table>

Transient absorption spectra of 1,1-di(2-pyridyl)DHD in acetonitrile at excitation wavelength 325 nm and time delays up to 1 nanosecond resulted in formation of two signals with maximums at 370 and 480 nm and shoulders at 400 and 530 nm (Figure 1). At shorter timescale, from 0.3 to 10 ps, the spectra show only minor changes. The 370 and 480 nm bands insignificantly rise with lifetime around 8 ps accompanied by minor decay of 400 (\(\tau\sim10\) ps) and 530 (\(\tau\sim3\) ps) nm. At longer timescale, from 10 ps to 1 ns, the band at 380 nm does not change in intensity while the 480 nm band and two shoulders at 400 and 530 nm decay simultaneously with lifetime 1.5±0.5 ns. The 1 ns transient absorption spectrum consists of the peak at 370 nm, peak at 480 nm, shoulder at 400 nm, and shoulder at 530 nm.
Fig. 1. Transient absorption spectra of 1,1-di(2-pyridyl)DHD in acetonitrile, $\lambda_{\text{irr}}$=325 nm.

The complex of 1,1-di(2-pyridyl)DHD-Cu$^{2+}$ in acetonitrile gave broad signal from 360 to 420 nm, sharp signal centered at 450 nm, and a shoulder at 530 nm (Figure 2). The sharp peak at 450 nm decays double exponentially with $\tau_1$~30 ps and $\tau_2$~1 ns; the shoulder at 530 nm decays with time constant around 1 ps; and the broad band between 380 and 420 nm decays with a two-exponential with time constant around 1 ps and 20 ps. At longer timescale, the spectra consist of sharp peak at 450 nm and broad band from 380 to 420 nm.
Fig. 2. Transient absorption spectra of 1,1-di(2-pyridyl)DHD-Cu$^{2+}$ in acetonitrile, $\lambda_{irr} = 325$ nm.

Transient spectra of 1,1-di(2-pyridyl)DHD-Cu$^{2+}$ showed formation of triplet state as a minor pathway. The major pathway involves formation of charge-separated species followed by electron transfer from pyridyl radical-anion to Cu$^{2+}$. Spectrum of charge-separated species cannot be seen in spectra due to very fast electron transfer to Cu$^{2+}$ ion. The formed radical-cation species is stable, and has lifetime more than 1 ns (Scheme 2).

Transient spectra of 1,1-di(2-pyridyl)DHD and its cupric salt have not many similarities. The spectra of 1,1-di(2-pyridyl)DHD showed in Figure 1 is complicated and the fit of spectrum into a sum of five Gaussian functions at 0.5 ps showed that it contains 5 bands with centers at
370, 380, 450, 480, and 530 nm. It is hard to do the peak assignment, but based on the spectra on Figure 1 the band at 380 nm appears in both spectra and could be assigned as absorption of a triplet state. In case of 1,1-di(2-pyridyl)DHD the triplet state is a long lived species (lifetime is more than 1 ns) while in 1,1-di(2-pyridyl)DHD-Cu$^{2+}$ it is a short lived species (~15 ps) (Scheme 3).

Scheme 2. Mechanism of pyrene-4,5-dione release from 1,1-di(2-pyridyl)DHD-Cu$^{2+}$
For 1,1-di(2-pyridyl)DHD the mechanism of photolysis probably engage two pathways showed in Scheme 3: first path involves formation of triplet state species; the second one involves formation of charge separated state of 1,1-di(2-pyridyl)DHD and followed by formation of a highly conjugated state. Both ways lead to formation of quinone and olefin. All intermediate species are long lived and cannot be monitored at femtosecond scale; lifetimes for these intermediates were estimated to be more than 2 ns.

**Scheme 3.** Mechanism of pyrene-4,5-dione release from 1,1-di(2-pyridyl)DHD
Since both transient spectra, 1,1-di(2-pyridyl)DHD and its cupric salt, demonstrated the presence of 380 and 450 nm bands, these peaks may be due to formation of charge separated state (450 nm - radical-cation and 380 – radical-anion). Assignment of transients which correspond to triplet states in spectra of 1,1-di(2-pyridyl)DHD does not seem possible in this timescale. The formations of broad peaks of intermediate species make it hard to determine the separate bands and to assign them. Although it may be that the peak at 480 nm is related to highly conjugated state. In order to prove it, nanosecond transient absorption spectroscopy of 1,1-di(2-pyridyl)DHD need to be done.

References

CONCLUSIONS

In this thesis, the photochemistry of the masked phototactivated quinone releasing agents, 1,2-DPyDHD, 1,1-di(2-pyridyl)DHD, and their release of pyrene-4,5-dione was studied. Using NMR and UV-Vis spectroscopy we proved the formation of the complexes of 1,2-DPyDHD and pyrene-4,5-dione with DNA molecules. The quinone release quantum yield of 1,2-DPyDHD and 1,1-di(2-pyridyl)DHD molecules is reported in this work. Also, using femtosecond transient absorption UV-Vis spectroscopy, the ultrafast photochemistry of aforementioned molecules and the DNA-adducts was studied, and the photochemical mechanisms were proposed.

This work has disproved the hypothesis that dihydrodioxins destroy DNA by releasing ortho-quinones. Pyrene-4,5-dione does not seem to be responsible for DNA damage; the transient absorption spectra of its mixture with HS-DNA have shown that pyrene-4,5-dione forms long-lived solvent separated radical-ion pair, but NMR and UV-Vis absorption spectra of pyrene-4,5-dione-DNA complex were identical before and after sample irradiation.

We found that DNA damage happens due to electron transfer from 1,2-DPyDHD to DNA stack. Ultrafast transient absorption spectroscopy experiment showed formation of long-lived pyrene radical-cation in mixture with DNA. Pyrene-ring of 1,2-DPyDHD transfers its electron to pyridine ring; the formed pyridine radical is the short-lived species and it transfers the electron to DNA stack, where it damages one of the nucleobases by a mechanism that as yet to be determined.