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Gas-phase protein salt bridge stabilities from collisional activation and electron transfer dissociation

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ABSTRACT

The gas phase structures of several proteins have been studied by electron transfer dissociation (ETD) with and without prior collisional heating after electrospraying these proteins from native-like solutions into a quadrupole ion trap mass spectrometer. Without prior collisional heating, we find that ETD fragmentation is mostly limited to regions of the protein that are not spanned by the salt bridges known to form in solution. When protein ions are collisionally heated before ETD, new product ions are observed, and in almost all cases, these new ions arise from protein regions that are spanned by the salt bridges. Together these results confirm the existence of salt bridges in protein ions and demonstrate that a sufficient amount energy is required to disrupt these salt bridges in the gas phase. More interestingly, we also show that different salt bridges require different collisional activation voltages to be disrupted, suggesting that they have variable stabilities in the gas phase. These stabilities appear to be influenced by the gas-phase basicities of the involved residues and the presence of nearby charged residues. We also find that higher collisional activation voltages are needed to enable the formation of new product from sites spanned by multiple salt bridges.

1. Introduction

Electrospraying protein solutions under native-like conditions can allow protein ions to maintain some features of their solution phase structure in the gas phase. Several methods and protein systems have been explored to provide evidence for this idea. An early example was the work by Chait and co-workers that demonstrated different charge-state distributions for myoglobin ions when they were electrosprayed from native-like or denaturing solutions [1]. Numerous subsequent studies have confirmed that proteins that are more folded in solution give rise to spectra dominated by lower charge states, while unfolded proteins in solutions result in higher charge states [2–4]. More recently, ion mobility coupled with mass spectrometry has been used to understand the relationship between protein gas phase and solution phase structures [5–7]. For instance, Loo and co-workers noted that the 20S proteasome maintains a gas phase structure whose diameter is similar to the value calculated from its crystallographic structure [8].

In contrast to charge-state distributions and ion mobility, which are somewhat passive indicators of gas-phase structure, gas-phase dissociation methods have also been used to provide insight into protein ion structure. Electron-based dissociation methods have been the most useful in this regard because they tend to break covalent bonds while maintaining non-covalent interactions. Loo and co-workers found that electron capture dissociation (ECD) was able to localize protein-ligand binding sites, suggesting that non-covalent interactions are maintained in gas phase [9]. Gross and co-workers showed the existence of non-covalent interactions of hemoglobin ions in the gas phase by using ECD combined with collision induced unfolding (CIU) and ion mobility measurements [10]. Activated-ion ETD was similarly used to study the structures of human hemoglobin and other protein complexes [11,12]. Breuker and co-workers have investigated gas-phase protein structure and unfolding using ECD [13–17]. Our group recently used electron transfer dissociation together with collision-induced dissociation (CID) to support the idea that protein ions can maintain known solution-phase salt bridges in the gas phase [18]. In that work, product ions from ETD of protein ions were found to arise only from regions not spanned by salt bridges. CID of undissociated charge-reduced ions confirmed that salt bridges were intact as CID disrupted the noncovalent salt bridges to generate new product ions in the regions spanned by these interactions.

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The possibility that electrostatic interactions, such as salt bridges, can be maintained in the gas phase is a not obvious because barriers for proton transfer between a protonated base and a deprotonated acid are low in the gas phase [19], even though the low dielectric in the gas-phase strengthens electrostatic interactions. Several studies, however, have provided evidence that salt bridges can exist in the gas phase. For example, using blackbody infrared radiative dissociation, Williams and co-workers showed bradykinin and its methylester have different activation energies, suggesting the presence of a salt bridge in one case but not the other [20]. In addition, the same group argued that peptides with more than one basic residue gave rise to more stable salt bridge structures, indicating that salt bridges can be stabilized by a nearby charge or dipole. Breuker and co-workers have also shown the importance of electrostatic interactions for stabilizing the native three-helix bundle of the KIX protein in the gas phase [13,14]. In other work, this group also demonstrated that salt bridges could hinder ubiquitin from unfolding in the gas phase, thereby influencing its dissociation during ECD [14].

In the current work, we set out to further investigate proteins' ability to retain their solution-phase electrostatic interactions and study the extent to which these interactions can be perturbed in the gas phase. In our previous work, we found that salt bridges known to form in solution cause ETD product ions of native-like protein ions to be observed mainly in regions that are not spanned by salt bridges [18]. Based on these previous observations, we hypothesized that native-like salt bridges that persist in the gas-phase could be disrupted by collisional heating/unfolding and subsequent ETD would then release new product ions from regions spanned by the residues involved in these salt bridges. In this work, we test this hypothesis using three model proteins and find that the salt bridges can be disrupted by collisional heating/unfolding, generating the predicted new product ions. Moreover, we find that different salt bridges require different levels of collisional activation to be disrupted, suggesting that they have different gas-phase stabilities.

2. Experimental

2.1. Materials

Ubiquitin (8.6 kDa) from bovine erythrocytes and azurin (13.9 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trp-cage (2.2 kDa) was purchased from AnaSpec, Inc. (Fremont, CA, USA). Ammonium acetate, water, and acetic acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Protein solutions for ESI–MS analysis were prepared by diluting stock solutions in deionized water to a final concentration of 5 μM in 5 mM ammonium acetate. The pH of the solution was then adjusted with acetic acid to the level used to obtain the NMR or X-ray crystal structures of the protein of interest.

2.2. Instrumentation and ETD and CA/ETD experiments

All mass spectral measurements were carried out on a Bruker AmaZon (Billerica, MA, USA) quadrupole ion trap mass spectrometer equipped with an electrospray ionization source. Typically, the needle voltage was kept at around 4.0 kV, and the capillary temperature was set to 100 °C. In an MS/MS experiment, ETD reagent anions from fluoranthene were generated in a chemical ionization source and injected into the ion trap to react with the stored ions for 100–130 ms. In collisional activation (CA)/ETD experiments, protein ions were collisionally heated before ETD. Collisional heating/unfolding was accomplished by varying the CID voltage by steps of 0.02 or 0.03 V from 0 V to voltages at which 10–15% of the protein ions started to dissociate. For all ETD and CA/ETD experiments, the ions were isolated using a very wide isolation width (~10 Da) to minimize any isolation induced collisional activation.

In evaluating the resulting ETD and CA/ETD spectra, only ions with abundances at least three times greater than the noise were chosen. Ion assignments were made using a mass tolerance of ± 0.1 m/z unit. In addition, because a wide isolation width was used, only product ions containing the predicted isotopic distribution were considered as properly identified. In some cases, the charge states and, thus, identities of more highly charged product ions were confirmed using proton transfer reactions [21].

2.3. Activation energy onset determination

During the CA/ETD experiments, the onset voltages for newly formed product ions were determined by increasing the collision voltage in steps of 0.02 or 0.03 V and finding the minimum voltage at which the new product ion was consistently measured. A new product ion was considered consistently measured when its signal-to-noise ratio exceeded 3 and when its normalized ion abundance was at least three times greater than its abundance at a voltage that was 0.02 or 0.03 V lower. Three replicates of each voltage setting were acquired, and typically 400 spectra were averaged at each voltage setting. The onset voltages were determined for each replicate and then averaged. To account for signal fluctuations over time, the ion abundances of the newly formed product ions were normalized to the most abundant product ion in the spectrum. In almost all cases, the most abundant product was the same ion regardless of collisional activation voltage used.

2.4. Protein structures

The structures of each studied protein, including salt bridge interactions and secondary structural elements, were obtained from either NMR or X-ray crystal structures present in the Protein Data Bank (PDB). The PDB files that were used for the studied proteins were 1AZU for azurin, 2JZZ for ubiquitin, and 1L2Y for Trp-cage. A constraint of 4 Å between the positive and negative charge centers on side chains was used to identify salt bridges. The identified salt bridge patterns were also verified using the appropriate tools from Proteopedia (http://www.Proteopedia.org/).

3. Results and discussion

3.1. Azurin

The first protein that we investigated was azurin, which we had previously shown maintained its native pattern of salt bridges in the gas phase [18]. Upon electrospraying azurin from a native-like solution, the 8+ and 7+ charge states of the protein were observed. As we demonstrated previously, the ETD spectrum of the protein ions (Fig. 1a) shows backbone dissociations mainly from regions that are not spanned by salt bridges (Fig. 1c). Based on the PDB structure of azurin, salt bridges exist in the solution phase between K24 and E104, D62 and R79, D62 and K74, and D77 and R79. In the ETD spectrum, the most abundant product ions are a series of z ions from z_3^{+} to z_{24}^{2+} corresponding to a protein region that is not spanned by salt bridges, suggesting that the salt bridges in the rest of the protein are preventing ion dissociation. Some ions (e.g. z_{25}^{2+}, z_{32}^{2+}, and z_{43}^{3+}) with low abundance are observed in a region spanned by the salt bridge between K24 and E104, suggesting that the salt bridge may not exist in a small fraction of the azurin ions in the gas phase, but there is no product ion resulting from the disruption of any of the other salt bridges. Interestingly, if the K24-E104 salt bridge is missing in some protein ions, it is somewhat surprising that no product ions are observed