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Determination of thermodynamic and kinetic properties of biomolecules by mass spectrometry

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Over the past two decades, mass spectrometry (MS) has transformed the life sciences. The advances in understanding biomolecule structure and function by MS is progressing at an accelerated pace. MS has also largely been applied to study thermodynamic and kinetic structure of biomolecules. Herein, we highlight the recent discussions about native mass spectrometry and studies about determining stable gas phase structures, hydrogen/deuterium exchange studies about reaction kinetics and determination of binding constants of biomolecules with their ligands.

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Introduction

Since the pioneering work of J.J. Thomson, MS has become a centerpiece technology for characterization of molecules and detailed structure elucidation. In our opinion, no other analytical tool has experienced such a tremendous growth in its uses. This has largely been catalyzed by the advent of soft ionization methods in MS, namely matrix-assisted laser desorption ionization (MALDI) [1] and electrospray ionization (ESI) [2]. In the present review, we focus on the use of soft ionization MS for determining thermodynamic and kinetic properties of biomolecules. The most important advances were realized with ESI-MS, and therefore we limit our discussion to ESI-MS based studies.

Biomolecules in the gas phase: can they retain their native structure?

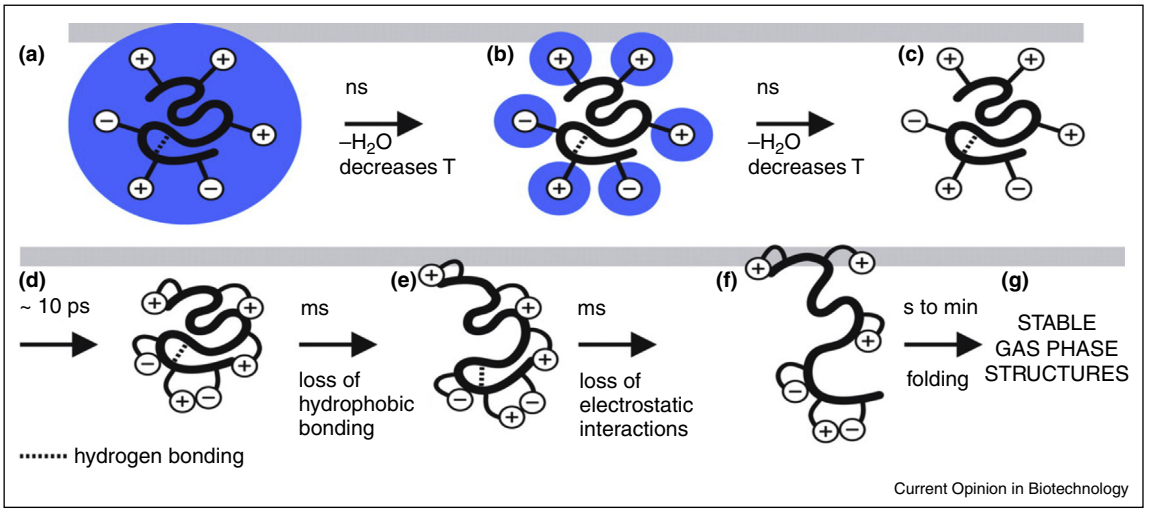
In a typical ESI experiment, an acidified organic solvent is employed, in which analytes are ionized by either proton or cation transfer. These conditions are not suitable to preserve the native structure of biomolecules in solution and lead to extensive unfolding, therefore referred to as 'denaturing' ESI. In another setting, solutions of biomolecules are sprayed from an aqueous volatile buffer whose pH is kept near to 7.4, mimicking physiological conditions. The neutral pH ensures the integrity of tertiary and quaternary structures. Mild instrumental conditions are also required, that is, low interface temperatures, low collision voltages, and optimized ion guide pressures to preserve biomolecule complexes [3–6]. Collectively, these non-denaturing conditions are referred to as 'native ESI-MS' [7]. The premise of native MS is that biomolecules can be kept intact, in their solution phase (SP) structure. However, the validity of this assumption is still debated and the crucial questions always raised are 'to what extent can MS provide information about SP structures?' and 'can the folded structures of a biomolecule be preserved in the gas phase (GP)?' [8••]. Although there has been great progress, current experimental evidence cannot answer these questions unambiguously. One of the hallmarks of native MS is that biomolecules have lower and narrower charge state distribution (CSD) compared to biomolecule ions generated from ESI using denaturing buffer systems. Folded, native-like molecules are more compact and therefore accommodate fewer charges, resulting in higher mass to charge ratios (m/z), as compared to unfolded ones which are detected at lower m/z ratios [9]. Hence, any change in the conformation of a biomolecule manifests itself with a clear change in the CSD in the mass spectrum. The fact that biomolecules have lower charge states in native MS is used as supporting evidence of the presence of native structures. However, can one really rely only on the CSD? Although it is generally valid that native structures produce lower CSD in the ESI spectrum, the answer to the above questions is 'not always' [10].

When a protein is dissolved in native buffer, it will be surrounded by water in the GP. According to the charge residue mechanism in ESI, surrounding water is lost by evaporation and fission in microseconds that leaves behind multiply charged, 'dry' protein ions [11,12]. Depending on the nature of the proteins, SP interactions undergo rearrangements in the GP. In the absence of water, hydrophobic contributions are lost. On the other hand, absence of the dielectric screening by water strengthens electrostatic interactions in the GP. Therefore, the secondary interactions re-equilibrate themselves and a biomolecule may assume a different structure that

does not originally exist in solution (Figure 1). This has been the subject of a recent controversy. Levis' group has suggested that ESI after femtosecond laser vaporization is softer than ESI alone [13]. They employed the CSD of cytochrome C to support their hypothesis. However, other studies disagreed with that argument [14]. It is therefore helpful to redefine the 'native structure' of the biomolecules to remove the confusion about 'conformation' and 'nativeness'. The native structure of a biomolecule is defined as the fully folded and biologically functional form of the molecule [15]. Combination of MS coupled to another, structure-sensitive or function-sensitive method could be useful to obtain direct evidence for the existence of a native structure in the GP. Lebrilla's group was the first to report evidence for enzymatic activity in GP complexes of lysozyme and oligosaccharides in the absence of solvent. However, the only 'activity' found was an endothermic proton transfer from a specifically bound substrate oligosaccharides in collision-induced dissociation (CID) experiments, which hardly qualifies as an enzymatic conversion [16]. Successful passage of virus particles [17] through the high vacuum of a quadropole mass spectrometer, following their ionization by ESI was reported. Collected virus particles were viable after all the ionization and ion transfer steps, which was shown by their ability to infect tobacco plants. In a recent investigation [18••] fluorescent properties of green fluorescent protein (GFP) were studied in the GP, a case where structure is directly linked to biological function. Although a narrow CSD and a collision cross section very close to that expected for correctly folded GFP, and a large stability against dissociation all support a near-native GP structure, no fluorescence emission was observed for gas-phase GFP. The lack of fluorescence in the GP was attributed to the loss of structural water. This study presents a nice example where the CSD does not necessarily guarantee a native structure. The role of structural water has recently been studied by the Russell group, which has looked at stepwise evolution of substance P peptide ion. The results reveal that upon ionization by ESI, doubly charged ion $[SP + 2H]^{2+}$ and triply charged ion $[SP + 3H]^{3+}$ carry significant amounts of water clusters $[(H_2O)_n]$ at cryogenic temperatures, while each produce dehydrated conformers at higher temperatures [19]. However, no evidence for structural changes as a function of the variable extent of hydration was observed indicating that the population of entirely dehydrated conformers resembles that of the hydrated species. In a similar fashion, Rizzo's group has studied gramicidin S peptide [20]. In their experiments, they first formed GP ions, cooled them cryogenically and then probed the structures by optical double resonance spectroscopy, revealing vibrational fingerprints of different conformers. Their data indicate that the first two water molecules induce a substantial change of the gramicidin-S structure by breaking two intra-molecular non-covalent bonds. The peptide structure remains largely intact upon further solvation. Both these studies are very promising as they shed light onto processes during the ESI and support the notion that SP structures could be preserved during ESI. However, both of these reported cases involved small peptides in which changes in the local environment might not induce a discernable change in the final structure. Therefore, this topic still calls for new studies with larger proteins.

Another way to look at this problem is to use a fragmentation method that will be specific to surface-exposed regions. Native electron capture dissociation (ECD) is

Figure 1



Stepwise evolution after ESI of the structure of a globular protein.
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