Chapter 1. Introduction

1.1. Background

Non covalent interactions are important for the structures and reactivity of biological molecules in the gas phase as well as in the solution phase.[1] It is well known that strong intramolecular hydrogen bonds determine gas phase structures of biomolecules.[2] Coulombic interactions of metal cations stabilize gas phase structures of biomolecules[3] and provide distinct reactions and dissociations.[4] This thesis examines gas phase structures and reactions of biomolecules, including metal complexes and molecular clusters.

A wide range of interactions of metal cations with biomolecules in the gas phase have been reported. For example, alkali-metallated ariginine cations form a salt bridged zwitterionic structure in the gas phase.[5] In the peptide complex, alkali metal forms multidentate bonds to carbonyl oxygen atoms in the peptide backbone and to chelating side chains.[6] Furthermore, the alkali metal cation is known to stabilize helical structures of peptides in the gas phase.[3] The alkaline earth metal cation induces deprotonation of either the amide group or the C-terminal carboxyl group in the gas phase.[7] This divalent metal can form at least six coordinate bonds with these groups.[8] The transition metal cations are known to yield deprotonation of amino groups, which results in multiple conformations of gas phase peptides.[8]

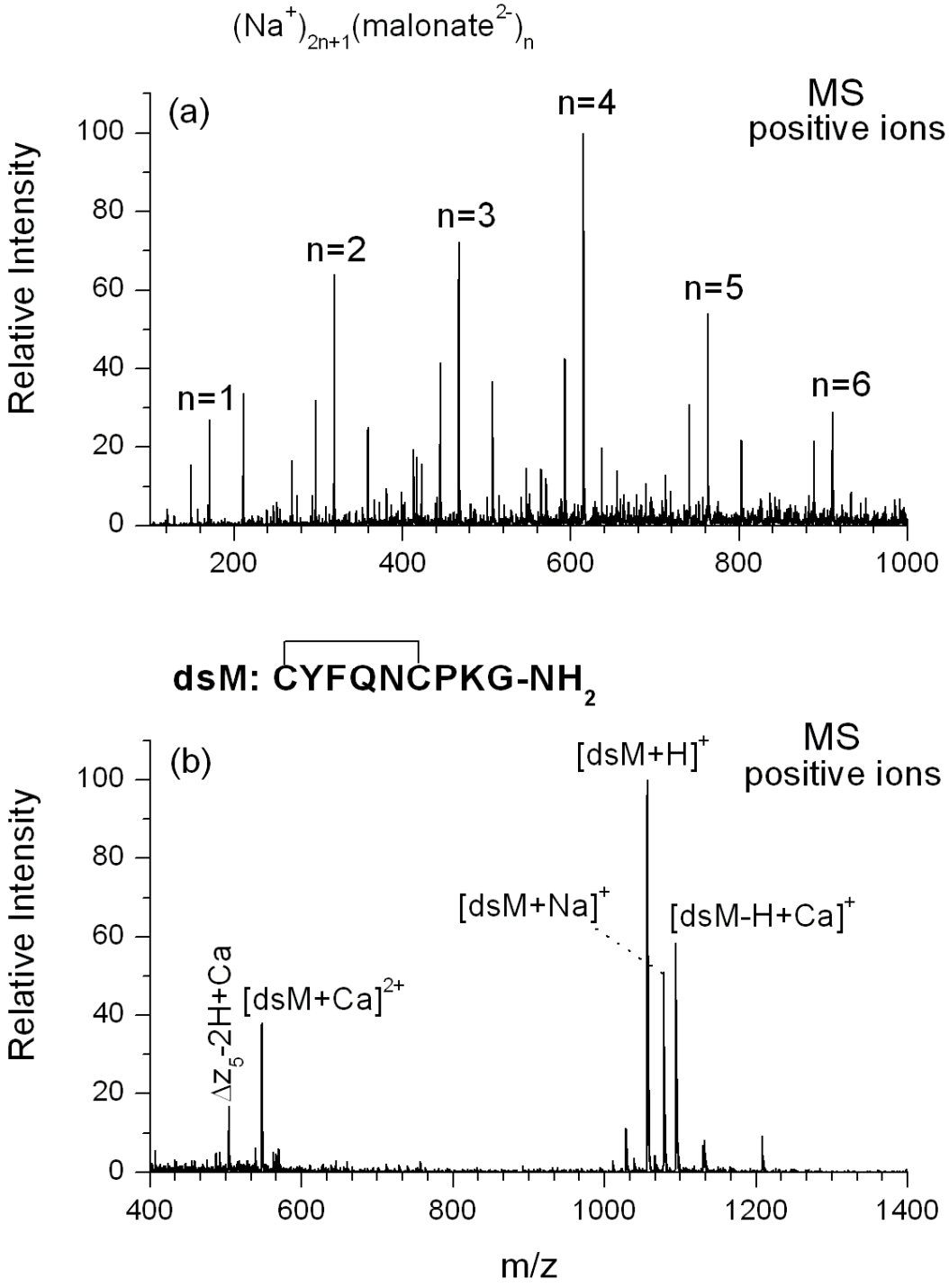
Complex chemistry using metallated molecular clusters has been widely investigated. The transfer of the alkali metal cation from the sodiated cyclic peptide complex (e.g., gramicidin S) to crown ether has been demonstrated.[9] Rodriguez-Cruz and Williams revealed solvent exchange reactions of hydrated clusters of divalent alkaline earth metal

ions and benzene molecules in the gas phase.[10] In numerous instances, transition metal cations have been shown to play unique roles in complex chemical reactions in the gas phase,[11] exemplified by the decarbonylation of ketones and aldehydes by Co^+.[12]

The development of soft ionization methods such as electrospray ionization[13] has introduced mass spectrometry as a powerful tool for obtaining the structural information of non-covalently bound biomolecule complexes. Soft ionization of the electrospray technique maintains non-covalent interactions of biomolecules (e.g., molecular clusters, metal-biomolecule complexes) through the intact transfer of ions from the solution phase to the gas phase.[1] Figure 1 shows ESI mass spectra of sodiated malonic acid and [Lys^8]-vasopressin (LVP) with calcium dichloride in the positive mode. Highly abundant sodiated malonate cluster cations (Figure 1a) and calcium bound LVP (Figure 1b) complex ions are shown with sodiated species in the spectra. In this thesis, all of the ions of biomolecules, including metal complexes, are generated through ESI processes.

Collision-induced dissocation (CID) has been widely employed to establish the structures of biomolecules using mass spectrometry (MS).[14] The CID technique is simple and effective for the investigation of biomolecules with ion trap mass spectrometry due to its unique MS^n capability.[15] For many peptides, CID yields dominating cleavage at the amide bonds, producing a series of ions that comprise b- and y-type fragments.[16] In contrast, the CID of metal complexes yields distinguishable dissociation pathways other than amide backbone cleavages. For example, the selective cleavage at an acidic residue is reported from singly charged sodiated peptide cations.[4] In chapters 2 and 3, we report highly selective elimination of H_2S_2 from singly charged alkali and alkaline earth metal bound peptide cations with disulfide linkages under low energy CID conditions. These

Figure 1. ESI-MS spectra of (a) sodiated malonate clusters and (b) LVP with calcium dichloride in positive mode.


$(Na^+)_{2n+1}(malonate^{2-})_n$
(a)
MS
positive ions
n=1
n=2
n=3
n=4
n=5
n=6
Relative Intensity
dsM: CYFQNCPKG-NH2
(b)
MS
positive ions
[dsM+H]+
[dsM+Na]+
[dsM-H+Ca]+
[dsM+Ca]2+
Δz5-2H+Ca
Relative Intensity
m/z

distinct CID pathways of metal complexes are initiated from metal stabilized complexes, with the intermediates and final products further stabilized by metal cations.

For molecular clusters that are strongly bound, CID can initiate complex chemistry that goes beyond simple monomeric dissociation. This is possible only when dissociation of the cluster requires more energy than the activation barrier for chemical reactions of non-covalently bound reactants. Chapters 4 and 5 describe the gas phase reactions of sodiated clusters of dicarboxylic acids generated via ESI.

Ion mobility spectrometry (IMS) is a unique tool for the structural investigations of molecular ions in the gas phase.[17] The separation of ions can be achieved in the drift tube of the instrument based on their mobilities under the weak electric field. Using the relation that ion mobility is inversely proportional to the collision cross section of the ion, information about the structure and the size of the ion can be obtained from the experimentally determined mobility value. Interfacing ESI with IMS expands the application of the instrument to structural investigations of nonvolatile biomolecules in the gas phase. In Chapters 6 through 8, polyatomic biomolecules are examined using electrospray ionization ion mobility spectrometry (ESI-IMS). Observed high correlation between mass and mobility of ion and experimentally determined collision cross sections can reveal information about structural details such as cyclic conformations of dicarboxylate anions through intramolecular hydrogen bonds.

1.2. Content of Thesis

1.2.1 Metal Complexes of Biomolecules

The Route 66 Method. In Chapters 2 and 3, we examine controllable and predictable fragmentation processes to identify disulfide linkages in peptides using what we call the

Route 66 method. This methodology is based on the highly selective elimination of H_2S_2 (66 mass units) from singly charged sodiated and alkaline earth metal bound peptide cations with disulfide linkages under CID conditions. The selective elimination of H_2S_2 leaves newly formed dehydroalanine residues in the peptide. Further activation of the product yields sequence information in the region that was previously short circuited by the disulfide bond. The process is initiated with a metal stabilized enolate anion at Cys, followed by cleavage of the S-C bond. Chapter 2 presents the application of the method using peptide hormones [Lys^8]-vasopressin and oxytocin to identify and locate the disulfide linkages.

Chapter 3 details the results of applying the Route 66 method to identify and locate the disulfide linkages in peptides from a peptic digest of insulin. Singly and doubly charged cationic Na^+ and Ca^{2+} complexes of the peptic digest peptides are generated using electrospray ionization mass spectrometry (ESI-MS). Collisional activation of doubly charged metal complexes of peptides with intermolecular disulfide linkages yields two sets of singly charged paired products, separated by 66 mass units resulting from selective S-C bond cleavages. Further activation of the singly charged product yields sequence information or elimination of 66 mass units if a second disulfide linkage is present in the peptide. Experimental and theoretical model peptide studies provide mechanistic details for the selective cleavage of the S-C bond.

Cluster Phase Chemistry. In Chapters 4 and 5, reactions of collisionally activated sodiated dicarboxylate clusters are examined using a homologous series of anionic and cationic gas phase clusters of dicarboxylic acids. The reaction is initiated by collision of the activated cluster with a single water molecule. Water molecules serve as proton donors for reacting dicarboxylate anions in the cluster and introducing energetically

favorable dissociation pathways that are not otherwise available. The more strongly bound small dicarboxylate, oxalate, and malonate anions preferentially react with water molecules rather than dissociate to lose disodium dicarboxylate monomers observed from larger dicarboxylate anions. The reactivity of several mixed dicarboxylate clusters is also reported in the positive mode. For example, malonate anion is shown to be more reactive than oxalate anion for decarboxylation when both are present in a cluster. The energetics of several representative cluster phase reactions are evaluated using computational modeling. Implications of these results for the atmospheric aerosol chemistry of dicarboxylic acids are discussed.

1.2.2. Mass-Mobility Correlation and Gas Phase Structures of Biomolecules

As part of an effort to further explore the utility of IMS in the identification of organic compounds on other planetary bodies, as well as to better understand the origin of strong correlations among seemingly dissimilar compounds, reduced ion mobilities are measured for different classes of biomolecules drifting in N_2 and CO_2. To provide a detailed understanding for the shapes of complex organic molecular ions, theoretical models to predict ion mobilities and related cross sections of gas phase molecular ions are necessary. Chapters 6 and 7 discuss ion-neutral interactions in order to gain insight into the structural details of molecular ions applying a 12-4 potential model. The reduced ion mobilities of amino acid cations and carboxylate anions are determined in both N_2 and CO_2 drift gases. A 12-4 potential model of the ion-neutral interaction is applied to data sets of mobilities of amino acid cations and carboxylate anions.

In Chapter 6, experimentally determined reduced ion mobilities of protonated 14 abiotic amino acid cations in both N_2 and CO_2 drift gases are reported. Observed

differences in IMS separability as a function of drift gas species are discussed. Mobilities in N_2 are considerably larger than their CO_2 counterparts. A strong correlation between the ion masses and their mobilities are discussed from the fit with a 12-4 potential model for the ion-neutral interaction in N_2 and CO_2. Comparing the model results for amino acids in the two drift gases indicates that the polarizability of the neutral has a dominant effect on the ion-neutral interaction. Furthermore, the model indicates that the effective radius of the ion is essentially independent of the drift gas species, while the effective radius of the neutral is strongly influenced by its structural symmetry.

Chapter 7 discusses the high correlation between mass and mobility of deprotonated carboxylate anions in both N_2 and CO_2 drift gases. In addition to analysis using the fit to the data set with a 12-4 hard core potential model, computational modeling is used to search energetically favored conformations for carboxylate anions under the experimental condition in order to support the analysis. The carboxylate anions with multicarboxyl groups form hydrogen-bonded cyclic confirmations, which cause higher ion mobility values than those that form extended conformations.

In Chapter 8, experimentally observed high mass and mobility correlation among tertiary and quaternary ammonium cations in N_2 is studied using a classical ion-neutral collision model and a computational trajectory method. The conventional trajectory method, which was developed for He drift gas, is modified for N_2 drift gas introducing an additional geometrical factor from the linear shape of N_2 and the ion-quadrupole interaction. Theoretical analysis is carried out to investigate the contribution of each potential term, ion quadrupole, ion-induced dipole, and Van der Waals potential to the ion-neutral interaction. For the smaller molecular ions, the importance of long-range interaction is emphasized, while short-range interactions dominate the collision cross

sections of the larger molecular ions. The importance of the shape asymmetry of the ammonium cations in determining observed correlation between mass and mobility is discussed.

1.3. Conclusion

Non-covalent interactions direct selective dissociation pathways and novel chemical reactions of biomolecules in the gas phase. These properties provide a new method in proteomics for identifying post translational modification (i.e., disulfide linkage) in peptides using non-covalently bound metal complexes and distinctive chemistry in small clusters that bridge the gap between the solution and gas phases. In addition, the gas phase structures induced by non-covalent ionic hydrogen bonds provoke characteristic gas phase properties of ion classes, such as high correlation between masses and mobilities. In conclusion, investigation of non-covalent interactions may improve understanding and aid in the design of reactions in various biological systems.

1.4. References

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