

PREFACE

The introduction of biological molecules into the gas phase by matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) has led to a revolution in biological mass spectrometry. The analytical aspects are a success story. Molecular weights can be determined with a high precision, peptide sequencing is now done with great success, and even higher-order structures of peptides and proteins can be accessed using mass spectrometry. Exceptionally high sensitivity, high mass resolution, and inherent speed are the key factors that positioned mass spectrometry at the forefront of analytical techniques for identification and characterization of biomolecules.

This success is based largely on the principles of mass spectrometry that have been developed since the mid-1970s for small organic molecules. However, studies of biomolecules in the gas phase have also revealed a number of challenges associated with the flexibility and the size of these species. For example, it was difficult to achieve efficient fragmentation of large molecules using traditional mass spectrometric approaches. Understanding of fundamental limitations of the existing ion activation techniques resulted in development of novel analytical approaches for studying fragmentation of large molecules in the gas phase. Improved identification of biomolecules in real-world applications is facilitated by understanding of their fragmentation mechanisms and the effect of the primary and the secondary structure on the observed fragmentation patterns.

Because of the large size, conformational flexibility, and the ability of biomolecules to hold multiple charges, studies of biomolecular gas-phase ion chemistry have opened a number of new and exciting areas of research. Multiply charged biomolecules are excellent targets for studying ion-ion chemistry and processes following capture of low-energy electrons. Various approaches are being

developed to gain phenomenological understanding of the formation and fragmentation of hydrogen-rich radical cations, molecular radical cations, and radical anions of peptides and proteins. Development of new approaches for studying thermochemistry of gas-phase biomolecules and their dissociation energetics is at the forefront of the field. Vibrational spectroscopy of biomolecular ions is another area of research that is currently undergoing an explosive growth. In parallel, new high-resolution spectroscopic techniques have been successfully applied to larger systems, providing feedback to mass spectrometric studies. Reactivity of mass-selected biomolecules with solid targets has a potential for preparation of novel surfaces relevant for a variety of applications in biology and biotechnology.

In addition, there are several basic aspects related to the physics of the various problems that have remained unanswered. For example, the question of ergodicity and/or statistical versus nonstatistical behavior in the breakup of biomolecules has been raised in connection with several methods, including electron capture dissociation (ECD) or photodissociation. The old questions that were raised many years ago concerning organic molecules are again at the forefront—do gas phase biomolecules undergo intramolecular vibrational redistribution (IVR) prior to dissociation? Are all vibrational modes involved in IVR? Is there site selectivity and charge-directed reactivity? The mere fact that a large protein fragments on the short timescale of mass spectrometry, which is an absolute necessity in terms of analysis and sequencing, is somewhat surprising in view of our previous knowledge of dissociation of relatively small organic molecules in the gas phase and its description using statistical theories [Rice–Ramsperger–Kassel–Marcus/quasiequilibrium theory (RRKM/QET) and the like].

This book is a collection of reviews on fundamental aspects underlying mass spectrometry of biomolecules. The various selected topics have been arranged in three parts: (1) structures and dynamics of gas-phase biomolecules; (2) activation, dissociation, and reactivity; and (3) thermochemistry and energetics.

Fundamental mass spectrometry has always been strongly linked to a variety of gas-phase spectroscopic techniques, which provide unique insights on the structure and dynamics of ions and molecules in the gas phase. High-resolution UV and IR spectroscopy discussed in Chapter 1 allows study of the structure and dynamics of individual conformers of neutral biomolecules, exploring the effect of the solvent on the intrinsic properties of these molecules, and molecular recognition by examining the behavior of gas-phase clusters of biomolecules. Chapter 2 gives an example of high-resolution photodetachment photoelectron spectroscopy studies of electron transfer in iron–sulfur (Fe–S) clusters. In particular, this technique is used to explore the effect of solvents and protein environment on the electronic properties of the cubane-type [4Fe–4S] cluster—the most common agent for electron transfer and storage in metalloproteins.

Ion–molecule reactions and H/D (hydrogen/deuterium) exchange studies have traditionally been used in mass spectrometry for structure determinations. Chapter 3 gives an overview of the application of these techniques to studies of structures and conformations of gas-phase biomolecules. While spectroscopic techniques are

currently limited to relatively small systems, mass spectrometry has been used to investigate quaternary structures of large protein complexes. Experimental approaches utilized in such studies are summarized in Chapter 4. Protein structures and folding in the gas phase is discussed in Chapter 5. Understanding protein dynamics in the absence of solvent—the driving force and the timescale of protein folding in the gas phase—is important for separating the effect of solvent from the effect of the intrinsic properties of proteins on their dynamics in solution.

The dynamics of the intramolecular vibrational energy redistribution (IVR) in gas-phase biomolecules is discussed in Chapters 6 and 7. Classical trajectory simulations using semiempirical PM3 potential energy surfaces described in Chapter 6 are instrumental for understanding ultra fast dynamics following photoionization of biomolecules and the validity of statistical theories of dissociation of these large floppy molecules. Studies of gas-phase ion chemistry of peptides and proteins revealed a variety of very interesting phenomena, some of which (e.g., electron capture dissociation and photodissociation) were described as nonergodic processes that circumvent IVR. The pros and cons of IVR and ergodic behavior in biomolecules based on the available experimental findings are discussed in Chapter 7.

Gas-phase fragmentation of protonated peptides is an important prerequisite for peptide and protein identification using tandem mass spectrometry (MS/MS). Understanding mechanistic aspects of peptide fragmentation as a function of peptide sequence and conformation summarized in Chapter 8 plays a central role in the interpretation of MS/MS spectra and refining strategies for database searching. Most mass spectrometric studies utilize closed-shell biomolecules (protonated or cationized on metals) generated using soft ionization techniques. Formation and dissociation of peptide radical cations described in Chapter 9 is a new rapidly growing field in gas-phase ion chemistry of biomolecules. These ions are formed by gas-phase fragmentation of complexes of the corresponding neutral peptide with transition metals and various organic ligands.

Collisional activation and multiphoton excitation are conventionally used for identification of biomolecules in a variety of mass spectrometric applications. Current status of multiphoton excitation, spectroscopy, and photodissociation of gas-phase biomolecules is summarized in Chapter 10. Chapter 11 presents classical trajectory simulations of the energy transfer in collisions of ions with atomic neutrals and surfaces. The phenomena observed following ion-surface collisions and the instrumentation involved in such studies are presented in Chapter 12 with particular emphasis on soft landing of biological molecules on a variety of surfaces. Soft landing can be utilized for a very specific modification of surfaces using a beam of mass-selected ions of any size and composition or for separating and preparing biomolecules on substrates in pure form for subsequent analysis.

Another method of ion activation in biological mass spectrometry relies on capture of low-energy electrons by multiply charged ions. Electron capture dissociation (ECD), discussed in Chapter 13, opens up a variety of unique dissociation pathways and provides information on the structure of the ion that is complementary to collisional or multiphoton excitation. Chapter 14 presents the

fundamental principles of ion-ion chemistry of biomolecules. Ion-ion reactions provide a means of manipulating charge states of multiply charged peptides and proteins. Charge reduction by reactions of multiply charged biomolecules with singly charged ions of opposite polarity has developed as a powerful tool for structural elucidation of peptides and proteins.

Mass spectrometry has been widely utilized for thermochemical determinations. However, studying thermochemistry and dissociation energetics of peptides and proteins is challenging because most of the well-developed experimental approaches that have been successfully employed in the studies of small and medium-size ions are simply not applicable to the fragmentation of large molecules. Chapter 15 presents an overview of mass spectrometric approaches that have been utilized for thermochemical determinations of biomolecules and discusses the current status and limitations of these techniques, focusing on determination of proton affinities and alkali metal affinities of biomolecules. Chapter 16 describes the experimental approaches developed for studying the energetics and entropy effects in peptide and protein dissociation reactions.

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