INTERACTIONS OF PEPTIDES WITH SIMPLE LEWIS ACIDS AND
FRAGMENTATION MECHANISMS OF ADDUCTS STUDIED BY TANDEM MASS
SPECTROMETRY

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Dissertation

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ABSTRACT

The dissociations of [Cu(II)AA(AA-H)]\(^+\) complexes with the amino acids (AA) Gly, Ala, Val, Leu, Ile, \(\tau\)-Leu, and Phe (no heteroatoms in the side chain) have been examined in the gas phase by tandem mass spectrometry (MS/MS), using collisionally activated dissociation (CAD) in an electrospray ionization ion trap mass spectrometer. These complexes contain an intact and a deprotonated AA ligand. The amino groups of both AA units, the carbonyl group of the intact amino acid, and the deprotonated hydroxyl oxygen coordinate Cu(II) in square-planar fashion. Under CAD conditions, the [Cu(II)AA(AA-H)]\(^+\) complexes undergo decarboxylation with simultaneous reduction of Cu(II) to Cu(I); during this process, a radical site is created at the \(\alpha\)-carbon of the decarboxylated ligand (\(N_1H_2C_\alphaHC_\betaH_2R\)). The radical site is able to move along the backbone of the decarboxylated amino acid to form two new radicals (\(\cdot N_1HC_\alphaH_2C_\betaH_2R\) and/or \(N_1H_2C_\alphaH_2\cdot C_\betaHR\)). From the complex of \(\tau\)-Leu, only \(C_\alpha\) and \(N_1\) radicals can be formed. The whole radical ligand can be lost to form [Cu(I)AA]\(^+\) from these three isomeric radicals. Alternatively, further radical induced dissociations can take place along the backbone of the decarboxylated amino acid ligand to yield [Cu(II)AA(AA-H2-CO2)]\(^+\), [Cu(I)AA(\(\cdot NH_2\))]\(^+\), and/or [Cu(I)AA(NH=\(C_\alphaH_2\))]\(^+\). Those products may undergo consecutive dissociation to form the final product [Cu(I)AA]\(^+\). The sodiated copper
complexes ([Cu(II)(AA-H+Na)(AA-H)]⁺) show the same fragmentation patterns as their non-sodiated counterparts; sodium ion is retained on the intact amino acid ligand and is not involved in the CAD pathways. On the basis of the CAD spectra, reasonable
dissociation mechanisms are proposed for the [Cu(II)AA(AA-H)]⁺ complexes and substantiated by calculated potential energy diagram of selected CAD channels of the
[Cu(II)Ala(Ala-H)]⁺ complex.

For copper complexes with amino acids with aliphatic or phenyl side chains, only the deprotonated amino acid ligand is involved in the dissociation reactions. On the other hand, complexes of copper and amino acids with functionalized side-chains additionally undergo inter-ligand reactions. As the size of the molecules coordinated to copper increases, the probability of inter-ligand reactions increases.

The above characteristics are revealed from the CAD reactions of [Cu(II)AA(AA-H)]⁺ complexes with the functionalized amino acids Trp, Tyr, His, Arg, and Lys, or the dipeptide GlyGly. The larger, functionalized side chains of these amino acids facilitate their coordination to copper ion. Under CAD conditions, most of these [Cu(II)AA(AA-H)]⁺ complexes undergo decarboxylation with concomitant reduction of Cu(II) to Cu(I), which transfers the radical site to the α-carbon of the deprotonated α-amino acid/peptide. The dominant reaction pathway after the reductive decarboxylation is either elimination of the radical ligand or inter-ligand hydrogen atom transfer from the COOH group to the α-radical site followed by a second decarboxylation and subsequent radical induced dissociations. The larger the amino acid/peptide size, the easier these reactions occur. Even the GlyGly/copper complex showed an intense inter-ligand interaction that was not
observed from aliphatic amino acid/copper complexes. Reaction mechanisms are proposed and corroborated by deuterium-labeling experiments.

In a separate study, a wide range of singly charged dilithiated peptides, \([\text{Pep} - \text{H} + 2\text{Li}]^+\), has been studied by tandem mass spectrometry on electrospray ionization quadrupole ion trap (ESI-QIT) and matrix-assisted laser desorption/ionization quadrupole-time-of-flight (MALDI-Q-ToF) mass spectrometers. In the \([\text{Pep} - \text{H} + 2\text{Li}]^+\) peptide derivatives, a proton in the peptide is replaced by a lithium cation (usually at the most acidic site); the other lithium cation is probably mobile along the backbone of the peptide upon excitation and induces fragmentations that produce sequence ions.

Theoretical calculations are carried out for the dipeptide complex \([\text{PheGly} - \text{H} + 2\text{Li}]^+\). The results show that there are several different isomeric structures for dilithiated PheGly, including lithium carboxylate and lithium amide structures with one or two salt bridges. The relative energies of these isomers are not significantly large and all of them can be populated upon typical CAD experiments. This explains the presence of protonated and singly lithiated fragments in the CAD spectra of the dilithiated peptides.

For peptides that have no extremely basic or acidic residues, CAD of their singly charged dilithiated derivatives leads to almost complete structurally diagnostic \(y_n^{**}\), \(e_n^{**}\), and \(a_n^*\) ions, which allow definitive sequence determination (the number of * indicates the number of metal ions in these singly charged fragments). The dissociations of \([\text{Pep} - \text{H} + 2\text{Li}]^+\) reveal that salt bridges with both carboxylate and deprotonated amide structures are populated upon CAD, consistent with the theoretical prediction. Such structures are found to promote sequence-specific fragmentations as well as the elimination of radicals to form dilithiated peptide \(\alpha\)-backbone radicals.
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TABLE OF CONTENTS

LIST OF FIGURES........................................................................................................................................... x

LIST OF SCHEMES.......................................................................................................................................... xiii

LIST OF TABLES............................................................................................................................................ xiv

CHAPTER

I. INTRODUCTION.............................................................................................................................................. 1

II. MASS SPECTROMETRY BACKGROUND...................................................................................................... 8

2.1. Mass Spectrometry Instrumentation ........................................................................................................ 8

2.2. Ionization Methods .................................................................................................................................... 10

2.2.1. Electrospray Ionization (ESI)........................................................................................................... 11

2.2.2. Matrix-Assisted Laser Desorption/Ionization (MALDI)................................................................. 14

2.3. Mass Analyzer ........................................................................................................................................... 16

2.3.1. Quadrupole Ion Trap (QIT) Mass Analyzer ....................................................................................... 17

2.3.2. Quadrupole Ion Trap Tandem Mass Analyzer.................................................................................. 20

2.3.3. Quadrupole Mass Analyzer................................................................................................................ 22

2.3.4. Time-of-Flight (ToF) Mass Analyzer ............................................................................................... 23

2.3.5. Quadrupole-Time-of-Flight (Q-ToF) Tandem Mass Analyzer......................................................... 26

III. MATERIALS, METHODS AND INSTRUMENTATION ................................................................................... 27

3.1. Materials .................................................................................................................................................... 27
3.2. Experimental Instrumentations .................................................................................27

3.2.1. Bruker Daltonics Esquire-LC ESI-QIT Mass Spectrometer ................................ 28

3.2.2. Micromass Q-ToF Ultima MALDI Mass Spectrometer .................................... 31

3.3. Methods ..................................................................................................................33

3.3.1. Copper(II) Catalyzed Reactions ...................................................................... 33

3.3.2. Theoretical Calculations ................................................................................. 34

3.3.3. Dilithiated Peptide Fragmentation ................................................................... 35

IV. CU(II)-CATALYZED REACTIONS IN TERNARY, SINGLY CHARGED CU(II) COMPLEXES OF AMINO ACIDS WITH HYDROCARBON SIDE CHAINS ...... 38

4.1. Cu(II)-Bound Dimer Complex with Ala ................................................................. 38

4.2. Cu(II)-Bound Dimer Complex with Gly ................................................................. 45

4.3. Cu(II)-Bound Dimer Complex with Leu, Ile and t-Leu ......................................... 45

4.4. Cu(II)-Bound Dimer Complex with Val and Phe ................................................... 49

4.5. Conclusions ............................................................................................................. 53

V. CU(II)-CATALYZED REACTIONS OF SIDE CHAIN FUNCTIONALIZED AMINO ACIDS (W, Y, H, R, K) AND GG ......................................................... 55

5.1. Cu(II)-Bound Homo-Dimer Complexes with W and Y ....................................... 55

5.2. Cu(II)-Bound Homo-Dimer Complex with His ..................................................... 68

5.3. Cu(II)-Bound Homo-Dimer Complexes with R and K ......................................... 73

5.4. Cu(II)-Bound Homo-Dimer Complex with GG .................................................... 81

5.5. Conclusions ............................................................................................................. 84

VI. STRUCTURAL CHARACTERIZATION OF PEPTIDES VIA TANDEM MASS SPECTROMETRY OF THEIR DILITHIATED MONOCATIONS ................................ 86

6.1. Random Backbone Cleavages .............................................................................. 87
6.2. Structures and Decomposition Mechanisms of the Dilithiated Complexes ..........90
6.3. Selective Cleavage at Internal K Residues ......................................................100
6.4. Side Chain Loss from S Residues ..................................................................104
6.5. Selective Cleavage at D Residues .................................................................107
6.6. YAGFLR ......................................................................................................107
6.7. Conclusions .................................................................................................110
VII. SUMMARY ..................................................................................................111
REFERENCES .....................................................................................................116
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Major components of a mass spectrometer</td>
<td>9</td>
</tr>
</tbody>
</table>
| 2.2. ESI ionization source;  
(a) scheme of an electrospray ionization source and | 13 |
(b) the desolvation/division process in ESI | 13 |
| 2.3. The matrix assisted laser desorption/ionization (MALDI) process | 15 |
| 2.4. Quadrupole ion trap mass analyzer;  
(a) electrodes layout and | 18 |
(b) quadrupole ion trap mass analyzer | 18 |
| 2.5. A quadrupole mass analyzer. The ions move in the z-direction | 21 |
| 2.6. Layout of time-of-flight (ToF) mass analyzer | 25 |
| 3.1. Scheme of the Bruker Daltonics Esquire-LC ESI-QIT MS instrument | 30 |
| 3.2. Scheme of the Micromass Ultima MALDI-Q-ToF MS instrument | 32 |
| 4.1. ESI-QIT mass spectrum of \([Cu(II)Ala(Ala-H)]^+\) | 39 |
| 4.2. CAD mass spectra of \([Cu(II)Ala(Ala-H)]^+\);  
(a) CAD (MS²) spectrum of m/z 240 and | 40 |
(b) CAD (MS³) spectrum of m/z 196 | 40 |
| 4.3. Energy profile of fragmentation reaction pathways for \([Cu(II)Ala(Ala-H)]^+\), relative energies in kJ/mol | 43 |
| 4.4. CAD mass spectra of  
(a) CAD (MS²) spectrum of \([Cu(II)Gly(Gly-H)]^+\) (m/z 212) and | 46 |
(b) CAD (MS³) spectrum of \([Cu(II)(Gly-H+Na)(Gly-H)]^+\) (m/z 234) | 46 |
4.5. CAD mass spectra of
(a) CAD (MS$^2$) spectrum of $[\text{Cu(II)Leu(Leu-H)}]^+$ (m/z 324), .................... 47
(b) CAD (MS$^2$) spectrum of $[\text{Cu(II)Ile(Ile-H)}]^+$ (m/z 324), and ............... 47
(c) CAD (MS$^3$) spectrum of $[\text{Cu(II)t-Leu(t-Leu-H)}]^+$ (m/z 324).................. 47

4.6. CAD (MS$^2$) mass spectra of
(a) CAD spectrum of $[\text{Cu(II)Val(Val-H)}]^+$ (m/z 296), ................................ 50
(b) CAD spectrum of $[\text{Cu(II)Phe(Phe-H)}]^+$ (m/z 396), and ......................... 50
(c) CAD spectrum of $[\text{Cu(II)Val(Phe-H)}]^+$ or $[\text{Cu(II)Phe(Val-H)}]^+$ (m/z 344). 50

5.1. ESI-QIT mass spectrum of $[\text{Cu(II)W(W-H)}]^+$................................. 56

5.2. CAD mass spectra of $[\text{Cu(II)W(W-H)}]^+$;
(a) CAD (MS$^2$) spectrum of m/z 470 and ......................................... 57
(b) CAD (MS$^3$) spectrum of m/z 396.................................................... 57

5.3. CAD mass spectra of $[\text{Cu(II)Y(Y-H)}]^+$ (m/z 424)............................ 62

5.4. CAD mass spectra of $[\text{Cu(II)His(His-H)}]^+$ (m/z 372)..................... 69

5.5. CAD mass spectra of $[\text{Cu(II)R(R-H)}]^+$; inserts show expansions of peaks.
(a) CAD (MS$^2$) spectrum of m/z 410 and ......................................... 74
(b) CAD (MS$^3$) spectrum of deuteron labeled complex m/z 423....................... 74

5.6. CAD mass spectra of $[\text{Cu(II)K(K-H)}]^+$ (m/z 354).......................... 79

5.7. CAD mass spectra of $[\text{Cu(II)GG(GG-H)}]^+$ (m/z 326)..................... 82

6.1. CAD (MS$^2$) mass spectra of YAGFL;
(a) protonated.......................................................... 88
(b) mono-lithiated......................................................... 88
(c) singly charged dilithiated................................. 88
(d) singly charged disodiated................................. 89
(e) singly charged trilithiated................................. 89

6.2. Conformations and relative energies (kJ/mol) of singly charged dilithiated FG complexes................................. 92

6.3. ESI-QIT CAD mass spectra of
(a) singly charged dilithiated GGKAA............................................ 103
(b) singly charged dilithiated YGGFLK........................................... 103

6.4. ESI-QIT CAD mass spectra of SIKVAV;
(a) monolithiated and.................................................. 105
(b) singly charged dilithiated.................................................. 105
6.5. CAD mass spectra of singly charged dilithiated DRVYIHPF acquired by
(a) ESI-QIT MS and  ........................................................................... 108
(b) MALDI-Q-ToF MS ........................................................................ 108

6.6. ESI-QIT CAD mass spectra of YAGFLR;
(a) singly charged dilithiated ............................................................. 109
(b) monolithiated ............................................................................. 109


## LIST OF SCHEMES

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. Reaction pathways of ([\text{Cu(II)Ala(Ala-H)}]^+); the acronym (rH) indicates a hydrogen atom rearrangement</td>
<td>41</td>
</tr>
<tr>
<td>5.1. Reaction pathways of ([\text{Cu(II)W(W-H)}]^+) (a)</td>
<td>59</td>
</tr>
<tr>
<td>Reaction pathways of ([\text{Cu(II)W(W-H)}]^+) (b)</td>
<td>60</td>
</tr>
<tr>
<td>5.2. Reaction pathways of ([\text{Cu(II)Y(Y-H)}]^+) (a)</td>
<td>63</td>
</tr>
<tr>
<td>Reaction pathways of (m/z) 260 from ([\text{Cu(II)Y(Y-H)}]^+) (b)</td>
<td>64</td>
</tr>
<tr>
<td>Reaction pathways of ([\text{Cu(II)Y(Y-H)}]^+) (c)</td>
<td>65</td>
</tr>
<tr>
<td>5.3. Reaction pathways of ([\text{Cu(II)His(His-H)}]^+) (a)</td>
<td>70</td>
</tr>
<tr>
<td>Reaction pathways of (C_\alpha^*) radical from ([\text{Cu(II)His(His-H)}]^+) (b)</td>
<td>71</td>
</tr>
<tr>
<td>5.4. Reaction pathways of ([\text{Cu(II)R(R-H)}]^+) (a). P: unlabeled; D: deuteron labeled</td>
<td>75</td>
</tr>
<tr>
<td>Reaction pathways of ([\text{Cu(II)R(R-H)}]^+) (b). P: unlabeled; D: deuteron labeled</td>
<td>76</td>
</tr>
<tr>
<td>5.5. Reaction pathways of ([\text{Cu(II)K(K-H)}]^+)</td>
<td>80</td>
</tr>
<tr>
<td>5.6. Reaction pathways of ([\text{Cu(II)GG(GG-H)}]^+)</td>
<td>83</td>
</tr>
<tr>
<td>6.1. Major sequence ions from dilithiated peptides</td>
<td>94</td>
</tr>
<tr>
<td>6.2. Dissociation pathways of ([\text{YAGFL - H + 2Li}]^+) to</td>
<td>95</td>
</tr>
<tr>
<td>(a) (y_n^{<strong>}) ((y_1^{</strong>} - y_3^{**})) ions</td>
<td>95</td>
</tr>
<tr>
<td>(b) (y_{n-1}^{<strong>}) ((y_4^{</strong>})) ions</td>
<td>96</td>
</tr>
<tr>
<td>(c) (c_{n-2}^{<strong>}) ((c_3^{</strong>})) ions</td>
<td>96</td>
</tr>
<tr>
<td>(d) (c_{n-2}^{<strong>}) ((c_2^{</strong>})) ions</td>
<td>97</td>
</tr>
<tr>
<td>(e) (a_n^*) ions</td>
<td>97</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. List of peaks (m/z) in the CAD spectra of [Cu(II)AA(AA-H)]⁺ complexes</td>
<td>52</td>
</tr>
<tr>
<td>6.1. Important fragment ions from singly charged dilithiated peptides after CAD</td>
<td>101</td>
</tr>
</tbody>
</table>
Metal ions have been frequently recognized as risk factors for neuro-degenerative disorders, although they are necessary components for the survival and development of biological systems. Copper ion, as one of the very important metal ions in our body, has been long found to be an essential element in living systems.\textsuperscript{1-6} Copper ions are frequently involved in oxidation, dioxygen transport, and electron transfer processes. It plays a significant role in maintaining normal neurological function, immune system, and respiration. Copper ion imbalance in living systems would cause disease. Copper mobilization and redox activity can cause severe damage resulting from the repetitive formation of reactive oxygen species. The damage is usually incurred by an initial reduction of the Cu(II) ion to Cu(I), followed by a Fenton-type reaction to form the hydroxyl radical, which is considered to be the most reactive ROS (reactive oxygen species) generated in vivo, and that causes diseases such as chronic inflammation, Alzheimer's disease, aging, and cancer. It has been shown that renal dysfunction can be induced by the copper level in the kidneys.\textsuperscript{7} Wilson's disease has been characterized as a hereditary disease involving excessive copper accumulation in the liver due to a decreased excretion of copper from the liver. Although iron is present at higher
physiological concentrations, copper can react with H$_2$O$_2$ to form hydroxyl radicals 60 times faster than iron does.$^{8,9}$ In order to gain a better understanding of how copper ions work in biological systems, more knowledge about the intrinsic chemistry of copper/protein complexes is necessary. Insight about this chemistry can be obtained by studying copper/amino acid or peptide model systems in the gas phase.$^{10-34}$

Metal/molecule interactions are usually metal specific and the gas phase reactivity of molecules attached to transition metal ions depends on the oxidation state of the metal ion. Cu(I), with a closed shell d$^{10}$ configuration, has very different chemical properties than Cu(II) does. For copper(I) and amino acid complexes, the main fragments originate from a loss of 46 u corresponding to the elimination of H$_2$O and CO.$^{20-22}$ Three reaction mechanisms have been proposed by theory and the mechanism of lowest critical energy involves successive insertions of Cu(I) into the C-C and C-OH bonds. Many reactions involving Cu(II) ion and amino acid/peptide complexes produce radical cations and fragment ions that are induced by radical reactions.$^{26-31}$

Hu et al.$^{32}$ studied the gas phase coordination properties of divalent ions with His-containing peptides. It was found that the 44 u loss from Cu(II)-EFVYVHPV complex involved the removal of CO$_2$ from the C-terminal carboxylate group, which is a different reactivity as compared to the H$_2$O and CO eliminations in Cu(I)/amino acid complexes. It was proposed that the hydrogen atom on the C-terminal end (COOH) is transferred to Cu(II) ion and the subsequent CO$_2$ loss from the C-terminal created a radical site at the $\alpha$-carbon. Such a radical can induce further fragmentation on the side chain of the peptide. Gatlin$^{27}$ and Seymour$^{28}$ et al. also reported that the copper(II) complexes formed from a deprotonated amino acid and 2,2$'$-bipyridine underwent reductive decarboxylation.
and formation of a radical site at the α-carbon, followed by fragmentation on the side chain of the amino acid through α-cleavage. We found, theoretically and experimentally, that dimeric copper/aliphatic amino acid complexes dissociate similarly to form reductive decarboxylation products, which fragment further by radical induced dissociation at the originally deprotonated amino acid ligand. The originally intact amino acid in these copper/amino acid complexes stays unchanged. This reaction is very specific for copper(II) complexes while other metal (Ni(II) and Co(II)) complexes react differently upon collisionally activated dissociation (CAD) conditions in the mass spectrometer.\textsuperscript{12}

Amino-acid derived radicals with the unpaired electron at the alpha-carbon atom have been proposed as intermediates in the oxidative damage of amino acids and proteins. In this dissertation, a novel method is introduced for forming alpha-amino acid radicals, viz. Cu(II)-catalyzed decarboxylation of ternary complexes [Cu(II)AA(AA-H)]\textsuperscript{+} (AA = amino acid). The unimolecular chemistry of the copper ion bound radicals produced is subsequently determined by CAD and multistage mass spectrometry. Reaction mechanisms are proposed based on the experimental results and are substantiated by deuterium labeling. These studies also found that inter-ligand radical reactions occur when the amino acids or peptides contained in the Cu(II) complexes are large or flexible enough. Such reactions probe the bimolecular reactivity of the α-radicals.

Peptide analysis plays an important role in studies aiming at understanding how proteins and biosystems work. Hence, the development of effective, rapid, and sensitive methods of peptide sequencing has always been an active research area in biological science.\textsuperscript{36-38} For structural analysis by mass spectrometry (MS), proteins are usually
separated on 2-D gels and subjected to tryptic digestion first. The digested products are then analyzed by MS to determine their masses. A search of the measured masses in a protein database may help to identify the peptides (and their protein precursor), if that protein had been characterized before.\textsuperscript{0,40} Otherwise, the sequences of unknown peptides can be deduced by tandem mass spectrometry (MS/MS).\textsuperscript{36-38} The majority of protein MS and MS/MS studies reported so far have employed fast atom bombardment (FAB),\textsuperscript{41} matrix-assisted laser desorption ionization (MALDI),\textsuperscript{42,43} or electrospray ionization (ESI)\textsuperscript{44} to ionize the digested peptides. FAB was the major ionization method of biological samples between 1982 and \textasciitilde 1992, while the newer MALDI and ESI methods are used largely today.

In general, the ionization methods mentioned produce protonated peptides, \([\text{Pep} + \text{H}]^+\),\textsuperscript{45} having the proton attached at the most basic site of the peptide. Upon collisional excitation, protons not attached to Arg residues become mobile, \textit{i.e.} they can move along the backbone or to the side chain substituents to induce fragmentation reactions that yield products characteristic of the peptide sequence.\textsuperscript{45-48} Protonated peptides contain three types of backbone bonds, viz. $^\alpha$C–C(=O), C(=O)–N, and N–$^\alpha$C, either of which may be cleaved upon fragmentation. According to the nomenclature introduced by Roepstorff\textsuperscript{48} and Biemann,\textsuperscript{50} the resulting fragment ions are classified, as $a_n$, $b_n$, and $c_n$ ions if they retain the N-terminus of the peptide or $x_n$, $y_n$, and $z_n$ ions if they retain the C-terminus of the peptide. From these sequence-indicative backbone fragments, the $b_n/y_n$ pairs arising from breakup of the peptide bond usually dominate MS/MS spectra obtained by collisionally activated dissociation (CAD) at low collision energies (eV range).\textsuperscript{45-48} The $b_n/y_n$ backbone ions are formed by migration of the ionizing proton to the various N
atoms of the amide bonds, which weakens these bonds and facilitates fragmentation via
attack of the emerging acylium ions, C(=O)+⋯HN, by a nearby nucleophile. Most often, the attacking nucleophile is the N-terminally adjacent carbonyl oxygen and the b_n product has a protonated oxazolone end group, while the complementary y_n ion is a truncated peptide. Since such cleavages can take place randomly at any position in the backbone, they lead to complementary series of fragments that reflect the peptide’s sequence.

If the peptide carries functionalized amino acid residues (e.g., Asp, His, or Lys), certain bonds near these residues may be cleaved selectively under certain conditions. Such selective cleavages are of interest because they produce simpler fragmentation patterns in CAD spectra, similar to enzymatic digests producing simpler and more predictable peptide mixtures. For example, it has been found that an internal lysine residue enhances the cleavage of the amide bond in the C-terminal position, especially in the absence of a mobile proton; the amine group of the lysine side chain initiates fragmentation, leading to b_n sequence ions with lactam structures. Similarly, in peptides with no mobile proton, acidic residues (Asp, Glu) have been found to enhance cleavage of the C-terminally adjacent amide bond. And the acidic proton of the side-chain initiates amide bond cleavage to create a b_n ion with an anhydride end group.

Peptides from tryptic digests often yield doubly protonated ions when ionized by ESI, i.e. [Pep + 2H]+. One proton is sequestered at the basic Lys or Arg C-terminal, while the other can migrate along the backbone to produce the same types of ions presented above for singly charged [Pep + H]+ ions. It is not uncommon that peptide
[Pep + H]$^+$ or [Pep + 2H]$^+$ ions do not produce enough backbone fragments upon MS/MS to allow for definitive sequence determination.\textsuperscript{37} For this reason, metalated peptides have been investigated as alternative precursor ions.\textsuperscript{57,58,63-76} Attachment of a metal cation (M$^+$) to peptides for sequencing purposes can be viewed as “derivatization” of the sample to alter and control the fragmentation observed in MS/MS experiments. Metal ions interact strongly with the basic sites of a peptide molecule, such as the carbonyl groups, N-terminus, and side chain substituents. Attachment of a metal ion to a backbone amide group increases the electrophilic character of the amide carbon the same way as proton attachment does, which leads to peptide bond cleavage to yield N-terminal b-type ions or C-terminal y-type ions. Using the nomenclature introduced for protonated fragments, these ions are named [b$_n$ – H + M]$^+$ or [y$_n$ – H + M]$^+$ ions because they carry M$^+$ in place of H$^+$ charges; for brevity, the acronyms b$_n^*$ and y$_n^*$ are used. Peptides cationized by singly charged metal ions undergo a unique fragmentation which produces truncated peptide complexes devoid of the C-terminal amino acid residue; these N-terminal fragments have been termed [b$_n$ + OH + M]$^+$ ions.\textsuperscript{69} Because of the favorable energetics, the MS/MS spectra of peptide [Pep + M]$^+$ ions are dominated by [b$_{n-1}$ + OH + M]$^+$ fragments and competitive fragmentations are either absent or suppressed significantly.\textsuperscript{58}

The mechanism of [b$_{n-1}$ + OH + M]$^+$ formation has been recently elucidated.\textsuperscript{77} This reaction proceeds via a mixed anhydride intermediate. With dipeptides, the same intermediate is traversed irrespective of sequence. For this reason, the MS/MS spectra of the [Pep + M]$^+$ ions from isomeric dipeptides are often indistinguishable.\textsuperscript{69,77,78} Our group recently found that this problem is bypassed if the peptides are derivatized to their carboxylate salts, for example, if [Pep – H + 2Li]$^+$ precursor ions are used.\textsuperscript{78,79} In these
complexes, the proton on the C-terminal COOH group is replaced by one lithium cation, while the second lithium cation provides the charge. Moreover, [Pep – H + 2Li]$^+$ from isomeric dipeptides give substantially different fragmentations patterns, displaying unique c- and y-type fragments that permit unequivocal differentiation.$^7$ This approach is extended in this dissertation to larger dilithiated peptides. The fragmentation characteristics of a number of [Pep – H + 2Li]$^+$ and select [Pep – H + 2Na]$^+$ ions are examined in detail by different MS/MS techniques in order to evaluate the usefulness of such precursor ions, vis à vis monometalated or protonated precursor ions, in the elucidation of peptide sequences. The experimental results show that singly charged dimetalated derivatives can lead to valuable sequence insight, which is particularly important when the corresponding protonated or monometalated precursors give MS/MS spectra with incomplete sequence ion series.$^7$
2.1. Mass Spectrometry Instrumentation

A mass spectrometer is an instrument that separates ions based on their mass-to-charge ratios (m/z). There are many types of mass spectrometers and new models are emerging on the market every year. There are four major components in every mass spectrometer: the sample inlet system, the ionization source, the mass analyzer, and the detector, as shown in Fig. 2.1. The sample inlet system allows the analyte to be introduced into the mass spectrometer for ionization. Sampling loops, syringes, and direct loading of sample and/or matrix systems are used for sample introduction. Once the analyte is introduced into the mass spectrometer, the ionization source will transport the analyte into the gas phase. Ions in solutions can be transferred into the gas phase by using a nebulizing gas, drying gas, and a strong electric field. From thick liquid and solid samples, laser ablation bombardment by ion beams are used for transporting ions in the gas phase. Once in the gas phase, ions are guided and accelerated by an electric field into the mass analyzer, where they are separated by their mass-to-charge ratios. Separated ions exit the analyzer in order of increasing or decreasing mass-to-charge ratios. The detector detects all the ions that impinge onto it and produces signals that are proportional...
Figure 2.1. Major components of a mass spectrometer.
to their abundances. Then the computer system processes and records the entire signals (mass-to-charge ratio, m/z, versus ion abundances) by using data-processing software.

Vacuum systems are very important in mass spectrometry. The high vacuum in mass spectrometers ensures that ions reach the detector without colliding into other molecules, so that the only reaction that can happen is the release of extra internal energy deposited upon ionization and acceleration. With a good vacuum system, good sensitivity and resolution can be obtained.

Each type of mass spectrometer has its own advantages and disadvantages depending on the kind of ionization method and the type of analyzer that are used. Different ionization methods and analyzers will provide different information about the sample. Usually the name of a mass spectrometer consists of the names of the ionization method and the analyzing method. Considering that ionization and mass analysis are the most important parts of mass spectrometry, the following section of this chapter provides detailed information about the ionization and mass dispersion methods used in the research presented in this dissertation.

2.2. Ionization Methods

A very important part of mass spectrometry analysis is the formation of analyte ions in the gas phase. Only if ions can be formed in the gas phase, mass spectrometry can be used as a characterization method for molecular system. Ionization methods can be categorized as "hard" or "soft" ionization methods. "Hard" ionization is an ionization method, which also coproduces abundant fragment ions as it ionizes the analyte; electron
impact (EI) falls into this category. Fragments can provide useful information about the structure of the analyte. "Soft" ionization methods produce abundant molecular ions or quasimolecular ions (ions with extra or missing pieces compared to the analyte molecule) without or with little fragmentation; electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) fall into this category. Unfragmented analyte ions provide molecular weight information about the analyte. Molecular ions usually refer to species formed by removing or adding an electron to the analyte; quasimolecular ions are those formed by adding an ion (usually proton or alkali metal ion) to or by removing an ion from the analyte molecule. ESI and MALDI are the major ionization methods used today for the formation of gas phase ions from nonvolatile samples, and quasimolecular ions are the major form of analyte ions that have been studied in this dissertation.

2.2.1. Electrospray Ionization (ESI)

Electrospray ionization is widely used to form gas phase ions from biomolecules. Positive and negative analyte ions are formed in solution by extraction of proton(s) from the solvent or by forming adducts with other cations, or by loss of positive ions (proton or metal cations), and these ions are transferred into the gas phase. A very important characteristic of ESI is that it often leads to multiply charged ions in the gas phase, which greatly increases the molecular weight range that can be sampled by typical mass spectrometers (m/z information obtained). Large biomolecules and synthetic polymers can be ionized by ESI. Because it is a "soft" ionization method, non-covalently bound complexes can stay intact under appropriate experimental conditions during the mass
It has been found that gaseous ions generated by electrospray ionization retain most of the characteristics of ions in solution.

For electrospray ionization, the analyte solution is introduced into the ionization source at atmospheric pressure to form gaseous ions. The liquid sample is injected through a needle under pressure (pump or back pressure). The voltage difference between the needle (acts as an electrode) and the counter electrode (usually the inlet of the next vacuum stage) is about several kilovolts. The strong electric field between the needle and the counter electrode leads to charge accumulation at the surface of the exiting liquid solution at the needle tip. There is a concentric flow of nebulizing gas (N₂) around the needle that ensures that the charged liquid solution forms a fine mist of tiny, highly charged droplets as it leaves the needle into the desolvation chamber (ionization source area). The charged droplets contain both positive and negative ions and the surface of the droplets is rich of ions of the polarity that is accelerated by the electric field. The heated drying gas (N₂) coming from another direction helps to further de-solvate the fine droplets. A scheme of an electrospray ionization source is shown in Fig. 2.2(a). Under these conditions, the charged droplets become smaller and smaller until charge repulsion is larger than surface cohesion so that coulombic repulsion leads to division into smaller droplets. This desolvation-division process continues until the field on the droplets becomes so large that analyte ions are ejected directly into the gas phase as shown in Fig. 2.2(b). Directed by the electric field and vacuum, analyte ions, some tiny droplets, and solvent molecules enter the next pumping stage of mass spectrometer.

The solvent plays an important role in the ionization process. A polar solvent or mixed solvents are usually used for ESI, but if the analyte is pre-charged (in salt form),
Figure 2.2. ESI ionization source;
(a) scheme of an electrospray ionization source and
(b) the desolvation/division process in ESI.
a non-polar solvent can also be used. Solvents with too high of a surface tension and non-polar solvents cannot be used (unless the analyte is pre-charged) because consistent flow of tiny droplets from such solvents cannot be easily achieved and thus steady signals cannot be obtained; for this reason, water, which is a solvent of high surface tension, must be mixed with other solvents like methanol for ESI. Because of the charge accumulated on the droplets, the droplets are drawn towards the counter electrode and also deformed which facilitates the formation of smaller droplets and the creation of a steady and constant electric current flow from the needle to the counter electrode.

One drawback of electrospray ionization is that many types of quasimolecular ions may be formed from the same analyte. For example, sodiated and potassiated adducts often accompany the formation of protonated adducts even if no sodium or potassium salt is added to the sample, since sodium and potassium salts are common contaminants from glassware.

2.2.2. Matrix-Assisted Laser Desorption/Ionization (MALDI)

Matrix-assisted laser desorption/ionization (MALDI) is another "soft" ionization method for large molecules. The analyte, matrix, and a salt (usually a monocation) are dissolved separately and these solutions are mixed together in a certain ratio. Usually, a large excess of matrix molecules is added. A minute amount of the mixed solution is then spotted on a target where, after the solvent evaporates, solid crystals are formed (the matrix, usually an acid with a conjugated ring, plays an important role in crystallization). The analyte and salt molecules are dispersed in a large excess amount of the matrix
Figure 2.3. The matrix assisted laser desorption/ionization (MALDI) process.
molecules (~ 1:1000 ratio) so that interactions between the analyte molecules are reduced to a minimum. The sample target is inserted into the ionization source, which is kept under vacuum. A laser beam strikes the crystallized sample on the target and mostly excites the matrix molecules. The matrix molecules must have a strong absorbance at the wavelength of the laser, which prevents the analyte molecules from being fragmented by the laser beam. The excited matrix molecules and matrix ions formed by the laser undergo proton transfer with analyte molecules and/or desorption into the gas phase as matrix/analyte clusters with salt (if any was added). The excess energy of the matrix results in further desolvation of matrix molecules from the clusters until the analyte ions are turned into gas phase ions. In the matrix/analyte/salt clusters, ionization may take place by metal ion adduction, if the analyte does not protonate readily. The analyte ions are finally guided into the mass analyzer by an applied electric field. The MALDI ionization process is shown in Fig. 2.3. Usually only singly charged ions are produced by matrix-assisted laser desorption/ionization as opposed to electrospray ionization; both methods cause little fragmentation.

2.3. Mass Analyzer

The mass analyzer is the place where the gas phase ions are separated by their mass-to-charge ratios (m/z). There are many types of analyzers and each type has its own strength over the others. The most pronounced differences among the mass analyzers are peak resolution (which is expressed as m/Δm), upper mass limit, focusing and transmitting properties, and available fragmentation stages. Structure information can
also be obtained for analyte ions generated by a soft ionization method \textit{i.e.} with little or no fragmentation) if tandem mass spectrometry is used. The types of analyzers employed here will be discussed in the following sections.

2.3.1. Quadrupole Ion Trap (QIT) Mass Analyzer

The QIT mass analyzer consists of three electrodes, which are a ring electrode and two end-cap electrodes as shown in Fig. 2.4(a)\textsuperscript{80,81}. There are orifices in the middle of the two end-cap electrodes where ions can enter and exit the ion trap mass analyzer. After being focused and guided into the ion trap through those tiny holes, ions are under the influence of a three-dimensional quadrupole field, which is formed by applying appropriate potentials to these three electrodes. Ions are retained in the center of the ion trap by collisions with a buffer gas (He) and then ejected out of the ion trap in the order of increased mass-to-charge ratios (m/z) by frequency ramping.

The details of how the ion trap works are discussed briefly. The three electrodes are almost hyperbolic in geometry and form an almost ideal three-dimensional quadrupole field in the trap when a radio frequency potential is applied to the ring electrode and the two end-caps are grounded (Fig. 2.4(b)). The trajectory of an ion in the ion trap is characterized by two frequencies that represent the motions of the ion in axial and radial directions, induced by the trapping field. When ions of one mass-to-charge ratio are trapped, they are focused towards the center of the trap in one direction and defocused in the other and then the situation reverses after approximately half a microsecond and it continues to alternate. The motion of the ions in the trap can be described by the
Figure 2.4. Quadrupole ion trap mass analyzer; (a) electrodes layout and (b) quadrupole ion trap analyzer.
following equation, where \( q_z \) is a dimensionless parameter, \( r \) is half the distance between the two end-caps, \( V \) is the amplitude of the voltage on the ring electrode, \( \omega \) is the oscillating RF frequency, and \( e \) and \( m \) are the charge and mass of an ion.

\[
q_z = \frac{4eV}{mr^2\omega^2}
\]

The resulting trajectory of the ion from its oscillations resembles a figure-of-eight, and ions undergo about 20,000 collisions per second with the helium buffer gas for about 1-30 ms.

Low mass ions have small excursions from the center of the ion trap and are close to the ion trap center. The larger the ions (m/z) are, the larger their excursion from the center of the ion trap. Ions are trapped in the center of the ion trap in a fashion of layered onion rings with small ions in the inner center and ions with increased mass to charge ratios on the outside. It has been reported that ion excursion from the trap center is less than 1 mm. This is how the ion trap works as an ion storage device.

After a certain period of time, ions in the trap are ejected by ramping the RF frequency on the ring electrode in a linear fashion, which causes the ions to exit the trap through the holes in the end-caps and impinge on the electron multiplier detector in order of increased mass-to-charge ratios. Only ca. half of the ions ejected from the ion trap reach the detector. Since each ion is ejected at a specific RF amplitude, and the starting RF amplitude and ramping rate are known, the signal can be recorded accordingly for each ion ejected from the ion trap. Smaller ions are ejected first so they experience a space charge perturbation and that results in poor spectral resolution. An auxiliary AC (alternating current) frequency is introduced and applied to the end-caps, which excites the ions resonantly in the axial direction while RF ramping. Ions are extracted from the
ion cloud and experience a charge-perturbation free period briefly before ejection. This process is called axial modulation. This way, ions of the same mass-to-charge ratio stay together upon ejection and produce a higher resolution mass spectrum.

2.3.2. Quadrupole Ion Trap Tandem Mass Analyzer

The quadrupole ion trap can also act as in-time tandem mass analyzer in contrast to a sector instrument in which in-space MS/MS is performed by placing one mass analyzer after another. For tandem and multistage mass spectrometry (MS\textsuperscript{n}), ion isolation is the first step. Upon choosing the isolation window, the ions below the chosen mass-to-charge ratio are ejected by ramping the RF frequency with axial modulation. The ions above the chosen range are removed by broadband waveform sweeping on the end-caps that lasts for about 5 ms. After isolation, ions are subjected to resonant excitation and hundreds of collisions with the He collision gas take place. The result is collisionally activated dissociation (CAD), also known as collisionally induced dissociation (CID), of the selected ions. Ejection of ions after the CAD process is achieved by ramping the RF frequency on the ring electrode and axial modulation. This is termed MS/MS (MS\textsuperscript{2}). Similarly, isolation of one of the fragment ions from CAD of the initially selected ions leads to an MS\textsuperscript{3} experiment. The isolation/fragmentation process can be continued if there are enough ions in the trap. N = 2-4 stages of isolation/fragmentation can be carried out routinely and studies with n = 13 have also been reported.
Figure 2.5. A quadrupole mass analyzer. The ions move in the z-direction.
2.3.3. Quadrupole Mass Analyzer

A quadrupole mass analyzer is composed of four parallel conducting rods that are held in a two by two configuration as shown in Fig. 2.5. Each pair of opposite rods is electrically connected and the polarities of the four rods alternate rapidly at a certain frequency; with DC and RF potentials applied to the rods, a two-dimensional quadrupole field is established in the x-y plane. Ions that are produced in the ionization source are guided into the quadrupole analyzer in the z direction. The DC and AC potentials are increased simultaneously at a constant ratio during the sweeping (scanning) process. These applied potentials can be represented by the following equations:

\[ \Phi_{x-y} = + (U + V \cos \omega t) \]
\[ \Phi_{y-z} = - (U + V \cos \omega t) \]

The potentials of the four poles vary in such a way that only ions of a specific mass-to-charge ratio have a stable trajectory in the entire quadrupole field and are allowed to reach the detector at a specific time. The rods with opposing polarity to the ions at a specific time will quench those ions that do not have a stable trajectory at that time. Low mass ions have relatively small momentum and change their course only slightly under the influence of the rapid changing polarities of the four rods; thus, they have higher opportunities to reach the detector. High mass ions have relatively large momentum and they divert further from the center of the four rods and have more chances to be quenched in the two dimensional field. So, not all ions are transmitted to the detector and thus the quadrupole mass analyzer has a relatively limited mass range and moderate mass.
resolution. If tandem mass spectrometry was to be performed, it should be coupled with another mass analyzer. In other words, a quadrupole mass analyzer can perform only a single stage of mass analysis, unlike the quadrupole ion trap.

2.3.4. Time-of-Flight (ToF) Mass Analyzer

A time-of-flight mass analyzer is basically a field-free drifting tube under high vacuum. Mass spectra are recorded according to the flight times of ions. When ions enter the flight drifting tube, they should have the same kinetic energy because they are accelerated under the same electric field. In the following equation, KE is the kinetic energy that ions gained from the acceleration potential V, v is the velocity of ions in the flight tube, and ze and m are charge and mass of the flying ions in the flight tube (usually singly charged).

\[ KE = \frac{mv^2}{2} = zeV \]

The length of the flight tube is a pre-set value, so that the flying time of an ion is related to its velocity. Low mass ions (small m/z) have larger velocities and arrive at the detector earlier than high mass ions. There are two modes that a time-of-flight analyzer can be operated when properly equipped, viz. linear mode and reflectron mode. In the linear mode, the detector is located at the end of the drifting tube. If ions dissociate during the drifting process, the parent ions, the produced neutral molecules, and the fragment ions will arrive at the detector at the same time. If ions of the same mass differ only slightly in their velocity, they will arrive at the detector at different times. In such cases, the recorded mass spectra have poor resolution and do not reflect the real masses of the ions.
In order to avoid this problem, a reflector is added in front of the first (or linear mode) detector, which is composed of a series of grids upon which different voltages can be applied. There is a second detector that is designated as reflectron detector arranged in the path of ions coming out of the reflector (as shown in Fig. 2.6). When the mass spectrometer is operated in the reflectron mode, ions of the same kinetic energy enter the drifting tube and reach the reflector. The voltage applied to the reflector is equal or slightly higher than the voltages applied to the ions right before they enter the drifting tube so that all ions are reflected out of the reflector. The larger the momentum of an ion is, the deeper this ion penetrates into the reflector. When changing their flying path and regaining their kinetic energy in the reflector, ions with the same mass and slightly different initial velocity are focused and arrive at the reflectron detector at the same time. The reflector greatly increases the resolution of a time-of-flight mass analyzer.

One distinguished advantage of the time-of-flight mass analyzer is that there is no upper limit for mass dispersion theoretically; hence, this mass analyzer does have a wide mass range and almost all ions are transmitted to the detector. Strictly speaking, the ToF analyzer is a one stage mass analyzer.

There are ToF analyzers in which the flight tube is orthogonal to the ion-focusing lens. At the low end of the flight tube, there is a reflector that reflects all ions that drift down the flight tube and repels them back to the detector to generate mass spectra. The mass dispersing principle is the same as for the common ToF mass analyzers. The flight path in orthogonal arrangements can be either v-shaped or w-shaped (higher resolution).
Figure 2.6. Layout of time-of-flight (ToF) mass analyzer.
2.3.5. Quadrupole-Time-of-Flight (Q-ToF) Tandem Mass Analyzer

The quadrupole-Time-of-Flight (Q-ToF) mass analyzer is a new type of analyzer that combines a quadrupole mass analyzer with a time-of-flight mass analyzer; this configuration is especially useful for tandem mass spectrometry experiments. This is a tandem mass analyzer in space. Ions that are generated in the ionization source are guided into the quadrupole analyzer. By setting specific voltages on the four rods, only ions with a certain mass-to-charge ratio are selected and focused by the two-dimensional quadrupole field. They pass into a collision cell for CAD with a collision gas (Ar), where product ions are formed. The product ions that exit the collision cell are guided into an orthogonally oriented time-of-flight mass analyzer and repelled down into the flight tube. Mass spectra are generated after the ions exit the flight tube and impinge onto the multi-channel plate detector.82
3.1. Materials

The solvents (water, methanol; HPLC grade) and glycine (Gly or G), tertiary leucine (t-Leu or t-L; a non-natural amino acid), ammonium hydroxide (NH₄OH), sodium trifluoroacetate (NaTFA), lithium hydroxide (LiOH), and lithium trifluoroacetate (LiTFA) were purchased from Sigma-Aldrich. Alanine (Ala or A), leucine (Leu or L), valine (Val or V), tyrosine (Tyr or Y), phenylalanine (Phe or F), tryptophan (Trp or W), histidine (His or H), lysine (Lys or K), arginine (Arg or R), and the peptides GG, LGG, GLA, LGF, GLF, GAY, YGG, GGGG, polyglycine, GGKAA, FFFFF, YGGFL, YAGFL, YGGFLK, YAGFLR, SIKVAV, polyalanine, and DRVYIHPF were purchased from Sigma, and AAG and AAAA from BACHEM. Cupric chloride (CuCl₂) was purchased from Fisher and isoleucine (Ile or I) from Eastman. All chemicals were used without further purification.

3.2. Experimental Instrumentations

The two mass spectrometers used for the experiments in this dissertation are described in the following sections.
3.2.1. Bruker Daltonics Esquire-LC ESI-QIT Mass Spectrometer

Most studies were conducted on the electrospray ionization quadrupole ion trap mass spectrometer (ESI-QIT MS), manufactured by Bruker Daltonics (Model: Esquire-LC, Billerica, MA). The ESI-QIT MS instrument is shown schematically in Fig. 3.1.81

Liquid samples are introduced into the ionization source by a syringe pump whose pumping rate is adjusted in the 100 - 300 ul/h range through a stainless steel needle that has a concentric flow of nitrogen nebulizing gas (1 - 10 psi) on the outside. The nebulizing gas helps to spray a fine mist of tiny droplets and thus achieve a steady ion flow. The ionization source is under normal air pressure, the needle is grounded and the entrance of the glass capillary (metallic surface) is held at - 4 kV of voltage. The needle and the glass capillary are orthogonal to each other, and by this arrangement, most of the liquid solution and neutral molecules are guided to the waste, which increases the signal-to-noise level in the system. The glass capillary has an inner diameter of about 1 mm and works both as ion transmitter and as a separator of two vacuum stages (atmospheric pressure at source region and ~ 1/10 air pressure in the area between the end of the capillary and skimmer one). It is surrounded by heated nitrogen drying gas (150 ºC, 8 -10 L/min), which exits through a hole above the entrance of the glass capillary into the ionization source. The drying gas forms a concurrent heated gas flow, in contrast to the nebulizing gas, and helps to desolvate the droplets. The drying gas temperature is kept at a moderate level to avoid ion fragmentation in the source area. Because of the electric field, the concentric nebulizing gas and concurrent drying gas, and the vacuum
difference, ions are ejected out of the tiny droplets and guided through the capillary into the next part of the mass spectrometer, the skimmer region. The desolvation process may not be complete in the ionization source area; further, some of the charged droplets and solvent and nitrogen molecules enter the skimmer region with the ejected ions. Because the skimmer region is under vacuum and different voltages, ions are guided through the orifices in the center of the two skimmers, where the droplet desolvation process can continue, while most of the neutral molecules are removed and pumped away by the vacuum system (shown in Fig. 2.2(a)). There are many ion/molecule collisions in this region, so it is very important to adjust the skimmer voltages in order to prevent unnecessary fragmentations.

The ions are guided through the octopoles and two lenses, which act as ion focusing devices into the ion trap where they are stored and analyzed. The principles of how the ion trap operates have been discussed in chapter II. By RF ramping, ions are ejected from the trap in order of increasing mass-to-charge ratios, and impinge onto the electron multiplier (detector) producing signals that are proportional to their populations. The electron multiplier can increase the signal by 5-8 orders of magnitude. Tandem mass spectrometry, which was used in most of the presented work, has been explained in detail in chapter II. Briefly, after a large mass range of ions is trapped, a wide range of RF frequencies on the ring electrode is ramped, combined with broadband waveform sweeping on the end-cap electrodes, to eject all but the ions of a given m/z value.

There is a gate in front of the detector, which is closed during the ejection period so that the detector is protected from unnecessary contamination. After ramping, the isolated ions are brought to resonant excitation by an auxiliary frequency applied to the end-cap
Figure 3.1. Scheme of the Bruker Daltonics Esquire-LC ESI-QIT MS instrument.
electrode. The ions acquire kinetic energy in this process and collide with the helium gas present in the trap, which causes fragmentation. If subsequently all ions are ejected and recorded, the MS/MS (or MS$^3$) spectrum is obtained. Or one of the fragment ions can be selected to undergo the same process as the original (precursor) ion for an MS$^3$ spectrum, etc.

The QIT mass spectrometers are very user-friendly. If a targeted mass or mass range is selected, almost all of the voltage and frequency parameters can be controlled by incorporated computer software. These parameters can be also accessed directly by operators who can input values so that optimized experimental conditions can be achieved.

3.2.2. Micromass Q-ToF Ultima MALDI Mass Spectrometer

The Q-ToF Ultima MALDI, manufactured by Micromass (Manchester, UK) is a hybrid quadrupole time-of-flight mass spectrometer with a MALDI source that ionizes samples by using laser irradiation. This instrument is capable of tandem mass spectrometry operation. The details of how each segment works have been explained in chapter II and the layout of the instrument is shown in Fig. 3.2. The analyte is mixed with a matrix compound to facilitate the production of ions in the gas phase. Once the analyte is loaded onto the ninety-six-well target plate and air-dried, the target is inserted into the ionization source that is under vacuum. Upon pulsed laser irradiation, the excitation of the matrix molecules induces desorption and ionization of the analyte in the gas phase. The so-formed ions are guided into the quadrupole mass analyzer. The quadrupole can be
Figure 3.2. Scheme of the Micromass Ulrima MALDI-Q-ToF MS instrument.
operated as an ion transport device for MS experiments (RF-only mode) or as a mass selection device for MS/MS experiments. A collision cell is situated after the quadrupole to induce fragmentation in the MS/MS experiments. The quadrupole collision cell assembly is followed by the orthogonal acceleration cell (OA) in which the ions are pushed downward into the ToF analyzer. The ions are pushed out of the OA following either a V or W (longer flight path for higher resolution) path traveling through the reflectron lens at the bottom of the instrument. Ions are then detected with a microchannel plate detector and ion counting system, and a computer processor generates a mass spectrum from the detected ion signals. The mass spectrum from a ToF experiment describes both the molecular weights and relative abundances of the components.

The MS/MS capability gives the MALDI-Q-ToF mass spectrometer great advantage over one dimensional MALDI-ToF MS because it can provide structural information for large molecules. MALDI-Q-ToF MS can also provide much higher-resolution mass spectra that significantly facilitate compound identification.

3.3. Methods

3.3.1. Copper(II) Catalyzed Reactions

The copper(II) bound dimer complexes \([\text{Cu(II)AA(AA-H)}]^+\) (AA = Gly, Ala, Val, Leu, Ile, t-Leu, Phe, Trp (W), Tyr (Y), His, Arg (R), Lys (K), or GlyGly (GG)) were formed in the gas phase by electrospray ionization and their consecutive fragmentations were examined by CAD in the quadrupole ion trap mass spectrometer. The solution
sprayed contained $10^{-4}$ M amino acid and $2.5\times10^{-5}$ M CuCl$_2$ in a water:methanol (50:50 by volume) mixture; with the hydrocarbon AAs, ammonium hydroxide was added to obtain final solutions containing 2% NH$_4$OH (by volume). The final solution was introduced into the ion source by a syringe pump at a rate of 100-300 $\mu$L/hr. The spraying needle was grounded and the entrance of the sampling capillary was set at -4 kV. Nitrogen was used as the nebulizing gas (1-10 psi) and drying gas (10 L/min, 150°C). He gas was used as the buffer gas in the ion trap. For CAD, the Cu(II) bound dimer precursor ions were isolated and excited to fragment in the ion trap for CAD with the RF frequency that was resonant with the dimer ion's frequency of motion in the trap. The excitation time was 40 ms and the RF amplitude ($V_{p-p}$) was 0.30-0.5 V. Thirty scans per spectrum were collected and the experiments were repeated at least three times.

Deuteron labeling experiments were performed by dissolving amino acids or peptide in mixed D$_2$O:MeOD (50:50 by volume, both from Aldrich) solvent at a concentration of $10^{-4}$ M and CuCl$_2$ at $2.5\times10^{-5}$ M. All other experimental conditions remained the same as above.

3.3.2. Theoretical Calculations

All computations were carried out with the Gaussian 98 program package. Geometry optimization of the Cu(II)-amino acid complexes and neutral ligands and vibrational frequency analysis were performed at the MP2(full)/6-31G(d) level, where "full" means that there were no electrons frozen in the MP2 calculations. Final energetics were obtained at the MP2(full)/6-311+G(2d,2p) level with use of the MP2(full)/6-31G(d)
geometries. The calculations were conducted by Dr. Gilles Ohanessian at Ecole Polytechnique, France.

3.3.3. Dilithiated Peptide Fragmentation

3.3.3.1. ESI Quadrupole Ion Trap Mass Spectrometry (QIT MS) Experiments

Dilithiated peptide complexes [Pep – H + 2Li]$^+$ were formed by ESI and their MS/MS characteristics were examined by CAD in the described quadrupole ion trap. The peptides were dissolved in methanol: water 1:1 (v:v), which was saturated with lithium hydroxide (LiOH) or lithium trifluoroacetate (LiTFA), at a concentration of 1 mg/mL. The solutions were introduced into the ion source by a syringe pump at a rate of 200 µL/hr. The spraying needle was grounded and the entrance of the sampling capillary was set at –4 kV. Nitrogen was used as the nebulizing gas (10 psi) and drying gas (8 L/min, 150 °C). [Pep – H + 2Na]$^+$ ions were formed analogously, using the corresponding sodium salts; these solutions also coproduced [Pep + H]$^+$ ions. In MS/MS mode, the desired precursor ion was first selected by ejecting all other ions from the trap; the selected ion was then accelerated to undergo CAD with the He buffer gas in the trap by an RF field that was turned on for 40 ms at an amplitude ($V_{pp}$) of 0.75-0.95 V. Thirty scans were averaged per spectrum.
3.3.3.2. MALDI Quadrupole/Time-of-Flight Mass Spectrometry (Q-ToF MS) Experiments

The Q-ToF Ultima MALDI mass spectrometer was used for acquiring MS/MS spectra of MALDI-generated \([\text{Pep} - \text{H} + 2\text{Li}]^+\) complexes. The peptides, LiTFA, and the matrix (2’,4’,6’-trihydroxyacetophenone, THAP) were dissolved in acetonitrile: water 3:7 (v:v) at concentrations of 10 mg/mL, 10 mg/mL, and 20 mg/mL, respectively. Sample solutions were made by mixing matrix: salt: peptide solutions in the ratio of 5: 1: 1. Approximately 1 \(\mu\)L of the well-mixed sample solution was spotted onto a 96-well target and allowed to air dry before introduction into the mass spectrometer. MS/MS spectra were measured by selecting the desired \([\text{Pep} - \text{H} + 2\text{Li}]^+\) ion with the quadrupole and subjecting it to CAD in a hexapole collision cell at a collision energy (lab frame) of 45-55 eV. The fragment ions were subsequently sent through the orthogonal ToF section for mass analysis and detection. Argon was used as collision gas at a pressure of 1.85 x 10^{-5} mbar.

3.3.3.3. Calculations

Molecular dynamics simulations on various forms of the dipeptide complex \([\text{FG} - \text{H} + 2\text{Li}]^+\) were performed using the InsightII program (Biosym Technologies, San Diego, CA) in conjunction with the AMBER force field. The energetically most favorable structures were optimized fully, using the same force fields, and grouped into families of ions with similar torsion angles. The most stable species in these families were then fully optimized at the HF/3-21G and B3LYP/6-31G(d) levels. The Gaussian program was
used in the latter calculations. The calculations were conducted by Dr. Béla Paizs at German Cancer Research Center, Germany.
CHAPTER IV

CU(II)-CATALYZED REACTIONS IN TERNARY, SINGLY CHARGED CU(II)
COMPLEXES OF AMINO ACIDS WITH HYDROCARBON SIDE CHAINS

4.1. Cu(II)-Bound Dimer Complex with Ala

A solution of CuCl₂ and Ala was injected into the electrospray instrument. The resulting mass spectrum is shown in Fig. 4.1. In this spectrum, the peaks at m/z 90 and 179 are protonated alanine and the proton-bound Ala dimer, respectively, and m/z 112 is sodiated alanine. The peak at m/z 240 is [Cu(II)Ala(Ala-H)]⁺, m/z 262 is [Cu(II)(Ala-H+Na)(Ala-H)]⁺, and m/z 196 is a fragment from the [Cu(II)Ala(Ala-H)]⁺ complex (vide infra). Copper-bound complexes dominate the spectrum. Cu-containing ions are easily identified, because they appear as doublets originating from incorporation of either ⁶³Cu or ⁶⁵Cu (~ 3:1 abundance ratio). The peak at m/z 240, [Cu(II)Ala(Ala-H)]⁺, was selected for CAD, which led to the spectrum shown in Fig. 4.2(a). The major fragment in this spectrum appears at m/z 196 and arises from the loss of 44 u from the parent ion. There are also peaks at m/z 195, 181, 168, and 152. The MS³ spectrum of m/z 196 is shown in Fig. 4.2(b). It shows the same fragmentation pattern as that obtained in the MS² experiment.
Figure 4.1. ESI-QIT mass spectrum of [Cu(II)Ala(Ala-H)]^+.
Figure 4.2. CAD mass spectra of [Cu(II)Ala(Ala-H)]$^{+}$; 
(a) CAD (MS$^2$) spectrum of m/z 240 and 
(b) CAD (MS$^3$) spectrum of m/z 196.
Scheme 4.1. Reaction pathways of [Cu(II)Ala(Ala-H)]⁺; the acronym rH indicates a hydrogen atom rearrangement.
Cu(II) complexes prefer square-planar geometries, as shown in scheme 4.1 for [Cu(II)Ala(Ala-H)]^+ (m/z 240). The intact alanine ligand binds Cu(II) with its amino and carbonyl groups, while the deprotonated alanine ligand binds Cu(II) with its amino group and carboxylate groups. Scheme 4.1 also provides plausible pathways to the observed fragments. Fragmentation begins with reductive decarboxylation; during this process, CO_2 (44 u) is lost from the deprotonated Ala, as Cu(II) is reduced to Cu(I) and a radical site is formed at the \( \alpha \)-carbon of the deprotonated Ala, \( \text{viz} \), a \( C_\alpha \) radical (m/z 196). Two isomeric radicals, \( N_1 \) (radical site on the amino nitrogen) and \( C_\beta \) (radical site on the \( \beta \)-carbon), can be formed from \( C_\alpha \) by hydrogen rearrangement. Two types of consecutive decompositions can take place from each of these radicals. One reaction is detachment of the entire partially reacted side chain (Ala-H-CO_2) from the dimeric complex to form monomeric Ala-Cu^+ (m/z 152). The other reaction is an elimination via \( \beta \)-bond scission in the partially reacted side chain. Through such \( \beta \)-scission, a hydrogen radical (from \( C_\alpha \), \( N_1 \), and/or \( C_\beta \)), a CH_3 radical (from \( N_1 \)) and a C_2H_4 molecule (from \( C_\beta \)) can be eliminated to form the products at m/z 195, 181, 168, respectively. These latter products can dissociate further to form the final product Ala-Cu^+ (m/z 152). There are a few additional minor fragments, which are not discussed because of their minuscule relative abundances.

Fig. 4.3 is the calculated energy profile for the fragmentation pathways of the copper-bound Ala dimer complex [Cu(II)Ala(Ala-H)]^+; relative energies are in kJ/mol. The parent ion [Cu(II)Ala(Ala-H)]^+ is the lowest energy ion, with the relative energy of 0.0 kJ/mol. The formation of the initial reductive decarboxylation product \( C_\alpha \), carrying
Figure 4.3. Energy profile of fragmentation reaction pathways for [Cu(II)Ala(Ala-H)]
+ CO2 + C2H4 + NH2, relative energies in kJ/mol.
the radical site at the $\alpha$-C of the decarboxylated ligand, is endothermic by only 33.9 kJ/mol. $C_\alpha$ can rearrange to $C_\beta$ and $N_1$ via direct (one-step) 1,2-hydrogen transfers or stepwise, as shown in Fig. 4.3 for $C_\alpha \rightarrow C_\beta$. In the stepwise $C_\alpha \rightarrow C_\beta$ rearrangement, a $\beta$-H atom first moves to Cu(I) ion and subsequently to the $\alpha$-C atom of the reacting ligand (solid line in Fig. 4.3). The transition state (TS) of the first step lies at 193.1 kJ/mol and leads to a Cu(I)hydride/enamine complex, which is $112.4 - 33.9 = 78.5$ kJ/mol less stable than $C_\alpha$; the TS of the second step is located at a somewhat higher energy, 200.2 kJ/mol, and leads to the $C_\beta$ radical, which is $55.2 - 33.9 = 21.3$ kJ/mol less stable than $C_\alpha$. The one-step 1,2-H rearrangement $C_\alpha \rightarrow C_\beta$ requires $\sim 15$ kJ/mol more energy than the stepwise reaction dashed line in Fig. 4.3 and, thus, is less competitive. The calculations show that Cu(I) ion facilitates nominal 1,2-H shifts by shuttling the H atom between the heavy atoms. According to the potential energy diagram of Fig. 4.3, $C_\beta$ dissociates spontaneously (via $\beta$-bond scission) to Ala-Cu$^+\cdot$-NH$_2 +$ C$_2$H$_4$ because it is formed well above its dissociation threshold. The rearrangement $C_\alpha \rightarrow N_1$ can proceed similarly via an intermediate Cu(I)hydride/imine complex, formed by migration of an N-H hydrogen to Cu(I) ion and subsequent transfer of this H atom to the $\alpha$-C atom of the decarboxylated ligand. The Cu hydride intermediate in this case lies $67.7 - 33.9 = 33.8$ kJ/mol above $C_\alpha$. The stepwise $C_\alpha \rightarrow N_1$ pathway should again be energetically more favorable than the direct 1,2-H rearrangement. It is noteworthy that the Ala-Cu$^{+}(H)$-NH$_2$-CH=CH$_2$ complex is significantly less stable than the Ala-Cu$^{+}(H)$-NH=CH-CH$_3$ complex (by $112.4 - 67.7 = 44.7$ kJ/mol), indicating that an imine ligand binds more strongly to Cu(I) than an isomeric enamine ligand.
4.2. Cu(II)-Bound Dimer Complex with Gly

Fig. 4.4(a) is the CAD spectrum of \([\text{Cu(II)Gly(Gly-H)}]^+\) (m/z 212). After reductive decarboxylation (m/z 168), the only major consecutive fragmentation is a 30-u loss to form m/z 138. There is almost no hydrogen radical loss from the decarboxylated product, which shows that the loss of the whole side chain from the partially reacted amino acid \((i.e. \text{of } \cdot \text{CH}_2\text{NH}_2)\) is a more competitive process. The sodiated product \([\text{Cu(II)(Gly-H+Na)(Gly-H)}]^+\) (m/z 234) was also selected for a tandem MS study; the resulting CAD spectrum is shown in Fig. 4.4(b) and is completely analogous to that of Fig. 4.4(a) except for the mass shifts due to the exchange of the acidic proton in the intact glycine ligand with a sodium ion. This finding is plausible considering that the originally intact amino acid ligand in \([\text{Cu(II)AA(AA-H)}]^+\) complexes with unfunctionalized AAs does not participate in the decarboxylation and consecutive radical induced dissociation processes. The salt bridge formed by H\(^+/\text{Na}^+\) replacement does not influence the fragmentation pattern of the parent ion, which is dominated by the radical site reactions.

4.3. Cu(II)-Bound Dimer Complex with Leu, Ile and \(\tau\)-Leu

The copper-Leu and copper-Ile complexes behave the same way as the copper-Ala complex under the experimental conditions used (Fig. 4.5(a) and 4.5(b)). Leu and Ile could be differentiated by the fragmentation patterns of their \([\text{Cu(II)Bpy(Xle-H)}]^+\) complexes (Bpy, bipyridine; Xle, Leu or Ile).\(^{28}\) These differences are also observed here for the corresponding \([\text{Cu(II)Xle(Xle-H)}]^+\) complexes. The C\(_\alpha\) radicals emerging after
Figure 4.4. CAD mass spectra of
(a) CAD (MS²) spectrum of [Cu(II)Gly(Gly-H)]⁺ (m/z 212) and
(b) CAD (MS²) spectrum of [Cu(II)(Gly-H+Na)(Gly-H)]⁺ (m/z 234).
Figure 4.5. CAD spectra of
(a) CAD (MS$^2$) spectrum of $[\text{Cu(II)Leu(Leu-H)}]^+$ (m/z 324),
(b) CAD (MS$^2$) spectrum of $[\text{Cu(II)Ile(Ile-H)}]^+$ (m/z 324) and
(c) CAD (MS$^2$) spectrum of $[\text{Cu(II)t-Leu(t-Leu-H)}]^+$ (m/z 324).
reductive decarboxylation undergo loss of 43 u (CH₃·CHCH₃) if Xle = Leu and loss of 29 u (·CH₂CH₃) if Xle = Ile. There is no noticeable amount of 15-u loss (·CH₃) from Ile, which is probably due to the fact that the reaction pathway releasing the larger radical (·CH₂CH₃) is energetically favored over the reaction pathway releasing the smaller, less stable radical (·CH₃). The dissociations of the t-Leu-copper complex under CAD conditions are shown in Fig. 4.5(c). The major difference between the CAD spectra of the t-Leu and Leu/Ile complexes is the significant amount of ·CH₃ loss (~15 u, m/z 265) from t-Leu complex. Since there is no Cβ hydrogen in t-Leu (NH₂CHCβ(CH₃)₃COOH), a Cβ radical cannot be formed from its Cu(II) complex. As a result, there is no m/z 210 peak in Fig. 4.5(c). In contrast, there is such a peak (minor) in both Fig. 4.5(a) and 4.5(b) because the Leu/Ile complexes are able to form a Cβ radical, which can decompose to Xle-Cu⁺-·NH₂ (m/z 210) + C₅H₁₀ via β-bond scission (cf. Scheme 4.1). There are two common products for all three complexes. The first arises from loss of the entire decarboxylated ligand (86 u), *i.e.* of the ligand with the unpaired electron, and generates m/z 194. The second results from elimination of the side chain of the decarboxylated Leu, Ile, or t-Leu ligand, which is cleaved via N₁ radical intermediates and creates fragment ions with a NH=CH₂ unit attached to Cu(I) (at m/z 223). Overall, the fragments generated from the Cα radical provide the most valuable structure information about the identity of the AA bound to copper(II). The sodiated copper complexes [Cu(II)(AA-H+Na)(AA-H)]⁺ have also been studied and showed identical fragmentation patterns to those of their non-sodiated counterparts (spectra not shown); this further confirms that sodium ion is retained on the intact amino acid ligand that is not involved in CAD reactions and that the latter reactions are radical-induced and, hence, insensitive to the introduction of a COO⁻.
Na$^+$ salt bridge. The salt bridge would have influenced the outcome of charge-induced dissociations.

4.4. Cu(II)-Bound Dimer Complex with Val and Phe.

Fig. 4.6(a) is the CAD spectrum of [Cu(II)Val(Val-H)]$^+$, which shows the occurrence of reductive decarboxylation and subsequent losses of 15 u (m/z 237, loss of $\cdot$CH$_3$ radical from C$_\alpha$), 43 u (m/z 209, loss of CH$_3$·CHCH$_3$ radical from N$_1$), 56 u (m/z 196, loss of CH$_2$=C(CH$_3$)$_2$ from C$_\beta$), and 72 u which is the loss of the entire decarboxylated Val ligand (m/z 180). Thus, the reaction pathways of [Cu(II)Val(Val-H)]$^+$ are completely analogous to those of [Cu(II)Ala(Ala-H)]$^+$.

For [Cu(II)Phe(Phe-H)]$^+$, as shown in Fig. 4.6(b), there is no C$_\alpha$ radical product detected (should be at m/z 271), and the product from the corresponding N$_1$ radical cation (m/z 257 according to the mechanism of Scheme 4.1) is minuscule. Here, the dominant fragment, m/z 244, is generated from the C$_\beta$ radical cation. The isomerization C$_\alpha$ $\rightarrow$ C$_\beta$ involves transfer of a benzylic hydrogen atom and produces a resonance-stabilized benzylic radical,$^8$ therefore, the formation of C$_\beta$ is both kinetically and thermodynamically favored, explaining the high relative abundance of m/z 244 which is formed by elimination of a styrene molecule (104 u) from C$_\beta$.

Experiments with the mixed dimer complex Cu(II)-Phe/Val were also carried out and the CAD spectrum is shown in Fig. 4.6(c). After the reductive decarboxylation, the major fragments are loss of 72 u, which proceeds from a decarboxylated Val ligand; and the loss of styrene (104 u), which precedes styrene loss from the C$_\beta$ radical resulting from
Figure 4.6. CAD (MS\(^2\)) mass spectra of
(a) CAD spectrum of [Cu(II)Val(Val-H)]\(^+\) (m/z 296),
(b) CAD spectrum of [Cu(II)Phe(Phe-H)]\(^+\) (m/z 392) and
(c) CAD spectrum of [Cu(II)Phe(Val-H)]\(^+\) or [Cu(II)Val(Phe-H)]\(^+\) (m/z 344).
a deprotonated Phe ligand. From this fragmentation behavior, it is concluded that the ion population probed contains the isomeric complexes [Cu(II)Phe(Val-H)]⁺ and [Cu(II)Val(Phe-H)]⁺, which is very plausible because the proton on the carboxyl groups exchanges very fast in slightly basic solution for both Val and Phe based on the pKₐ values of Phe (1.83) and Val (2.32). The spectrum of the mixed complex, Fig. 4.6(c), shows that styrene loss is the decomposition preferred by the deprotonated Phe ligand (after initial CO₂ expulsion), as was the case for the homodimeric complex (Fig. 4.6(b)). On the other hand, the mixed complex portion that contains a deprotonated Val, rather losses the decarboxylated ligand - 72 u in Fig. 4.6(c), while the corresponding homodimeric complex preferably loses the side chain of the decarboxylated ligand (~ 56 u in Fig. 4.6(a)). This change is attributed to differences in the internal energy distributions of homo- and heterodimeric complexes.

Table 4.1 summarizes the fragments (m/z) observed in the CAD spectra of [Cu(II)AA(AA-H)]⁺ complexes. Loss of CO₂ is the major fragmentation pathway for all amino acids involved in this study. Hydrogen radical elimination after the decarboxylation preceeds abundantly from the Ala and Val copper complexes. The products from the C_α radical have moderate intensity except for the Phe copper complex. The products from the C_β radical have moderate intensity except for the Leu/Ile copper complexes (no C_β radical exists for the Gly/t-Leu copper complexes). All complexes dissociate to form products via the N₁ radical except those with Gly and Phe ligands. All of the complexes dissociate to form [Cu(I)AA]⁺ ions.
Table 4.1. List of peaks (m/z) in the CAD spectra of [Cu(II)AA(AA-H)]⁺ complexes.

<table>
<thead>
<tr>
<th>AA</th>
<th>Complex of CO₂</th>
<th>Loss of CO₂</th>
<th>H-elimination from Cα, Cβ, or N₁</th>
<th>'Product from Cα</th>
<th>'Product from Cβ</th>
<th>'Product from N₁</th>
<th>[Cu-AA]⁺</th>
</tr>
</thead>
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<tr>
<td>Gly</td>
<td>212</td>
<td>168</td>
<td>167&lt;sup&gt;a&lt;/sup&gt;</td>
<td>z&lt;sup&gt;b&lt;/sup&gt;</td>
<td>z&lt;sup&gt;b&lt;/sup&gt;</td>
<td>z&lt;sup&gt;b&lt;/sup&gt;</td>
<td>138</td>
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<tr>
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<td>196</td>
<td>195</td>
<td>z&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168</td>
<td>181</td>
<td>152</td>
</tr>
<tr>
<td>Val</td>
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<td>252</td>
<td>251</td>
<td>237</td>
<td>196</td>
<td>209</td>
<td>180</td>
</tr>
<tr>
<td>Leu</td>
<td>324</td>
<td>280</td>
<td>279&lt;sup&gt;a&lt;/sup&gt;</td>
<td>237</td>
<td>210&lt;sup&gt;a&lt;/sup&gt;</td>
<td>223</td>
<td>194&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ile</td>
<td>324</td>
<td>280</td>
<td>279&lt;sup&gt;a&lt;/sup&gt;</td>
<td>251</td>
<td>210&lt;sup&gt;a&lt;/sup&gt;</td>
<td>223</td>
<td>194&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>t-Leu</td>
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<td>265</td>
<td>z&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>392</td>
<td>348</td>
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<td>z&lt;sup&gt;b&lt;/sup&gt;</td>
<td>244</td>
<td>257&lt;sup&gt;a&lt;/sup&gt;</td>
<td>228</td>
</tr>
</tbody>
</table>

<sup>a</sup> Italic numbers indicate minor products.

<sup>b</sup> "-" means not possible or not formed.

<sup>c</sup> Products formed via C-C or C-N bond cleavages. Hydrogen eliminations are shown in a separate column.
4.5. Conclusions

The dissociations of $[\text{Cu(II)AA(AA-H)}]^+$ complexes (AA: Gly, Ala, Val, Leu, Ile, $t$-Leu, and Phe) have been examined by CAD experiments in an electrospray ionization mass spectrometer. In the single-stage mass spectra, copperated complexes of the amino acid dominate, but there are protonated and sodiated amino acids as well. In the complexes, the amino groups of both amino acids, the carbonyl group of the intact amino acid, and the deprotonated hydroxyl oxygen coordinate Cu(II) in square-planar fashion. Under CAD conditions, the $[\text{Cu(II)AA(AA-H)}]^+$ complexes undergo decarboxylation with simultaneous reduction of Cu(II) to Cu(I); during this process, a radical site is created at the $\alpha$-carbon of the decarboxylated ligand ($N_1H_2\cdot C_\alpha HC_\beta H_2R; \ R = \text{side chain}$). The radical site is able to move along the backbone of the decarboxylated amino acid to form two new radicals ($\cdot N_1 HC_\alpha H_2 C_\beta H_2 R$ and/or $N_1 H_2 C_\alpha H_2 \cdot C_\beta HR$). From the complex of $t$-Leu, only $C_\alpha$ and $N_1$ radicals can be formed. The whole radical ligand can be lost to form $[\text{Cu(I)AA}]^+$ from those three isomeric radicals. Alternatively, further radical induced dissociations can take place along the backbone of the decarboxylated amino acid to yield $[\text{Cu(II)AA(AA-2H-CO}_2)]^+$, $[\text{Cu(I)AA(\cdot NH}_2)]^+$, and/or $[\text{Cu(I)AA(NH=C}_\alpha H_2)]^+$. Those products may undergo consecutive dissociation to form the final product $[\text{Cu(I)AA}]^+$. The sodiated copper complexes ($[\text{Cu(II)(AA-H+Na)(AA-H)}]^+$) show the same fragmentation patterns as their non-sodiated counterparts; sodium ion is retained on the intact amino acid ligand and is not involved in the CAD pathways.

On the basis of the CAD spectra, reasonable dissociation mechanisms are proposed for the $[\text{Cu(II)AA(AA-H)}]^+$ complexes and substantiated by the calculated potential energy diagram for select CAD channels of the $[\text{Cu(II)Ala(Ala-H)}]^+$ complex. The
theoretical calculations provide information about the hydrogen migration pathways. After decarboxylation, a hydrogen from the amino group or the β-carbon migrates to the copper ion, from where it is shuttled to C\textsubscript{α} to form radicals at the amino nitrogen (N\textsubscript{1}) or the β-carbon (C\textsubscript{β}), respectively. Such pathways require lower activation energies than direct 1,2-H atom rearrangements and may also take place with α-peptide radicals generated in vivo where Cu(II) ions are available. The overall results show that the combination of theory and experiment is a powerful tool to elucidate reaction mechanisms in gas phase.
CHAPTER V

CU(II)-CATALYZED REACTIONS OF SIDE CHAIN FUNCTIONALIZED AMINO ACIDS (W, Y, H, R, K) AND GG

5.1. Cu(II)-Bound Homo-Dimer Complexes with W and Y

A solution of CuCl₂ and W was injected and sprayed into the instrument. Fig. 5.1 shows the resulting mass spectrum. The ions at m/z 205 and 409 are the protonated tryptophan, and the proton-bound dimer of tryptophan, respectively; m/z 227 and 431 are sodiated W and the Na⁺-bound dimer of W, respectively. The peak at m/z 470 represents [Cu(II)W(W-H)]⁺, while m/z 492 is [Cu(II)(W-H+Na)(W-H)]⁺, i.e. the corresponding H⁺/Na⁺ exchange product. Proton-bound complexes dominate the spectrum. The ion at m/z 470, [Cu(II)W(W-H)]⁺, was selected for a CAD experiment and the spectrum acquired is shown in Fig. 5.2(a). Based on this spectrum, the major fragments from m/z 470 are m/z 426, which is the loss of 44 u from the parent ion, and m/z 396, which is the loss of 74 u from the parent ion. There are also two minor peaks at m/z 382 and 352, which become dominant in the MS³ spectrum of ion m/z 426 (not shown). The absence of m/z 396 in the MS³ spectrum of m/z 426 indicates that the loss of 74 u occurs directly from the parent ion and is a competitive reaction pathway with respect to the reductive decarboxylation reaction. MS³ on m/z 396 leads to the spectrum shown in Fig. 5.2(b).
Figure 5.1. ESI-QIT mass spectrum of [Cu(II)W(W-H)]⁺.
Figure 5.2. CAD mass spectra of [Cu(II)W(W-H)]⁺;
(a) CAD (MS²) spectrum of m/z 470 and
(b) CAD (MS³) spectrum of m/z 396.
The loss of 129 u is the only dominant fragmentation pathway; it corresponds to the elimination of an imine form of the tryptophan side chain, as shown on the spectrum and explained later in Scheme 5.1. The side chain of W provides an additional binding site for Cu(II) ion. Since Cu(II) prefers higher coordination numbers in solution (as compared to Cu(I)), it is very likely that the [Cu(II)W(W-H)]\(^+\) complex contains a hexacoordinated metal ion. Such a structure is depicted in Scheme 5.1, in which the intact W ligand coordinates the copper ion with its amino, carbonyl, and the indole side chain groups, while the deprotonated W ligand complexes Cu(II) with its amino group, deprotonated carboxyl oxygen, and aromatic side chain substituent. The Cu(II)-indole side chain bonds could be elongated due to the rigidity of the side chain of W.

The proposed fragmentation mechanisms are given in Scheme 5.1. In part (a), the copper/W complex first undergoes reductive decarboxylation (- 44 u), which releases a CO\(_2\) molecule from the deprotonated W ligand, and, at the same time, converts Cu(II) to Cu(I) and generates a radical site at the \(\alpha\)-carbon of the deprotonated W. Unlike the radical induced reactions discussed with the hydrocarbon amino acid/copper complexes in chapter IV, inter-ligand radical reactions dominate here probably because of the larger size and structure of the W side chain. One reaction pathway after the reductive decarboxylation is hydrogen atom abstraction from the carboxyl group on the intact W by the radical site at the \(\alpha\)-carbon of the deprotonated W, followed by decarboxylation (- 44 u) and transfer of the radical site to the \(\alpha\)-carbon of the originally intact W (m/z 426). An alternative reaction pathway is hydrogen atom abstraction from the indole nitrogen on the side chain of the intact W followed by the elimination of a 74-u radical from this W to yield an imine ligand bound Cu(I) (m/z 352). Consecutive dissociation of the mentioned
Scheme 5.1. Reaction pathways of [Cu(II)W(W-H)]⁺ (a).
Scheme 5.1. Reaction pathways of $[\text{Cu(II)W(W-H)}]^+$ (b).
imine (-129 u) gives rise to m/z 223; this fragmentation sequence is revealed by the
abundant m/z 223 peak in the MS$^3$ spectrum of m/z 352 (not shown). As mentioned
above, there is a competitive reaction pathway to the reductive decarboxylation (part (b)
of Scheme 5.1), where the incipient ⋅OCO- radical arising upon the reduction of Cu(II) to
Cu(I) does not undergo CO$_2$ loss but abstracts the N-H hydrogen atom from the indole
group of the intact W ligand (inter-ligand H transfer), initiating a cascade of radical
induced transformations that cleave a 74-u radical from this ligand and leave an indole-
imine bound to Cu(I) (m/z 396). The incipient ⋅OCO- radical can similarly abstract the N-
H hydrogen from the indole group of the deprotonated W ligand (intra-ligand H transfer
in this case) to ultimately create the same m/z 396 fragment. Inter- and intra-ligand
pathways from a homodimeric Cu(II)/W complex cannot be distinguished
experimentally. The intra-ligand reaction should, however, be associated with significant
strain and is, therefore, less likely than the inter-ligand reaction.

The copper/Y complex dissociates via almost the same (inter-ligand reactions) as the
copper/W complex, but also undergoes more radical induced cleavages on the
deprotonated Y ligand (as with the hydrocarbon amino acids). Fig. 5.3 reproduces CAD
spectrum of [Cu(II)Y(Y-H)]$^+$ (m/z 424). Major fragments appear at m/z 380, which is the
loss of 44 u from the parent ion, and m/z 350, which is the loss of 74 u from the parent
ion. The absence of m/z 350 in the MS$^3$ spectrum of 380 indicates that the loss of 74 u
from the parent ion is a competitive and not a consecutive reaction pathway with respect
to the reductive decarboxylation reaction, as shown in Scheme 5.2. In the [Cu(II)Y(Y-
H)]$^+$ complex, the intact Y ligand can coordinate the copper ion with its amino, carbonyl,
and aromatic side chain substituents, and the deprotonated Y ligand can coordinate Cu(II)
Figure 5.3. CAD mass spectrum of [Cu(II)Y(Y-H)]⁺ (m/z 424).
Scheme 5.2. Reaction pathways of [Cu(II)Y(Y-H)]⁺ (a).
Scheme 5.2. Reaction pathways of m/z 260 from [Cu(II)Y(Y-H)]\(^+\) (b).
Scheme 5.2. Reaction pathways of [Cu(II)Y(Y-H)]+ (c).
with its amino, deprotonated carboxyl oxygen, and the aromatic side chain substituents, which overall provide six coordination sites to Cu(II) ion; the Cu(II)-phenyl group interactions could be weak due to the rigidity of the side chains of Y.

The dissociation mechanisms outlined in Scheme 5.2, parts (a)-(c), can explain the observed fragments. Reductive decarboxylation with loss of 44 u (CO₂) from the deprotonated Y ligand leads to the Cₐ radical of the Y complex, see part (a) of Scheme 5.2. In analogy to the radical induced reactions taking place with the hydrocarbon amino acid/copper complexes (discussed in chapter IV), Cₐ can rearrange to N₁, from which the p-hydroxybenzyl radical (107 u) can be cleaved to produce m/z 273 and, after consecutive elimination of NH=CH₂ (29 u), m/z 244. If Cₐ rearranges to C₉ instead, β-bond scission to release p-hydroxystyrene (120 u) becomes possible, leading to a Cu(I) complex bound to an intact Y ligand and to a ·NH₂ radical (m/z 260). There are three possible reactions that can take place from the latter complex, namely elimination of a ·NH₂ radical, NH₃ molecule, or 74-u radical to yield m/z 244, 243, and 186, respectively, as shown in Scheme 5.2(a). The eliminations of NH₃ and 74 u (H₂N-CHCOOH) can be rationalized by inter-ligand H rearrangement from the OH group of the intact tyrosine ligand to the ·NH₂ radical ligand and subsequent radical-induced cleavage of the remaining tyrosine part to cyclohexadienone (106 u) and α-glycyl radical (74 u) units that remain attached to Cu(I), see Scheme 5.2(b). Elimination of the NH₃ or α-glycyl radical ligands from the latter trimeric complex leads to m/z 243 and 186, respectively. The elimination of the cyclohexadienone ligand is not observed, suggesting that it is bound more strongly to Cu(I) than either NH₃ or α-glycyl radical. Another reaction pathway
proceeding after the reductive decarboxylation (from C$_\alpha$) is inter-ligand hydrogen transfer from the hydroxyl group on the intact Y ligand to the radical site at $\alpha$-carbon of the deprotonated Y ligand, followed by the elimination of 74 u from the originally intact Y to form the fragment at m/z 306, which contains a cyclohexadienone ligand, as shown in Scheme 5.2(a).

There is another competitive reaction pathway (Scheme 5.2(c)) upon the dissociation of the copper/Y complex, similar to the one encountered with the Cu(II)/W complex, where the incipient carboxylate radical emerging upon the reduction of Cu(II) to Cu(I) extracts the hydrogen atom of the intact Y hydroxyl group. The ⋅O radical site generated at the Y side chain after this inter-ligand H atom rearrangement can induce a cascade of radical reactions that split the originally intact Y ligand to a cyclohexadienone and an $\alpha$-glycyl radical unit, as discussed above; elimination of the $\alpha$-glycyl radical (74 u) from this intermediate gives rise to m/z 350, cf. Scheme 5.2(c), which contains a Cu(I) ion complexed to a cyclohexadienone and an intact Y ligand. The MS$^3$ spectrum of m/z 350 reveals that further activation of this fragment generates the Y-Cu$^+$ complex (m/z 244) by loss of the cyclohexadienone ligand; hence, the latter ligand must have a lower Cu$^+$ binding energy than tyrosine.

It should be mentioned at this point that the dissociation sequences presented in Scheme 5.1 (for the Cu(II)/W complex) and 5.2 (for the Cu(II)/Y complex) are corroborated by MS$^n$ (n = 3-4) data, as has been mentioned in select cases so far. The proposed mechanisms are also supported fully by the reactions of deuterated isotopomers, in which all heteroatom-bound protons of the dimeric Cu(II)/AA complexes have been replaced by deuterons.
The major differences between W or Y ligands on one and amino acid ligands with hydrocarbon side chains on the other hand are that the side chains of W and Y can bind copper ion and contain heteroatom-bound hydrogen atoms that can be transferred in inter-ligand reactions. A further incentive for such reactions is that they can promote radical induced dissociations within the W and Y side chains that create smaller imine (from W) or ketone (from Y) ligands which can remain attached to the metal ion.

5.2. Cu(II)-Bound Homo-Dimer Complex with His

Fig. 5.4 depicts the CAD spectrum of [Cu(II)His(His-H)]⁺ (m/z 372, the three letter code will be used for His in order to differentiate it from hydrogen (H)). The major fragment peak appears at m/z 328, which is generated by the loss of 44 u from the parent ion. There are smaller peaks at m/z 284, 255, and 174, which are also the dominant peaks in the MS³ spectrum of the m/z 328 ion (not shown). The spectrum of Fig. 5.4 reveals that reductive decarboxylation is the dominant dissociation process; two more reactions proceed with considerable yield, namely inter-ligand hydrogen transfer and subsequent loss of a second CO₂ molecule from the originally intact His ligand (m/z 284) and further decomposition of the latter product by elimination of CH₂=NH (m/z 255), as shown in Scheme 5.3(a). The ion at m/z 284 formed after the second decarboxylation carries an unpaired electron at the α-C atom of the originally intact His ligand and, thus, is a Cα-type radical ion; it is abbreviated by Cα' to distinguish it from the Cα radical ion resulting after the first (reductive) decarboxylation. With the d-seven isotopomer of [Cu(II)His(His-H)]⁺ (all O-H and N-H protons replaced by deuterons), m/z 284 shifts to m/z 291, which is reproduced at the lower left corner of Scheme 5.3(b). The most weakly bonded Cu(I)
Figure 5.4. CAD mass spectrum of \([\text{Cu(II)His(His-H)}]^+\) (m/z 372).
Scheme 5.3. Reaction pathways of $[\text{Cu(II)His(His-H)}]^+$ (a).
Scheme 5.3. Reaction pathways of C$_{\alpha}'$ radical from [Cu(II)His(His-H)]$^+$ (b).
ligand in C\textsubscript{\textalpha}' should be the radical-containing CH\textsubscript{2}-CH-ND\textsubscript{2} chain; such \( \alpha \)-radical ligands were preferentially eliminated from the Cu(II)/W and Cu(II)/Y complexes \textit{vide supra} and should also interact weakly with copper ion in the fragments formed from the Cu(II)/His complex. Consequently, collisional activation of C\textsubscript{\textalpha}' would readily break this Cu(I)/ligand bond to form the more flexible C\textsubscript{\textalpha}' conformer shown on the top left corner of Scheme 5.3(b), in which inter-ligand H (D) transfer can readily take place. The \cdot N radical site created can initiate \( \beta \)-C-C bond cleavage, splitting the ligand into a formaldimine molecule (CH\textsubscript{2}=NH or CHD=ND from d\textsubscript{7}-C\textsubscript{\textalpha}') and an imidazolyl radical, either of which may be detached to yield m/z 255 (or its m/z 260 isotopomer) and m/z 203 (or its m/z 209 isotopomer), respectively. However, the same neutral losses could also occur via intra-ligand H (D) transfer, as shown on the lower part of Scheme 5.3(b). The intra-ligand rearrangements would probably be facilitated by initial H (D) transfer to Cu\textsuperscript{+}, as discussed in charter IV. Intra- and inter-ligand pathways create the same product and are indistinguishable experimentally with the Cu(II)/His complex studied. Since intra-ligand hydrogen transfers require quite high activation energies (see Fig. 4.3), the inter-ligand reaction path is assumed to be the favored pathway to m/z 255/203 (and their isotopomers). Substantiating evidence for this supposition comes from the consecutive dissociation of m/z 255 and its d\textsubscript{5}-isotopomer (m/z 260), which must involve inter-ligand H (D) atom rearrangement. The corresponding MS\textsuperscript{3} spectra (not shown) reveal that these ions decompose extensively by loss of a second formaldimine unit; m/z 255 loses CH\textsubscript{2}=NH (29 u) to form m/z 226, \textit{cf}. Scheme 5.3(a), while m/z 260 (lower right corner of Scheme 5.3(b)) loses a 31-u neutral (most likely CHD=ND). These losses can only be accounted for by an inter-ligand H (D) atom transfer of one N-H (or N-D) hydrogen atom.
to the -CH₂ site if the imidazolyl radical, followed by formaldimine expulsion. The MS³ spectrum of m/z 255 also contains a major fragment at m/z 174, which is formed by cleavage of the imidazolyl radical ligand (81 u); expectedly, this fragment shifts to m/z 177 with the d₇ sample. It is noteworthy that most fragments from the copper/His complex have the imine nitrogens of the two imidazole moieties coordinated to copper ion, which reflects the higher copper ion affinity of the imidazole side chain of His.⁸⁶

5.3. Cu(II)-Bound Homo-Dimer Complexes with R and K

The CAD spectra of the copper/R complex and the deuteron labeled copper/R complex are shown in Fig 5.5. Fig 5.5(a), which corresponds to the copper/R complex, shows that the dominant fragment is not the reductive decarboxylation product (m/z 366) but m/z 193. The next most abundant peak, m/z 237, results from the R-Cu(I) ion. The reductive decarboxylation product m/z 366, the consecutive decarboxylation product m/z 322, and m/z 192 have moderate intensities. The ion at m/z 237 does not appear in MS³ spectra (not shown) of either m/z 366 or 322 proving that the R-Cu(I) ion is produced directly from the parent ion m/z 410 and that its formation is competitive to the reductive decarboxylation. The CAD spectrum of the deuteron labeled copper/R complex is shown in Fig 5.5(b) for comparison. The most abundant peak is m/z 200, followed by m/z 199, 244, and 379, while m/z 198, 243, and 335 have moderate intensities. Except for the mass shifts expected from deuteron/hydrogen exchanges as compared to Fig 5.5(a), there are two additional peaks, in Fig 5.5(b), viz. m/z 199 and 243, as clearly shown in the inserts; these will be discussed in detail in the following section.
Figure 5.5. CAD (MS²) mass spectra of [Cu(II)R(R-H)]⁺; inserts show expansions of peaks.
(a) CAD spectrum of m/z 410 and
(b) CAD spectrum of deuteron labeled complex m/z 423.
Scheme 5.4. Reaction pathways of [Cu(II)R(R-H)]⁺ (a).
P: unlabeled; D: deuteron labeled.
Scheme 5.4. Reaction pathways of [Cu(II)R(R-H)]\(^+\) (b).

P: unlabeled; D: deuteron labeled.

P: m/z 410
D: m/z 423

P: m/z 237
D: m/z 244
The reaction pathways proposed to explain the observed fragments are given in Scheme 5.4, in which \(\alpha\)-H atoms (H atoms attached to \(\alpha\)-carbons) are shown in red in order to distinguish them from mobile (heteroatom-bound) hydrogens. Scheme 5.4(a) shows the reductive decarboxylation product \(C_\alpha\) (P: m/z 366; P designates the unlabeled complex) followed by 1) consecutive decarboxylation to form \(C'_\alpha\) (P: m/z 322) via inter-ligand mobile hydrogen transfer from the COOH group and successive elimination from \(C'_\alpha\) of either the radical (P: m/z 193) or the neutral molecule (P: m/z 192) ligand resulting from the decarboxylation processes; or 2) \(\alpha\)-H atom extraction from the intact R and successive release of the newly formed (R-H)⋅ radical (P: m/z 193) from the copper complex.

For the inter-ligand H atom transfers discussed, the \(\alpha\)-amino radical ligand may dissociate to reach the rearranging H atoms, or the C=O⋅⋅Cu\(^+\) and NH\(_2\)⋅⋅Cu\(^+\) bonds on the intact R ligand may dissociate to reach the \(\alpha\)-radical site. Either of these bonds should be weaker than those formed between the guanidine moiety and Cu\(^+\) and, thus, breakable upon CAD conditions.\(^{86,87}\) It is reasonable to assume that the --CH-NH\(_2\) (\(\alpha\)-amino radical) substituent coordinates less strongly than the other functional groups and preferably detaches to initiate inter-ligand H rearrangements. Based on Scheme 5.4(a), the \(\alpha\)-H extraction product from \(C_\alpha\) via pathway 2 is identical with the product formed by radical loss from \(C'_\alpha\) via pathway 1. These products become distinguishable with the labeled complex, where the \(\alpha\)-H extraction product from pathway 2 (D: m/z 199; D designates the deuteron labeled complex) is one mass unit smaller than the radical loss product from pathway 1 (D: m/z 200). The relative abundances of m/z 198, 199, and 200 from the
deuterated sample reveal that pathway 1 (source of m/z 198 and 200) proceeds with substantially higher yield than pathway 2 (source of m/z 199). Further, the abundance ratio of m/z 198 and 200 provides evidence that C\textsubscript{α}' primarily loses its radical ligand, consistent with the supposition made above that an α-amino radical binds Cu(I) much more weakly than the corresponding closed shell amine.

The product at m/z 237 in Fig. 5.5(a), which is formed directly from the parent ion \textit{(vide supra)} is rationalized in Scheme 5.4(b) by a competitive reaction pathway with respect to reductive decarboxylation of the copper complex upon CAD. The incipient COO\textsuperscript{-} radical generated upon reduction of Cu(II) to Cu(I) is proposed to extract either a guanidino NH\textsubscript{2} hydrogen atom or an α-hydrogen (in red) from the intact R ligand. The resulting intermediates then lose the newly formed radicals to form the Cu(I)R complex (P: m/z 237). Both pathways lead to the same product by elimination of different radicals. Labeling provides evidence that α-hydrogen atoms participate in this dissociation (D: m/z 243) with a comparable yield as NH\textsubscript{2} hydrogens at the guanidine side chains (D: m/z 244).

The CAD spectrum of the copper/K complex is shown in Fig 5.6. Again the dominant peak is not from decarboxylation (m/z 310) but m/z 165. Other abundant peaks are observed at m/z 209, which is the K-Cu(I) ion, and m/z 266, the second decarboxylation product. The first (reductive) decarboxylation product, m/z 310, is a minor product.

The proposed mechanisms for the observed reaction pathways are included in Scheme 5.5; only major reactions will be discussed. The Scheme shows the reductive decarboxylation product C\textsubscript{α} (m/z 310), the consecutive decarboxylation product C\textsubscript{α}' (m/z
Figure 5.6. CAD mass spectrum of [Cu(II)K(K-H)]\(^+\) (m/z 354).
Scheme 5.5. Reaction pathways \([\text{Cu(II)K(K-H)}]^+\).
266) formed after inter-ligand hydrogen transfer from the COOH group of the intact K ligand, and the further dissociation product of Cα' by loss of its radical ligand. The diaminopentyl radical cleaved from Cα' to form m/z 165 is also a ligand in Cα; its elimination from Cα gives rise to the K-Cu(I) ion at m/z 209. The very low relative intensity of m/z 310 (Cα) points out that the consecutive fragmentations of this radical ion are particularly facile. The predominant fragments in the CAD spectra of the Cu(II)/R and Cu(II)/K complexes are ions arising after the second decarboxylation via inter-ligand H migrations. The longer and more flexible side chains of R and K obviously facilitate these radical reactions in the gas phase.

5.4. Cu(II)-Bound Homo-Dimer Complex with GG

As the size of the copper ligand increases, inter-ligand reactions become possible, even without functionalized side chains. As attested in Fig 5.7 (CAD spectrum of GG), the loss of 44 u (m/z 282) is the major reaction pathway, followed by another 44-u loss (m/z 238). There are other, less abundant products at m/z 151, 180, 195, and 224. The major reaction pathways are rationalized in Scheme 5.6. Reductive decarboxylation to the Cα radical is the dominant reaction pathway. Cα can undergo inter-ligand hydrogen transfer and consecutive decarboxylation from the originally intact GG to produce Cα' (m/z 238). Radical induced elimination of a β-amino acetyl radical (58 u) from Cα' generates a fragment with a formaldimine (CH₂=NH) ligand (m/z 180), and final detachment of this imine leads to m/z 151. Alternatively, Cα may undergo radical-induced elimination of a β-amino acetyl radical (58 u) to yield a Cu⁺ complex
Figure 5.7. CAD mass spectrum of [Cu(II)GG(GG-H)]⁺ (m/z 326).
Scheme 5.6. Reaction pathways of $[\text{Cu(II)GG(GG-H)}]^+$.
coordinated by GG and CH$_2$=NH (m/z 224), which may subsequently lose the more weakly bound (smaller) CH$_2$=NH ligand to form the GG-Cu$^+$ complex (m/z 195).

5.5. Conclusions

For copper complexes with hydrocarbon amino acids, only the deprotonated amino acid ligands are involved in the dissociation reactions. Complexes formed with copper and amino acids with functionalized side chains react differently. Now, inter-ligand reactions occur, and as the size of the molecules coordinated to copper increases, the chances for inter-ligand reactions increase.

The dissociations of [Cu(II)AA(AA-H)]$^+$ complexes (AA: W, Y, His, R, K, or GG) have been examined in detail by CAD experiments using an electrospray ionization mass spectrometer. The functionalized side chains of these amino acids and their larger size facilitate the coordination of these molecules to copper ion. Under CAD conditions, most of the [Cu(II)AA(AA-H)]$^+$ complexes studied here undergo decarboxylation with simultaneous reduction of Cu(II) to Cu(I) and production of a radical site at the $\alpha$-carbon of the deprotonated $\alpha$-amino acid/peptide ligands. The dominant reaction pathway after this initial reductive decarboxylation is either consecutive decarboxylation via inter-ligand hydrogen transfer, followed by the radical induced dissociations, or reductive elimination of an amino acid radical. The larger the amino acid/peptide sizes are, the easier these reactions occur. For example, the GG/copper complex showed intense inter-ligand interactions that were absent upon the dissociation of hydrocarbon amino acid/copper complexes.
The side chains of W and Y can form stable neutral molecules via radical-induced reactions; the formation of such molecules, which are good Cu(I) ligands, is therefore thermodynamically favored during the dissociation of copper complexes of W and Y. For His, the imidazole nitrogen on the His side chain has a high copper ion affinity and most fragmentation products contain Cu(I) ions bound to the nitrogens of the two imidazole moieties. The longer and more flexible side chains of R and K facilitate similar radical reactions in the gas phase upon CAD. More significantly, guanidino- and α-hydrogens of the originally intact R ligand become involved in inter-ligand radical reactions and these processes compete effectively with the decarboxylation process. Similar competitive inter-ligand rearrangements take place with side chain hydrogens of W and Y. Deuteron labeling and MS³ (and select MS⁴) experiments strongly support the proposed reaction mechanisms.
Singly charged dilithiated or disodiated ions from the following peptides (Pep) were investigated: AGG, LGG, GLA, LGF, GLF, GAY, YGG, GGGG, AAAA, GGGGG, GGKAA, FFFFF, YGGFL, YAGFL, GGGGGG, YGGFLK, YAGFLR, SIKVAV, AAAAAAA, AAAAAAAA, and DRVYIHPF. These precursors decompose to form fragment ions that contain either one or two metal ions. For simplicity, such fragments are designated by one or two asterisks, respectively. Thus, a $b_n^*$ ion carries one metal ion and has the composition $[b_n - H + M]^+$ ($M = \text{Li or Na}$), whereas a $y_n^{**}$ ion carries two metal ions and has the composition $[y_n - 2H + 2M]^+$. 

A common characteristic of the $[\text{Pep} - H + 2\text{Li}]^+$ complexes studied here is that upon low-energy CAD they lose $\text{H}_2\text{O}$, $\text{NH}_3$, and CO$_2$ and produce a-, b-, c-, y-, and z-type ions with one (*) or two (**) metal ions attached to them. The dilithiated peptides may also undergo losses of their aromatic side chain(s) to form distonic radical ions carrying Li$^+$ charge(s) and one unpaired electron at one $\alpha$-C atom of the peptide backbone. Further, selective cleavages at certain peptide residues are also observed. Different aspects of the detailed fragmentation pathways of dilithiated peptides will be
presented in the following sections. The decomposition pathways of the corresponding disodiated species are very similar and will be discussed only briefly.

6.1. Random Backbone Cleavages

The CAD pathways of protonated peptides that do not have extremely basic or acidic residue(s) occur randomly along the peptide backbone, leading to contiguous series of fragment ions that reveal the sequence. Our results show that singly charged dilithiated peptides without extremely basic or acidic residues follow the same trend.

The ESI-QIT CAD spectra of protonated (a), monolithiated (b), singly charged dilithiated (c), singly charged disodiated (d), and singly charged trilithiated (e) YAGFL (alanine-leucine-enkephalin) are contrasted in Fig. 6.1. The b4/a4 fragments resulting from the loss of the amino acid leucine from the C-terminus dominate the CAD spectrum of protonated YAGFL in Fig. 6.1(a). The CAD products of monolithiated YAGFL differ significantly from those of the protonated peptide, in that \([b_n + OH + Li]^+\) ions dominate the spectrum (n = 3-4) and the \(b_4^*/a_4^*\) fragments have much lower relative abundances than \(b_4/a_4\) from the protonated precursor, cf. Fig. 6.1(b) vs. 1(a). With the singly charged dilithiated YAGFL precursor (Fig. 6.1(c)), the CAD spectrum becomes markedly different again, displaying dominant \(a_4^*\) ions and relatively abundant \(c_n^{**}\) ions. The \([\text{Pep} – H + 2\text{Li}]^+\) ions studied were found to produce upon CAD in the trap almost complete \(c_n^{**}\), \(y_n^{**}\), and \(a_n^*\) series, accompanied by less abundant \(b_n^*/ b_n^{**}\) ions (not all ions are labeled in Fig. 6.1(c) to avoid crowding). The CAD spectrum measured using MALDI-Q/ToF MS/MS (not shown) contains the same fragments but with more complete
Figure 6.1. CAD (MS²) mass spectra of YAGFL:
(a) protonated,
(b) monolithiated, and
(c) singly charged dilithiated.
Figure 6.1. CAD (MS$^3$) mass spectra of YAGFL; (d) singly charged disodiated and (e) singly charged trilithiated.
sequence ion series (even $y_{1,**}$ is observed), for the ToF mass analyzer discriminates less against low mass ions as compared to the ion trap. CAD was also performed on singly charged disodiated YAGFL; the resulting spectrum (Fig. 6.1(d)) shows an essentially identical fragmentation pattern as [Pep – H + 2Li]$^+$ (Fig. 6.1(c)), with formation of $a_1^*$, which has one metal cation attached to it, as the most abundant fragmentation channel. It has been shown previously that monolithiated and monosodiated peptides decompose through common dissociation pathways under constant experimental conditions.$^{58}$ The results presented here provide evidence that singly charged dilithiated and disodiated peptides decompose through common pathways as well when activated similarly.

Homolytic cleavage reactions also occur in the metalated peptides studied here, as attested by the losses of 107 u ($\rho$-hydroxybenzyl radical) observed in Figs. 6(c)-(d). On the other hand, the dominant fragment in the CAD spectrum of trilithiated YAGFL, Fig. 6.1(e), results from the loss of 4-methylene-2,5-cyclohexadiene-1-one (106 u), i.e. the side chain of Y with one less of hydrogen atom, as shown in the spectrum. In this case, the hydrogen in the hydroxyl group is probably replaced by the third lithium ion, which at the same time coordinates to other carbonyl groups as well. Upon excitation, the 106-u neutral molecule is eliminated while the lithium cation remains attached to the peptide.

6.2. Structures and Decomposition Mechanisms of the Dilithiated Complexes

The [Pep – H + 2Li]$^+$ ions studied correspond to Li$^+$ complexes of the Li$^+$ salts of the peptide. Depending on which acidic proton in Pep is replaced by Li$^+$ ion, several isomeric structures exist for the [Pep – H + 2Li]$^+$ product. In order to determine the most
stable conformers, a number of plausible geometries of the dipeptide complex \([\text{FG} - \text{H} + 2\text{Li}]\) were optimized by theory. From the families of structures scanned by molecular mechanics/molecular dynamics, those shown in Fig. 6.2 were found to have the lowest energies. Further optimization and energy minimization of these structures using density functional theory at the B3LYP/6-31G(d) level resulted in the conformations and relative energies summarized in Fig. 6.2. In the most stable structure, 1 (relative energy 0 kJ/mol), the carboxylate hydrogen is replaced by one lithium cation to form a salt bridge, and the second lithium ion is attached to the carbonyl oxygen of the amide bond and the carboxylate oxygen. The two lithium ions are at the same time coordinated by the phenyl ring of the F residue. Structure 2 is almost the same as 1, but the lithium cation attached to both of the carboxylate oxygen atoms does not interact with the phenyl ring; 2 is about 3 kJ/mol higher in energy than structure 1. Structures 3 and 4 are Li-amide structures, with the deprotonation sites being secondary amide nitrogens instead of carboxylic groups. In structure 3 (33 kJ/mol relative to structure 1), one lithium ion interacts with both the C-terminal carbonyl oxygen and the deprotonated amide nitrogen; the other lithium cation is attached to the deprotonated amide oxygen and the phenyl ring of F. Structure 4 has almost the same energy (36 kJ/mol) as 3; in this isomer, one lithium ion is coordinated by the C-terminal carbonyl oxygen, the deprotonated amide nitrogen, and the aromatic ring and the other lithium cation is attached to the deprotonated amide bond oxygen and the N-terminal amino nitrogen. Structure 5 is a Li-amide and Li-carboxylate structure, in which, according to its name, both the amide nitrogen and the carboxyl group are deprotonated, while the amide oxygen is protonated. In structure 5, one lithium
Figure 6.2. Conformations and relative energies (kJ/mol) of singly charged dilithiated FG complexes.
cation is attached to both carboxylate oxygen atoms and the other to one carboxylate oxygen, the deprotonated amide nitrogen atom, and the phenyl ring of the F residue. The charge-separated structure of FG shown in 5 is a resonance structure of the enol tautomer of the amide group. The energy of structure 5 is only 41 kJ/mol relative to structure 1; such an energy window is readily accessible in an ion trap instrument and explains the formation of protonated fragments upon CAD of some singly charged dilithiated peptides.

Plausible structures for the major sequence ions from dilithiated YAGFL are shown in Scheme 6.1; the ions shown contain two AA residues. The \( y_n^{**} \) fragments are truncated peptides. Based on the computational results presented above, the most stable and, hence, most likely structure of these ions is a lithium carboxylate structure, such as 1 or 2. The \( c_n^{**} \) series corresponds to the \( \text{Li}^+ \) complexes of lithium amides. Here, the \( \text{Li}^+ \) ions are attached to a shorter peptide with an amidated C-terminus. Either the C-terminal or one of the internal amide groups may be deprotonated (Scheme 6.1). As for the monolithiated \( a_n^* \) series, it represents lithiated shorter peptides with imine chain ends, viz. their C-termini carry \(-\text{C}(=\text{O})-\text{N}=\text{CHR}\) groups.

Scheme 6.2 provides mechanistic rationalizations for the major fragments formed from collisionally activated YAGFL. The DFT calculations predict that the \( \text{Li}^+ \) ions are bound near the carboxylate terminus in the most stable geometry of \([\text{YAGFL} - \text{H} + 2\text{Li}]^+\). It is proposed that one lithium ion becomes mobile upon CAD, moving to different binding sites along the backbone or at the side chains where, as a Lewis acid, it can promote fragmentation of different bonds. With this assumption, Scheme 6.2(a) shows a reaction pathways to \( y_n^{**} \) ions, adapted from the established mechanisms operating upon
Scheme 6.1. Major sequence ions from dilithiated peptides.
Scheme 6.2. Dissociation pathway of \([\text{YAGFL} - \text{H} + 2\text{Li}]^+\) to (a) \(y_n^{**}\) ions (\(y_1^{**}-y_3^{**}\)).
Scheme 6.2. Dissociation pathway of \([\text{YAGFL} \ - \ H \ + \ 2\text{Li}]^+\) to (b) \(y_{4\star\star}\) and (c) \(c_{3\star\star}\) ions.
Scheme 6.2. Dissociation pathway of [YAGFL – H + 2Li]+ to (d) c_{n=2}^{**} (c_2^{**}) and (e) a_n^{*} ions.
the decomposition of protonated peptides. As the mobile Li⁺ ion approaches the amide nitrogen of a peptide bond, the carbonyl carbon of this bond becomes more electrophilic, facilitating its attack by the N-terminally adjacent carbonyl oxygen to form a protonated oxazolone. The oxazolone and the detaching Li⁺ amide are held together by hydrogen bonding. After proton transfer from the oxazolone to the Li⁺ amide piece, the C-terminal part of the peptide is released as a yⁿ⁺⁺ ion. Scheme 6.2(a) illustrates the formation of y₂⁺⁺ ions via this pathway. Li⁺ transfer reactions are possible within the ion/molecule complexes emerging after amide bond cleavage. Thus, if the Li⁺ ion involved in the dissociation is transferred to the N-terminal oxazolone piece, a bⁿ⁺ ion is formed, as illustrated for b₃⁺ in Scheme 6.2(a).

N-terminal c-type ions are observed up to cⁿ⁻²⁺⁺, which is the c₃⁺⁺ ion from dilithiated YAGFL. In the mechanism proposed in scheme 6.2(c), dissociation to cⁿ⁻²⁺⁺ begins from a lithium carboxylate structure. Charge-induced bond cleavages, as shown in the Scheme, lead to the expulsion of three stable molecules, viz. CO₂, a ketene, and an imine, and the c₃⁺⁺ ion. The formation of c₃⁺⁺ could be promoted by Li⁺ attachment at the carboxyl oxygen N-terminal to the N-Cα bond being broken. In the final product, both Li⁺ ions have moved away from the C-terminus. The structure shown for c₃⁺⁺ is not necessarily the lowest-energy isomer; in the latter, both metal ions are most likely bound at the deprotonated amide group (where the negative charge density is high) and simultaneously interact with other basic sites, such as carbonyl and aromatic groups.

The mechanism outlined in Scheme 6.2(c) cleaves the N-Cα bond in the (n-1)th AA residue (counting from the N-terminus), giving rise to a cⁿ⁺ ion with n-2 residues; it cannot produce a cⁿ⁻¹⁺⁺ ion, in accord with the absence of such fragments in the CAD
spectra of dilithiated peptides. The formation of $c_{n-2}^{**}$ ions, on the other hand, can be explained by tautomerationization of the lithium carboxylate to a lithium amide structure, prior to charge-induced bond cleavages; Scheme 6.2(d) illustrates this case, in which a ketene, an imine, and an isocyanate are eliminated to yield a $c_2^{**}$ ion from dilithiated YAGFL. The pathway depicted in Scheme 6.2(c) gives rise to the largest $c^{**}$-type ion observed (i.e. $c_{n-2}^{**}$), while that in Scheme 6.2(d), and analogous pathways starting from other deprotonated amide tautomers, give rise to smaller $c^{**}$-type fragments.

The much higher relative abundance of $y_2^{**}$ vs. $b_3^*$ in Fig. 6.1(c) points out that the lithium carboxylate of the dipeptide FL has a higher Li$^+$ affinity than the oxazolone-terminated YAG piece. Doubly lithiated $b_n^{**}$ ions are also observed occasionally, albeit with much lower abundance than $b_n^*$; for example, $b_3^{**}$ appears just above noise level in Fig. 6.1(c). The formation of such ions indicates that the ion/molecule complex produced upon amide bond cleavage is long-lived, allowing for several H$^+$/Li$^+$ exchanges between the complex constituents. Note that the $y_2^{**}$ structure shown in Scheme 6.2(a) is the nascent isomer generated during amide bond cleavage. As the bond is broken and $y_2^{**}$ departs, rearrangement to the energetically most favorable structure 1/2 (Fig. 6.2) is expected to occur.

The mechanism presented in Scheme 6.2(a) is termed the $b_n/y_n$ pathway, in analogy to the $b_n/y_n$ pathway operating in protonated peptides when they dissociate to form $b_n$ and $y_n$ sequence ions. The $b_n/y_n$ mechanism does not apply to the cleavage of the first N-terminal amide bond, where an oxazolone cannot be formed. This cleavage is explained by an alternative pathway, shown in Scheme 6.2(b), and adapted from the
a₁/yₙ₋₁ pathway of protonated peptides.⁴⁵,⁵¹,⁵² Again, dissociation is promoted by attachment of the mobile Li⁺ ion near the amide nitrogen, which elongates the corresponding C(=O)-NH bond, creating an unstable acylium cation that loses CO to form a proton-bound complex between an immonium ion and the detaching Li⁺ amide. Proton transfer to the amide gives rise to yₙ₋₁**(y₄** from YAGFL) and a neutral imine.

Lithium amide structures can also be invoked to rationalize the aₙ* series, as shown in scheme 6.2(e). Here, the amide anion induces bond cleavages towards the C-terminal side (in contrast to the mechanism in scheme 6.2(d)), leading to the expulsion of CO and, after proton transfer between the dissociating segments, to the formation of aₙ* and a neutral lithium carboxylate salt.

Table 6.1 summarizes the CAD spectra of the [Pep - H + 2Li]⁺ ions studied. Most fragments contain one or two Li⁺ ions, in agreement with the pathways presented in Scheme 6.2. Protonated species (i.e. no Li⁺ content) are, however, also observed, especially from some of the smaller peptides. This finding affirms that the dissociations proceed through ion/molecule complexes, as has been mentioned, in which H⁺/Li⁺ exchange reactions are possible.

6.3. Selective Cleavage at Internal K Residues

Selective backbone cleavages take place only next to specific residues, under defined conditions. They thus reveal specific structural (sequence) motifs. Our group has recently reported that peptide ions with no mobile proton undergo selective cleavage C-terminal to an internal lysine residue. This reaction is catalyzed by the NH₂ group in
Table 6.1. Important fragment ions from singly charged dilithiated peptides after CAD.a-c

<table>
<thead>
<tr>
<th>Peptide</th>
<th>$a_n^{(*)}$</th>
<th>$b_n^{(*)}$</th>
<th>$c_n^{**}$</th>
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<tr>
<td>YGGFL</td>
<td>256($a_3^*$), 262($a_3^{**}$),</td>
<td>284($b_3^*$), 290($b_3^{**}$),</td>
<td>244($c_2^*$), 250($c_2^{**}$),</td>
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<td></td>
<td>403($a_4^*$), 409($a_4^{**}$),</td>
<td>431($b_4^*$), 437($b_4^{**}$),</td>
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</tr>
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<tr>
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<td>220($b_3^*$), 228($b_3^{**}$),</td>
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<td></td>
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<td>291($b_3^*$), 297($b_3^{**}$),</td>
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<td>315($y_4^{<strong>}$), 386($y_5^{</strong>}$),</td>
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<tr>
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<td>121($b_3^<em>$), 127($b_3^{**}$), 178($b_3^</em>$), 184($b_3^{<strong>}$), 235($b_4^*$), 241($b_4^{</strong>}$), 292($b_5^*$), 298($b_5^{**}$),</td>
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<td>178($b_2^<em>$), 184($b_3^{**}$), 235($b_4^</em>$), 241($b_4^{**}$),</td>
<td>138($c_2^<em>$), 144($c_2^{**}$), 195($c_3^</em>$), 201($c_3^{<strong>}$), 258($c_4^{</strong>}$), 145($y_2^{<strong>}$), 202($y_3^{</strong>}$), 259($y_4^{**}$),</td>
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<td>Peptide</td>
<td>-18 u</td>
<td>Fragment Information</td>
<td>Masses (m/z)</td>
<td>Comments</td>
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<tr>
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<td>144(c₂**)</td>
<td>d</td>
<td>Isobaric with a₃.</td>
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<tr>
<td>FFFFF</td>
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<td>88(y₁**), 145(y₂**)</td>
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<td>GAY -18 u</td>
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<td>GLF -18 u</td>
<td>149(a₂*), 155(a₂**), 200(c₂**), 178(y₁**), 235(y₂**), 149(a₂*), 155(a₂**), 200(c₂**), 178(y₁**), 235(y₂**)</td>
<td>178(y₁**), 235(y₂**)</td>
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<td>LGG -18 u</td>
<td>143(a₂), 149(a₂*), 155(a₂**), 177(b₂*), 143(a₂), 149(a₂*), 155(a₂**), 177(b₂*), 200(c₂**), 178(y₁**), 235(y₂**)</td>
<td>178(y₁**), 235(y₂**)</td>
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<td>143(a₂), 155(a₂**), 177(b₂*), 143(a₂), 155(a₂**), 177(b₂*), 200(c₂**), 178(y₁**), 235(y₂**)</td>
<td>178(y₁**), 235(y₂**)</td>
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<tr>
<td>YGG -18 u</td>
<td>193(a₂), 199(a₂*), 193(a₂), 199(a₂*), 88(y₁**), 145(y₂**), 193(a₂), 199(a₂*), 88(y₁**), 145(y₂**)</td>
<td>88(y₁**), 145(y₂**)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-18 u indicates that water loss is the dominant process.
- The dominant sequence fragment is given in bold.
- Italicized are fragments with < 20% relative intensity (in respect to the most abundant sequence ions).
- Isobaric with a₃.
- Isobaric with c₁**.
- For LGF, the most abundant ion is m/z 188 (loss of water and H₂N-CH(CH₂CH(CH₃)₂)-C(=O)-N=CH₂).78
Fig. 6.3. ESI-QIT CAD spectra of
(a) singly charged dilithiated GGKAA and
(b) singly charged dilithiated YGGFLK.
lysine's side chain (see Scheme 3 in ref. 58). When singly charged dilithiated GGKAA is subjected to CAD in the ion trap instrument, the formation of \( b_3^* \) ions is significantly enhanced (Fig. 6.3(a)). Now, \( b_3^* \) is the dominant peak in the spectrum, in contrast to Fig. 6.1(c), in which \( b_3^* \) is an insignificant peak. All other fragment ions from \([\text{GGKAA} - \text{H} + 2\text{Li}]^+\) have very low abundances except for \( a_4^* \) and \( y_4^{**} \). On the contrary, a lysine residue at the C-terminal of the peptide, as in YGGFLK, does not induce a selective cleavage, as is evident from Fig. 6.3(b). The dominant peaks in the CAD spectrum of YGGFLK** (dilithiated) are \( c_n^{**} \), \( y_n^{**} \), and \( a_n^* \), while the \( b_n^* \) series is insignificant. These characteristics, which allow definitive sequence determination of the peptide, are very similar to those of YAGFL** (Fig. 6.1(c)) and YGGFL** (Table 6.1) which lack a K residue.

6.4. Side Chain Loss from S Residues

Peptides that have serine residues are believed to undergo sidechain cleavages upon excitation in the mass spectrometer.\(^{37,88}\) Fig. 6.4(a) depicts the CAD spectrum of monolithiated SIKVAV, in which the dominant peak is the \([b_5 + \text{OH} + \text{Li}]^+\) ion that arises from the loss of the C-terminal residue V.\(^{58}\) This fragment, the parent ion, and \( a_4^* \) lose a 30-u neutral, which originates from the side chain of serine, as proved by Adam \textit{et al.}\(^{88}\) using deuteron labeling experiments. The \( b_3^* \) ion is the most abundant \( b_n^* \) fragment due to the selective cleavage caused by the internal Lys residue, as mentioned above. Compared to the CAD spectrum of monolithiated SIKVAV, that of singly charged dilithiated SIKVAV shows more sidechain losses from the serine residue, as indicated in
Figure 6.4. ESI-QIT CAD spectra of SIKVAV;
(a) monolithiated and
(b) singly charged dilithiated.
Fig. 6.4(b). It is evident that the major reaction pathway upon excitation of SIKVAV** is loss of 30 u (CH$_2$O) from the parent ion, which corresponds to the loss of the serine side chain. The a$_n$* ions (n = 4-5) also undergo 30-u losses; in fact, the a$_n$*-30 peaks are more abundant than the a$_n$* peaks. The losses of small molecules are also quite abundant compared to other fragmentation pathways. C-terminal y$_n$** ion are also formed with considerable relative abundances from singly charged dilithiated SIKVAV, while y$_n$** ions are barely observed from monolithiated SIKVAV. The CAD spectrum acquired on the MALDI-Q-ToF mass spectrometer (not shown) is almost the same, except that the sequence ions and their consecutive fragments (- 30 u) appear with higher relative abundances. The relative abundance of b$_3$* (selective cleavage) is low, but it still is higher than those of any other b-type ions formed. The low proportion of b- and c-type fragments from SIKVAV** probably results from the other reaction channels having more competitive kinetics. It is noticed that water loss from both monolithiated and dilithiated SIKVAV and their fragments is not as abundant as from protonated serine-containing peptides.$^{37,58}$ Conversely, the elimination of CH$_2$O (30 u) is insignificant for protonated SIKVAV, but considerable for monolithiated and, especially, dilithiated SIKVAV. The elimination of CH$_2$O could be a charge-remote reaction and, thus, enhanced in the absence of a mobile proton. It is also possible that a Li$^+$ ion replaces the OH proton of the serine side chain in the reacting isomer leading to CH$_2$=O expulsion, thereby facilitating this reaction. The OH→OLi conversion would be more probable in the dilithiated peptide, in agreement with the increased yield of 30 u losses from this parent ion.
6.5. Selective Cleavage at D Residues

Protonated peptides with no mobile proton have been known to undergo selective
cleavages C-terminal to acidic residues. Protonated DRVYIHPF complex was examined by using both ESI-QIT and MALDI-Q-TOF mass
spectrometry. The CAD spectrum of DRVYIHPF** acquired by ESI-QIT MS is shown
in Fig. 6.5(a). The prevailing reaction pathways of this complex are eliminations of stable
small molecules, H$_2$O and NH$_3$. The next most noticeable peak is y$_7$** which arise from
selective C-terminal to the aspartic acid residue. Upon CAD in the MALDI-Q-ToF
instrument at 55 eV collision energy, an almost complete y$_n$** series is obtained (Fig.
6.5(b)) and other ion series rise slightly above noise level, which provides more
information about the peptide sequence. CAD in the MALDI-Q-ToF instrument deposits
broader internal energy distributions and higher average internal energies than CAD in an
ion trap which, in certain cases (as shown here), leads to structurally more revealing data.

6.6. YAGFLR

For most peptides discussed so far, singly charged dilithiated ions produce enough
CAD fragments for sequence analysis. There are occasionally cases, however, where
monolithiated peptides provide much more structural insight than dilithiated peptides.
Protonated Arg-containing peptides usually undergo 59-u losses from the Arg side chain.
Although many more fragments are formed, most of them have very low relative
abundances and are difficult to interpret. In such cases, singly charged dilithiated
Fig. 6.5. CAD mass spectra of singly charged dilithiated DRVYIHPF acquired by
(a) ESI-QIT MS and
(b) MALDI-Q-ToF MS.
Fig. 6.6. ESI-QIT CAD spectra of YAGFLR;
(a) monolithiated and
(b) singly charged dilithiated.
precursors may not be suitable either, as illustrated for YAGFLR** in Fig. 6.6(a). Only a few fragments are produced. Surprisingly, monolithiated YAGFLR provides an almost complete \([b_n + \text{OH} + \text{Li}]^+\) series as well as partial \(a_n^*\) and \(b_n^*\) series and, thus, is more useful for sequence determination. The dominant fragmentation pathways still involve losses of \(\text{H}_2\text{O}\) and of 59 u from the Arg side chain, as shown in Fig. 6.6(b). Nevertheless, attachment of one lithium cation to YAGFLR also leads to a sufficient number of sequence indicative fragments, unlike the dilithiated or protonated parent ions.

6.7. Conclusions

A wide range of singly charged dilithiated peptides has been studied by tandem mass spectrometry methods on both ESI-QIT and MALDI-Q-ToF instruments. In this type of peptide derivatives, a proton in the peptide is replaced by a lithium cation (usually at the most acidic site); the other lithium cation provides the charge and probably becomes mobile upon excitation, attaching at various locations of the peptide backbone, where it can induce fragmentation to produce sequence ions.

Theoretical calculations on the dipeptide complex \([\text{FG} - \text{H} + 2\text{Li}]^+\) have shown that there are several different low-energy isomers for dilithiated FG. From the five structures calculated, the lithiated Li-carboxylate structures 1 and 2 are the most stable ones and very close in energy (1, 0 kJ/mol; 2, 3 kJ/mol). The structures next lowest in energy are the lithiated Li-amide structures 3 (33 kJ/mol) and 4 (36 kJ/mol). The highest energy is found for the protonated Li-carboxylate and Li-amide structure 5 (41 kJ/mol relative to structure 1). Since the energy differences are not significantly large, all of these structures
can be sampled during CAD. This explains why protonated fragments are also present in the CAD spectra of the singly charged dilithiated peptides.

The MS/MS fragmentations of the dilithiated peptide complexes have been examined in detail and it has been shown that such precursor ions are very useful in the elucidation of peptide sequences. The study of a series of tripeptides has further revealed that fragmentation at the C-terminal side of glycine residues does not take place efficiently and may be completely absent. For peptides that have no extremely basic or acidic residues, CAD of the singly charged dilithiated peptides leads to almost complete structurally diagnostic \( y_n^{**}, c_n^{**}, \) and \( a_n^* \) ions, which allows for definitive sequence determination. The dissociations of \([\text{Pep} - \text{H} + 2\text{Li}]^+\) reveal that salt bridges with both carboxylate and deprotonated amide structures are populated. Such structures are found to promote sequence-specific fragmentations as well as the elimination of radicals to form dilithiated peptide \( \alpha \)-backbone radicals. As a whole, the derivatized singly charged dilithiated peptides show promising results for sequence analyses of peptides.
CHAPTER VII

SUMMARY

The dissociations of [Cu(II)AA(AA-H)]⁺ complexes (AA: Gly, Ala, Val, Leu, Ile, t-Leu, and Phe) have been examined by CAD experiments in an electrospray ionization mass spectrometer. In the single-stage mass spectra, copperated complexes of the amino acid dominate, but there are protonated and sodiated amino acids as well. In the complexes, the amino groups of both amino acids, the carbonyl group of the intact amino acid, and the deprotonated carboxyl oxygen coordinate Cu(II) in square-planar fashion. Under CAD conditions, the [Cu(II)AA(AA-H)]⁺ complexes undergo decarboxylation with simultaneous reduction of Cu(II) to Cu(I); during this process, a radical site is created at the α-carbon of the decarboxylated ligand (N₁H₂·C₆HC₆H₂R). The radical site is able to move along the backbone of the decarboxylated amino acid to form two new radicals (·N₁HC₆H₂C₆H₂R and/or N₁H₂C₆H₂·C₆HR). From the [Cu(II)AA(AA-H)]⁺ complex of t-Leu, only C₆ and N₁ radicals can be formed. The whole radical ligand can be lost to form [Cu(I)AA]⁺ from those three isomeric radicals. Alternatively, further radical induced dissociations can take place along the backbone of the decarboxylated amino acid to yield [Cu(II)AA(AA-2H-CO₂)]⁺, [Cu(I)AA(·NH₂)]⁺, and/or [Cu(I)AA(NH=C₆H₂)]⁺. Those products may undergo consecutive dissociation to form
the final product [Cu(I)AA]^+. The sodiated copper complexes ([Cu(II)(AA-H+Na)(AA-H)]^+) show the same fragmentation patterns as their non-sodiated counterparts; sodium ion is retained on the intact amino acid ligand and is not involved in the CAD pathways.

On the basis of the CAD spectra, reasonable dissociation mechanisms are proposed for the [Cu(II)AA(AA-H)]^+ complexes and substantiated by the calculated potential energy diagram for the select CAD channels of the [Cu(II)Ala(Ala-H)]^+ complex. The theoretical calculations provide information about the hydrogen migration pathways. After decarboxylation, a hydrogen from the amino group or the β-carbon migrates to the copper ion, from where it is shuttled to Cα to form radicals at the amino nitrogen (N1) or the β-carbon (Cβ), respectively. These results show that the combination of theory and experiment is a powerful tool to elucidate reaction mechanisms in the gas phase.

For copper complexes with hydrocarbon amino acids, only the deprotonated amino acid ligands are involved in the dissociation reactions. On the other hand, complexes of copper and amino acids with functionalized side chains additionally undergo inter-ligand reactions. As the size of the molecules coordinated to copper increases, the probability of inter-ligand reaction increases.

The dissociations of [Cu(II)AA(AA-H)]^+ complexes with AA: Trp (W), Tyr (Y), His, Arg (R), Lys (K), or GlyGly (GG) have also been examined by CAD experiments in an electrospray ionization mass spectrometer. The longer, functionalized side chains of these amino acids facilitate their coordination to copper ion. Under CAD conditions, most of the [Cu(II)AA(AA-H)]^+ complexes studied undergo decarboxylation with concomitant reduction of Cu(II) to Cu(I), which transfers the radical site to the α-carbon of the deprotonated α-amino acid/peptide. The dominant reaction pathway after the reductive
decarboxylation is either elimination of the radical ligand or inter-ligand hydrogen atom transfer from the COOH group to the \( \alpha \)-radical site followed by a second decarboxylation and subsequent radical induced dissociations. The larger the amino acid/peptide size, the easier these reactions occur. This size effect is evident with the GlyGly/copper complex, which showed an intense inter-ligand interaction that was not observed from the hydrocarbon amino acid/copper complexes.

From the side chains of Trp and Tyr, the elimination of stable neutral molecules is observed upon the dissociation of the copper complexes. For His, the imidazole ring on the side chain has such a high copper ion affinity that most fragmentation products appear to arise from structures in which nitrogen atoms of the imidazole moieties are coordinated to copper ion. The longer and flexible side chains of Arg and Lys lead to unique radical reactions in the gas phase upon CAD of the corresponding dimeric copper complexes. From the Cu(II)/Arg complex, the acidic as well as the \( \alpha \)-hydrogen of the originally intact Arg ligand is involved in inter-ligand hydrogen transfer reactions, and the extraction of an \( \alpha \)-hydrogen atom is a competitive reaction pathway with respect to the second decarboxylation process. Deuteron labeling experiments corroborated these reaction mechanisms.

A wide range of singly charged dilithiated peptides, \([\text{Pep} - \text{H} + 2\text{Li}]^+\), has been studied by tandem mass spectrometry on ESI-QIT and MALDI-Q-ToF MS instruments. In this type of peptide derivatives, a proton in the peptide is replaced by a lithium cation (usually at the most acidic site); the other lithium cation is probably mobile along the backbone of the peptide upon excitation and induces fragmentations that produce sequence ions.
Molecular dynamics simulations on various isomers and conformers of the dipeptide complex \([\text{PheGly} - \text{H} + 2\text{Li}]^+\) were performed using the InsightII program in conjunction with the AMBER force field. The most stable species in these families were then fully optimized at the HF/3-21G and B3LYP/6-31G(d) levels. The results show that there are several different isomeric structures for dilithiated FG. The lowest energy isomers have Li-carboxylate structures (1 and 2) and very similar energies (2 lies 3 kJ/mol above 1). The structures next lowest in energy are the Li-amide structures 3 and 4, which are separated by 33 and 36 kJ/mol, respectively, from 1. The structure containing Li-carboxylate and Li-amide groups is less stable (+ 41 kJ/mol relative to structure 1). The relative energies of structure 1-5 are not significantly large and all of them can be populated upon typical CAD experiments. This explains the presence of protonated and singly lithiated fragments in the CAD spectra of the dilithiated peptides.

The MS/MS fragmentations of dilithiated peptides show that such precursor ions are very useful in the elucidation of peptide sequences. For peptides that have no extremely basic or acidic residues, CAD of their singly charged dilithiated derivatives leads to almost complete structurally diagnostic \(y_n^{**}\), \(c_n^{**}\), and \(a_n^*\) ions, which allow definitive sequence determination. The dissociations of \([\text{Pep} - \text{H} + 2\text{Li}]^+\) reveal that salt bridges with both carboxylate and deprotonated amide structures are populated upon CAD. Such structures are found to promote sequence-specific fragmentations as well as the elimination of radicals to form dilithiated peptide \(\alpha\)-backbone radicals. Overall, singly charged dilithiated peptides show promising results for their use as precursor ions in sequence analyses of peptides.
This dissertation only provides a minute portion of breadth of research concerning the gas phase chemistry of simple Lewis acid/peptide complexes that can be performed by mass spectrometry. Other aspects of interactions of peptides with simple Lewis acids providing different intrinsic chemistry information have been studied and are not included in this dissertation.\textsuperscript{12,89-93} Mass spectrometry has been essential for exploring gas phase chemistry and such research ultimately leads to better or new MS-based structural characterization methods. The combination of theory and mass spectrometry has further created a powerful tool for the study of gas phase reaction mechanisms, so that they can be elucidated more accurately and become predictable.
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