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Gas-phase processes and measurements of macromolecular properties in solution: On the possibility of false positive and false negative signals of protein unfolding

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Abstract

Electrospray ionization mass spectrometry is increasingly applied to study protein behavior in solution, including characterization of their higher order structure, conformational dynamics and interactions with small ligands and other biopolymers. However, actual measurements of fundamental ionic parameters (mass and charge) take place in vacuum, and an array of gas phase processes occurring prior to protein ion detection and characterization may certainly affect them. While most previous studies were concerned primarily with the effect of gas phase processes on mass measurement (e.g., integrity of macromolecular complexes in the absence of solvent, non-specific interactions, etc.), the focus of our attention is the ionic charge. Charge state distributions of protein ions in ESI MS are often used to characterize large-scale dynamic processes in solution (such as protein unfolding). Formation of metastable protein aggregates either in the bulk of solution or in the electrosprayed droplets, their consequent transfer to the gas phase and asymmetric dissociation may give rise to a population of highly charged ions. Presence of such ions in ESI mass spectra usually indicates loss of native structure in solution. Therefore, studies of large-scale conformational dynamics in solution by monitoring protein ion charge state distributions should be carried out at low protein concentrations in order to minimize the occurrence of false positive signals of protein unfolding. The opposite phenomenon, absence of highly protonated ionic species in ESI spectra of unfolded proteins, does not occur even in the case of highly acidic proteins lacking a sufficient number of basic residues.

Keywords: Electrospray ionization; Charge state distribution; Non-covalent complex; Asymmetric dissociation; Proton affinity

1. Introduction

In the past decade electrospray ionization mass spectrometry (ESI MS) has become one of the most popular tools to study macromolecular behavior in solution [1,2]. Arguably, the most popular experimental strategy that utilizes ESI MS to probe protein structure and dynamics is hydrogen/deuterium exchange (HDX) [3,4], although various other methods are becoming increasingly reliant on ESI MS as a method of detection. These include chemical cross-linking, selective chemical labeling, radiolytic foot-printing and covalent trapping of intermediate states [2]. It is important to remember, however, that MS measurements are carried out in the absence of solvent, and such a dramatic change of the environment surely affects macromolecular properties in multiple (and often unexpected) ways [5]. Therefore, prior to applying any MS-based method to probing macromolecular properties in solution, the following questions must be addressed. Are there any processes occurring in the gas phase prior to MS detection that may influence the measured ionic parameters? If so, what effect will such interference have on the measurements of macromolecular properties in solution? Finally, what can be done in order to minimize these effects?

Even though all ions without exception suffer loss or alteration of at least some of their properties upon transition from solution to the gas phase, it does not necessarily invalidates the characterization of their behavior in solution using experimental methods that rely on MS detection. For example, studies of higher order protein structures by chemical cross-linking or selective chemical labeling can be carried out in most cases

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without any regard for protein behavior in the gas phase. This contrasts sharply with the studies of protein dynamics that employ a combination of HDX in solution and protein ion fragmentation in the gas phase [6], where intrinsic mobility of protons within solvent-free polypeptide ions may lead under certain conditions to hydrogen scrambling [7]. Another example highlighting the need for very careful planning of experiments and interpretation of their results is an ESI MS titration applied to measurements of formation constants of non-covalent complexes in solution [8]. What is clear now is the fact that in each case a good understanding of the physical principles governing behavior of macromolecular ions in the gas phase is needed in order to design optimal experimental schemes and avoid data misinterpretation.

Analysis of protein ion charge state distributions in ESI MS is another biophysical method that has been steadily gaining popularity as a technique allowing large scale conformational transitions in solution to be monitored [9,10]. The link between the degree of compactness of polypeptide chains in solution and the extent of multiple charging of corresponding ions in the gas phase was established over a decade ago [11] and this phenomenon was used extensively in the past as a means to monitor protein folding and unfolding events in solution. Loss of native structure (either complete or partial) is almost always easily detectable in ESI MS, as it manifests itself by the appearance of bimodal charge state distributions with clearly distinguishable contributions from both low and high charge density protein ions (Fig. 1). The former correspond to folded (compact) protein molecules, which can accumulate only few charges upon their transition from solution to gas phase due to limited solvent-exposed surface area (SASA). Loss of structure (which in the case of proteins is synonymous with loss of compactness and, therefore, dramatic increase of SASA) allows a significantly higher number of charges to be accumulated. Therefore, changing solvent conditions from near-native to denaturing almost always has a profound effect on the appearance of the ESI mass spectra of proteins, and the appearance of bimodal charge state distributions in ESI mass spectra almost always signals the emergence of non-native state(s) in solution.

In addition to indicating the presence of non-native states in solution, the evolving shape of the high charge (low *m*/*z*) end of the protein ion charge state distribution often hints at transitions among various non-native states. Indeed, not only does a progressive departure from the native conditions lead to continuous elevation of the total abundance of highly charged ions, but also a prominent shift in the intensity distribution can be seen in the low-*m*/*z* part of the spectrum (Fig. 1). Such a shift (towards protein ions carrying more charges and, therefore, representing conformations with less residual structure) clearly indicates that highly unstructured conformers become more favored species in solution as the denaturant concentration becomes sufficiently high. Since the contributions from various non-native states almost always overlap [10], their distinction requires that the ESI MS data be processed using chemometric methods, such as factor analysis [12]. Briefly, a large array of ESI MS data is acquired over a wide range of solution conditions

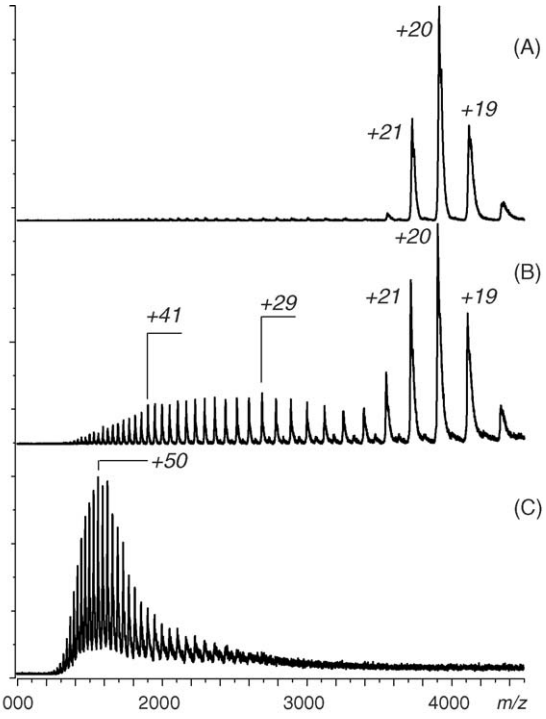

Fig. 1. ESI mass spectra of an 80 kDa protein transferrin acquired on a magnetic sector instrument under near-native (10 mM ammonium acetate, pH 7.0, panel (A)), mildly denaturing (10 mM ammonium acetate, pH adjusted to 5.0, panel (B)) and strongly denaturing (water/methanol/acetic acid, 47:50:3, v:v:v, panel (C)) conditions. Emergence of non-native (partially unfolded) states is evident in (B) as the charge state distribution becomes bimodal. Further unfolding of the protein (population of significantly less compact states) is manifested in (C) by a dramatic increase of the abundance of highly charged protein ions.

to ensure adequate sampling of the protein conformational space. Singular value decomposition analysis of this array yields a number of independent components, which (apart from the noise) are responsible for the observed variations of the charge state distributions. This number is equal to a number of protein conformers whose geometries are different enough to allow at least some distinction to be made as far as their individual contributions to the overall charge state distributions. A set of basis functions is then constructed, each representing a "pure signal" of a certain conformer and the entire data array is fitted using this set, yielding ionic profiles of individual protein states over the range of solution conditions [12]. At least in the case of the natively folded proteins and their complexes, average charge accumulated by ESI-generated ions can be used to estimate their SASA [13]. It remains to be seen whether similar estimates of SASA of non-native (partially unfolded) states can be carried out using the extent of multiple charging of protein ions corresponding to non-native conformations.

As is the case with many other MS-based techniques aiming at characterization of macromolecular properties and behavior in solution, the analysis of protein ion charge state distributions in ESI MS can be also affected by various processes occurring in the gas phase. One has to be mindful of the fact that protein compactness is not the only factor that determines the appearance of charge state distributions in ESI MS. Sometimes changes in solvent composition may trigger certain gas phase processes,