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Effects of Metal Ion Adduction on the Gas-Phase Conformations of Protein Ions

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Abstract

Changes in protein ion conformation as a result of nonspecific adduction of metal ions to the protein during electrospray ionization (ESI) from aqueous solutions were investigated using traveling wave ion mobility spectrometry (TWIMS). For all proteins examined, protein cations (and in most cases anions) with nonspecific metal ion adducts are more compact than the fully protonated (or deprotonated) ions with the same charge state. Compaction of protein cations upon nonspecific metal ion binding is most significant for intermediate charge state ions, and there is a greater reduction in collisional cross section with increasing number of metal ion adducts and increasing ion valency, consistent with an electrostatic interaction between the ions and the protein. Protein cations with the greatest number of adducted metal ions are no more compact than the lowest protonated ions formed from aqueous solutions. These results show that smaller collisional cross sections for metal-attached protein ions are not a good indicator of a specific metal-protein interaction in solution, because nonspecific metal ion adduction also results in smaller gaseous protein cation cross sections. In contrast, the collisional cross section of α -lactalbumin, which specifically binds one Ca^{2+} , is larger for the *holo*-form compared to the *apo*-form, in agreement with solution-phase measurements. Because compaction of protein cations occurs when metal ion adduction is nonspecific, elongation of a protein cation may be a more reliable indicator that a specific metal ion-protein interaction occurs in solution.

Introduction

Metal ion-biomolecule interactions are important in many different biological processes, including storage and transfer of electrons and catalytic activity, and these interactions can also stabilize biologically relevant folded and misfolded conformations of biomolecules [1-3]. It is estimated that one quarter to one third of all proteins require metal ions to function properly [4,5]. One of the most well known examples is hemoglobin, which has a central iron ion in the heme group that is necessary for oxygen transport from the lungs to all other cells in the body [6]. EF-hand proteins, such as calmodulin, bind calcium ions in order to regulate a variety of functions, including inflammation, metabolism, skeletal- and smooth-muscle contraction, memory, and immune response [7,8]. Conformational changes in biomolecules that occur upon metal ion binding are typically investigated using X-ray crystallography [9-11] or nuclear magnetic resonance (NMR) [11,12]. However, mass spectrometry (MS) has emerged as a rapid and sensitive analytical tool to investigate protein conformation and conformational changes from shifts in the charge-state distribution [13,14], or in combination with hydrogen/deuterium (H/D) exchange [15,16], chemical labeling and crosslinking [17,18], or ion mobility spectrometry (IMS) [19-24].

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Through H/D exchange experiments, calmodulin was shown to adopt a structure with less solvent accessible surface area when the protein binds up to four Ca^{2+} adducts [13]. IMS measurements indicate two gas phase ion populations of calmodulin with the abundance of the compact conformer increasing relative to the elongated conformer with increasing numbers of Ca^{2+} adducts [20]. Metal cation adduction to heparin, a pharmaceutical drug that is an anticoagulant, also resulted in a smaller collisional cross section (ccs) of the octasaccharide anions compared to the ion with no metals bound, in agreement with solution-phase measurements [23].

The gas-phase structures of small peptides bound to metal ions have been studied extensively [19,21,22,25-32]. Coordination of a metal ion often results in peptide ions adopting more compact structures compared to the fully protonated form. Infrared multiphoton dissociation spectra of polyalanine complexed to a trivalent metal ion, $((\text{Ala})_n - \text{H} + \text{M})^{2+}$, indicate that these ions have a compact conformation or conformations in which all carbonyl oxygen atoms coordinate to the metal ion, and interaction of the N-terminus with the metal ion is also favored [26]. Divalent metal ion adduction to insulin chain A anions stabilizes more compact conformations compared to the non-metalated or sodiated form that has the same net charge [19]. However, polyalanine peptide-divalent metal ion complexes can have more elongated conformations compared to the fully protonated form, which has been attributed to the peptide adopting a helical conformation that is stabilized by coordination of the metal ion to the C-terminus [21,22].

Although the presence of salts in solution can be essential to observe some macromolecular complexes in the gas phase [33-35], there are relatively few studies about the effects of nonspecific ion-protein interactions on the structures of larger gaseous protein ions [36,37]. Nonspecific anion adduction to macromolecular complexes has recently been shown to preserve compact conformations of macromolecular complexes, and many of these anions can be readily removed by activating the ions without altering the conformation of these ions to an extent observable by traveling wave IMS (TWIMS) [36]. Recent experiments have shown that acid adduction to cytochrome *c*, ubiquitin, and α -lactalbumin ions with partially-folded conformers results in these molecules adopting more compact conformations [37].

Here, the effects of nonspecific metal ion adduction on the gas-phase conformations of various protein cations and anions are investigated using TWIMS-MS. These results show that nonspecific metal ion adduction to proteins typically results in ions having more compact conformations than the non-metallated forms with the same charge state, and that this effect is the greatest for intermediate protein charge states and for increasing metal ion charge. These results suggest that a compaction of the gas-phase ion upon attachment of metal ions may not be a reliable indicator of any conformational changes that occur in solution as a result of specific metal ion binding, whereas an increase in collisional cross section upon metal ion binding may be a more significant indicator of specific conformational changes that occur in solution.

Experimental

All experiments were performed using a Synapt G2 High Definition mass spectrometer (Waters, Milford, MA, USA). Ions are formed by nanoelectrospray (nanoESI) from borosilicate capillaries that are pulled to a tip i.d. of $\sim 2 \mu\text{m}$ with a Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA, USA). The capillary is loaded with ~ 2 -10 μL of analyte solution, and a platinum wire is inserted into the solution. Electrospray is initiated by positioning the borosilicate capillary ~ 2 mm from the source inlet and applying a potential of 1.0-1.5 kV to the wire. No backing pressure is applied to the

borosilicate capillary; the nanoESI solution flow rate is controlled by the applied voltage. Under these conditions, nanoESI flow rates were measured to be $\sim 30 \text{ nL} \cdot \text{min}^{-1}$. The sampling and extraction cone potentials were 40 V and 3 V, respectively.

The TWIMS mobility cell was operated with a constant wave velocity of 700 m/s, wave height of 40 V, helium flow rate of 180 mL/min, IMS (N_2) flow rate of 90 mL/min, and measurements were made with the TOF analyzer in “V” (single reflectron) mode. Drift time distributions were smoothed twice using a Savitzky Golay algorithm with a one unit window. Drift times were converted to a ccs scale using the procedure outlined by Robinson and coworkers [38,39] with ions formed from denaturing solutions of ubiquitin (9+ through 13+), cytochrome *c* (13+ through 18+), and myoglobin (16+ through 22+) and the ccs values obtained for these ions from static-field drift tube experiments using N_2 as the drift gas carrier [39]. There is a 6.2% and 7.5% difference between the ccs calculated using this calibration procedure and values obtained using a static drift tube for cytochrome *c* 6+ and α -lactoglobulin 7+ [39], respectively, indicating the extent of the uncertainty associated with this calibration method. The calculated ccs using the exact hard sphere (EHSS) and projection (Proj) methods for the native conformations of ubiquitin and cytochrome *c* were obtained by converting the calculated ccs determined by Clemmer and coworkers [40-42] using He as the drift gas carrier to that expected if N_2 was used instead. This conversion factor was obtained by plotting the ccs values of ubiquitin 9+ to 11+, cytochrome *c* 14+ to 18+, and *apo*-myoglobin 19+ to 22+ obtained in static field IMS experiments using N_2 as the drift gas carrier against those when He is the drift gas [39]. The data was fit to a linear equation with an R^2 value of 0.998. For ubiquitin, the Proj and EHSS values with He as the drift gas of 930 and $1162 \text{ \AA}^2$ [40] correspond to values of 1192 and 1492, respectively, with N_2 . Converting ccs values from He to N_2 can introduce an error of at least 1.4% [43].

All chemicals were purchased from Sigma Aldrich (St. Louis, MO) and were used without further purification except for α -lactalbumin, which was desalted using a Bio-Rad Micro Bio Spin 6 column (Bio Rad Labs, Hercules, CA, USA). Unless otherwise specified, solutions for analysis were prepared at a final concentration of 10 μM protein in denaturing solutions (48.5/48.5/3.0 methanol/water/acetic acid) and aqueous solutions with and without 1 mM of a chloride salt.

Results and Discussion

Nonspecific Metal Ion Adduction to Protein Cations

Under the conditions of these experiments, the 6+ charge state of ubiquitin is the most abundant ion produced by ESI from a 10 μM aqueous solution, and the average and maximum charge states are 5.6 and 7, respectively (Figure 1a). With 1.0 mM NaCl (Figure 1b), the average (5.0) and maximum (6) charge states are slightly lower, and adduction of multiple sodium ions is observed, with more Na^+ adducting to lower charge state ions, as has been previously reported [44,45]. Similar results were obtained from an aqueous solution containing KCl. In contrast, ESI of an aqueous solution of ubiquitin containing 1.0 mM CaCl_2 (Figure 1c) results in average (7.0) and maximum (8) charge states that are higher than that obtained from just water, and there is more extensive Ca^{2+} adduction to the higher charge state ions. For example, up to 20 Ca^{2+} attach to the 8+ charge state, whereas a maximum of four Ca^{2+} adduct to the 4+ charge state. Similar results were obtained from an aqueous solution containing MgCl_2 . ESI of an aqueous solution of ubiquitin containing 1.0 mM LaCl_3 (Figure 1d) also produces significantly higher average (7.5) and maximum (10) charge states than those obtained from pure water, and the charge enhancement is greater than that from the CaCl_2 solution. Greater La^{3+} adduction occurs for higher charge state ions, with up to seven La^{3+} attached to the 9+ charge state. Similar results were obtained with an aqueous solution containing EuCl_3 . The greater adduction of Ca^{2+} and La^{3+} to

higher charge states of ubiquitin suggests that the higher charge state ions are a result of multiple Ca^{2+} or La^{3+} ions adducting to folded ubiquitin during electrospray [46].

Effects of Nonspecific Cation Adduction on Protein Ion Conformation

Ion mobility experiments were performed to investigate the effects of nonspecific cation adduction on the resulting gaseous protein ion conformations. The drift profiles for ubiquitin $6+$, a charge state that is produced by ESI from each of these solutions, are shown in Figure 2. Features in the drift profiles are labeled with Roman numerals, with higher numbers corresponding to longer drift times and hence larger ccs values. The drift distribution for (ubiquitin + 6H) $^{6+}$ has three features, at $\sim 1560 \text{ \AA}^2$ (II), $\sim 1725 \text{ \AA}^2$ (III), and $\sim 1800 \text{ \AA}^2$ (IV). The drift profile for (ubiquitin + 6H) $^{6+}$ is similar to that reported previously with TWIMS-MS [37,47], but differs from results for this ion from static-field IMS experiments [40,41]. More elongated conformations are observed with TWIMS-MS, consistent with these ions being heated prior to or during the TWIMS separation [47]. The distributions for (ubiquitin + 6H) $^{6+}$ formed from solutions containing each salt are indistinguishable from that for this ion produced from a solution without any salt, indicating that these salts at millimolar concentrations do not affect the conformation of this ion to an extent that can be measured using TWIMS-MS.

In contrast, the drift profiles of ubiquitin $6+$ with adducted cations are significantly different than those obtained for (ubiquitin + 6H) $^{6+}$. Ubiquitin $6+$ with a single monovalent cation (M^+) has a greater abundance of conformer III and less of feature IV compared to the fully protonated ion. With three M^+ adducts, a new, more compact feature at $\sim 1424 \text{ \AA}^2$ (I) appears. Features I and II are more abundant with five M^+ adducts, and the abundance of III and IV decrease with increasing numbers of M^+ adducts. Feature I is the only remaining feature with 13 Na^+ adducts.

A greater decrease in the ccs of ubiquitin $6+$ occurs with attachment of just one divalent (M^{2+}) or trivalent (M^{3+}) metal ion compared to adduction of three M^+ . With one M^{2+} adduct, II is the most abundant feature (Figure 2, middle). Feature I increases and II decreases in abundance with increasing numbers of M^{2+} adducts. With one M^{3+} adduct, II is the most abundant feature, and I is the dominant feature with three M^{3+} adducts (Figure 2, right). The low-abundance distributions at ccs values lower than feature I in the profiles of ubiquitin $6+$ with seven Ca^{2+} adducts and three La^{3+} adducts are unresolved ions at the same m/z , including dimers of ubiquitin with twice the charge. There are only minor differences in the drift profile for cation adducts with the same charge state, i.e., Na^+ vs. K^+ ; Ca^{2+} vs. Mg^{2+} ; or La^{3+} vs. Eu^{3+} , indicating that the metal ion charge state, and not the identity, is the most important factor which affects the conformation of these nonspecifically adducted protein ions.

To simplify comparisons between ions with differing number of adducts and different protonation and charge states, an average ccs of ubiquitin $6+$ was determined by weighting each feature in the drift profile by peak area to evaluate the overall effect of metal cation adduction on the gas-phase conformation of this charge state, and these values are shown as a function of the number of metal ion adducts in Figure 3a. (ubiquitin + $3\text{M}^{3+} - 3\text{H}$) $^{6+}$ has an average ccs that is $\sim 19\%$ lower than (ubiquitin + 6H) $^{6+}$. A similar lowering in the average ccs occurs with seven M^{2+} and 14 M^+ adducts for this same $6+$ charge state. For a given numbers of adducts, the average ccs consistently follows the trend: $\text{M}^{3+} < \text{M}^{2+} < \text{M}^+$. The smaller average ccs with increasing charge of the metal ion is consistent with salt-bridge interactions between the metal ion and acidic sites in the protein as well as charge-solvation interactions between the metal ion and heteroatoms in the protein inducing the more compact conformers observed here. Attachment of HClO_4 , HI, or H_2SO_4 to multiply

protonated protein ions can also induce compact conformations as a result of ionic interactions between the acid molecule and basic sites in the protein [37].

The 4+ is the lowest charge state generated by ESI from these aqueous solutions, and (ubiquitin + 4H)⁴⁺ is a single compact conformer or family of conformers with a ccs of 1262 Å² (Figure 3a). This value is ~6% greater than the ccs previously measured for this ion (after conversion to N₂ as the drift gas) [40,41], and 6% greater than the calculated ccs of the crystal structure of native ubiquitin using the Proj method calibrated for nitrogen as the drift gas (~1192 Å²), which can be considered a lower limit to the actual ccs of native ubiquitin [40,41]. The ccs values of metal ion adducts to the 4+ charge state are within 2% of the value for (ubiquitin + 4H)⁴⁺, suggesting that this is a lower limit to the extent to which ubiquitin ions can be compacted.

For ubiquitin in the 6+ charge state, increasing metal ion adduction causes elongated conformers to become more compact. The maximally adducted ions for M⁺, M²⁺, and M³⁺ all adopt a single, compact conformation or family of conformers, with ccs values between 1398 to 1425 Å². These ccs values are at least 11% greater than that measured for (ubiquitin + 4H)⁴⁺ and are bracketed between the calculated ccs of the native crystal structure obtained using the Proj and EHSS methods. These results indicate that ionic interactions between the cations and the protein results in more compact conformations of ubiquitin 6+, but the levels of adduction observed here do not make the 6+ as compact as the 4+, the most compact charge state formed from the same solution.

Similar experiments were performed for other proteins, and results for cytochrome *c* are shown in Figure 3b. The 6+ ion is the lowest charge state generated by ESI from an aqueous solution containing this protein, and the calculated average ccs for this ion is between the calculated ccs obtained for the native crystal structure of cytochrome *c* calculated using the EHSS and Proj methods [42]. The average ccs decreases from 2159 Å² for (cytochrome *c* + 7H)⁷⁺ to 1805 Å² with three M³⁺ adducts, whereas 7 M²⁺ adducts are necessary to reach a similar ccs (1810 Å²) (drift profiles shown in Figure S1). A similarly compact structure is not formed with 13 M⁺ adducts, the greatest number of adducts observed for the 7+ charge state. The average ccs of maximally adducted cytochrome *c* 7+ approaches that of (cytochrome *c* + 6H)⁶⁺ and the calculated ccs(N₂) for the native protein structure, but does not reach this compact state, presumably owing to the higher charge, which results in greater Coulombic repulsion in the ion, and can contribute to greater ion heating in TWIMS [47]. As was the case for ubiquitin, the average ccs of the metallated protein ions of a single charge state with a given number of adducts is consistently smaller for metal ions with higher charge. Similar results were obtained for lysozyme 8+, -lactalbumin 7+, and *hemo*-myoglobin 9+ (drift profiles of these protein ion charge states with various extents of metal ion adduction and the fully protonated form are shown in Figure S2). These results indicate that nonspecific electrostatic interactions between the metal ion and protein result in more compact conformations of the metallated protein cations with ccs values that approach those of the most compact form of the protein in both the gas phase and in solution.

Effect of Nonspecific Metal Ion Adducts and Protein Charge State on Conformation

The average ccs for fully protonated ions and ions with two Na⁺, Ca²⁺, or La³⁺ adducts for all charge states of ubiquitin and cytochrome *c* that are formed from aqueous solutions with and without each respective chloride salt are shown in Figure 4. The average ccs values for fully protonated ions formed from a denaturing solution for these charge states are within 8% of previously reported values [39] and are also shown for comparison. For all charge states, the metallated protein ions are more compact than the fully protonated ion of the same charge generated from either a purely aqueous or denaturing solution. Ubiquitin 6+ and cytochrome *c* 9+ ions with two La³⁺ attached have the greatest difference in ccs

compared to the corresponding fully protonated form with the same charge state, with an approximately 14% and 16% smaller average ccs value, respectively. The average ccs values of the protein ions with multivalent ions adducted are at least ~5% smaller than the fully protonated ion for other charge states of ubiquitin (5+, 7+ to 9+) and cytochrome *c* (7+, 8+, 10+, and 11+). In contrast, there is little difference (< 2%) between the average ccs of the fully protonated and metallated ions for the lowest charge states for both ubiquitin (4+) and cytochrome *c* (5+ and 6+).

High charge states of ubiquitin (8+ to 10+) and cytochrome *c* (9+ to 11+) are formed by ESI from aqueous solutions that contain 1.0 mM CaCl_2 or LaCl_3 , and these charge states are not formed from the aqueous solutions without these salts. The 9+ and 10+ ubiquitin ions formed from the LaCl_3 solution have average ccs values that are at least 6% higher than the highest charge state (7+) formed from the purely aqueous solution, whereas ubiquitin 8+ with 2 La^{3+} or 2 Ca^{2+} adducts have smaller average ccs values than (ubiquitin + 7H)⁷⁺. The high charge state ions formed for cytochrome *c*, except for (cytochrome *c* + 3H + 3Ca)⁹⁺, also have more than a 5% larger ccs than the highest charge state, (cytochrome *c* + 8H)⁸⁺, formed from the water only solution. These results indicate that metal ion adduction to proteins during ESI can result in high charge state protein ions that can have more open conformations than the highest charge state formed from a purely aqueous solution, but all metallated ions are more compact than the fully protonated forms with the same charge generated from either aqueous or denaturing solutions. These high charge state metallated ions are advantageous because they dissociate more efficiently upon electron capture to provide significant sequence coverage even when these ions are formed from solutions in which the protein has a native structure [46].

Nonspecific Cation Adduction and Protein Anion Conformations

Unlike results for protein cations, the presence of multivalent cations in an aqueous solution does not alter the anion charge states generated by ESI for α -lactalbumin, cytochrome *c*, and ubiquitin (data not shown), even though substantial metal ion adduction occurs. To determine how cation adduction affects protein anion conformation, drift time distributions were measured for these anions with various numbers of metal ions adducted. For both α -lactalbumin and cytochrome *c*, the metallated protein ions are more compact compared to the conformation of the non-metallated deprotonated ion for a given charge state with even more compact conformations observed for higher charge metal ions. For example, the drift profile of (α -lactalbumin + Ca – 9H)⁷⁻ and profiles for this ion with various extents of nonspecific Na^+ , Ca^{2+} , and La^{3+} adducts are shown in Figure 5a. Drift times are not converted to collision cross sections because no calibrant ccs values for protein anions are available. There are two features for (α -lactalbumin + Ca – 9H)⁷⁻ with drift times of ~7.7 ms (I) and ~9.8 ms (II), and these profiles are identical for this ion formed from aqueous solutions both with and without NaCl, CaCl_2 or LaCl_3 . Feature I increases and II decreases in abundance with increasing numbers of metal ion adducts. For a given number of adducts, the relative abundance of feature I follows the trend $\text{La}^{3+} > \text{Ca}^{2+} > \text{Na}^+$. Similar results were also obtained for cation adduction to cytochrome *c* 6- (Figure S3). These results indicate that electrostatic interactions between metal ions and the protein can also stabilize compact conformers of gaseous protein anions. However, more elongated conformations were observed for metallation of the ubiquitin 5- compared to the non-metallated form (Figure 5b). There are two features in the drift profiles of ubiquitin 5- with drift times of ~7.5 ms (I) and ~9.2 ms (II). Adduction of just one La^{3+} ion results in feature II becoming the most abundant, and this feature also is the most abundant when the protein has adducted three Ca^{2+} or seven Na^+ ions. These results indicate that nonspecific electrostatic interactions between the metal ion and the protein can stabilize more open conformations of the protein anion as well. Therefore, IMS-MS of protein anions adducted to metal ions may not be a

good indicator of changes in protein conformation that occur upon specific metal-protein interactions in solution, because nonspecific binding of metal ions can cause either compaction or elongation of gaseous protein ions. Careful control experiments with similar ions that do not specifically bind to the protein in solution would be essential in order to draw conclusions about the effects of metal ion binding to protein conformations in solution based on the IMS-MS negative ion data.

Specific Cation-Protein Interactions

For each protein and metal ion investigated here, nonspecific attachment of one or more metal ions results in more abundant compact forms of the protein cation. This indicates that a decrease in the ccs value upon specific metal ion binding may not necessarily indicate specific conformational changes have occurred in solution. However, an increase in the ccs value may be a more reliable indicator that such a change has occurred. This is illustrated with γ -lactalbumin, which binds one Ca^{2+} specifically. An ESI mass spectrum of this protein from an aqueous solution containing $100\ \mu\text{M}$ CaCl_2 results in an abundant ion corresponding to $(\gamma\text{-lactalbumin} + 5\text{H} + \text{Ca})^{7+}$; only 2.4% of the total ion abundance of the $7+$ charge state is the Ca^{2+} -free form (Figure 6a). The drift profile of $(\gamma\text{-lactalbumin} + 7\text{H})^{7+}$ has an abundant feature with a ccs of $\sim 1698\ \text{\AA}^2$, and a low intensity feature at $\sim 1990\ \text{\AA}^2$. In striking contrast, the more elongated conformation at $\sim 1990\ \text{\AA}^2$ is the dominant feature for $(\gamma\text{-lactalbumin} + 5\text{H} + \text{Ca})^{7+}$, and the more compact conformation at $\sim 1698\ \text{\AA}^2$ is significantly less abundant. *Apo*- γ -lactalbumin exists in a molten globule state with a high degree of α -helical secondary content, and Ca^{2+} adduction to the molten globule form results in the folding and stabilization of the native structure of the protein [48,49]. The crystal structures of *holo*- and *apo*- γ -lactalbumin forms indicate that the molten globule form has a $\sim 5\%$ smaller accessible surface area than the Ca^{2+} -bound native form [49]. These results indicate that specific metal ion-protein interactions that result in an increase in the protein surface area in solution can result in a larger ccs in the gas phase. Because our results suggest that nonspecific metal ion adduction results in more compact gaseous cations, an increase in ccs values for protein ions that specifically bind a metal ion in solution may be a more reliable indicator of a specific conformational change that occurs in solution.

Conclusions

A reduction in the collisional cross section of a metal-bound gaseous protein ion upon specific binding of the metal to the protein has been used to infer a specific structural change to the protein has occurred in solution and that elements of this structure remain intact in the gas phase [18,21]. However, nonspecific metal ion binding to the proteins investigated here consistently results in a decrease in the collisional cross sections of the protein cations. For metal adducted protein anions, nonspecific attachment of metal ions to anions can result in more unfolded or more compact forms being preferentially stabilized. These results suggest that, in the absence of careful control experiments, a smaller cross section measured for metal adducted protein cations is not a reliable indicator of a conformational change that has occurred in solution as a result of specific metal-protein complexation because nonspecific adduction could also result in metal ion-adducted protein ions with smaller cross sections. In contrast, nonspecific binding of metal ions rarely leads to more open forms of gaseous protein cations, and therefore, an increase in collision cross section upon metal adduction may be a more reliable indicator of solution-phase conformational changes that occur upon specific metal ion binding in solution. For protein anions, nonspecific metal ion adduction does not consistently result in protein ion compaction or elongation. For IMS-MS data to be most useful for measuring conformational changes to proteins that occur upon specific metal ion binding in solution, careful control experiments with similar ions of the same charge

state that do not specifically bind to the protein in solution should be performed to determine how these nonspecific metal ion protein adducts affect the conformation of the gaseous ions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. ESI mass spectra of aqueous solutions containing 10 μ M ubiquitin in (a) pure water or aqueous solutions containing 1.0 mM (b) NaCl, (c) $CaCl_2$, or (d) $LaCl_3$.

Figure 3.

Average collision cross sections in N_2 ($\text{\AA}^2$) for (a) ubiquitin 6+ and (b) cytochrome *c* 7+ as a function of the number of Na^+ (●), K^+ (○), Ca^{2+} (■), Mg^{2+} (□), La^{3+} (▲), or Eu^{3+} (△) adducts. N(EHSS) and N(Proj) are ccs values calculated by Clemmer and co-workers^{40,42} for the native protein crystal structure using the projection method (Proj) and the exact hard-sphere scattering method (EHSS) for the native (N) conformations and converted for using N_2 as the drift gas. Plotted for comparison are values for (a) ubiquitin 4+ with increasing number of Ca^{2+} adducts, and (b) the cytochrome *c* 6+ with increasing number of Na^+ adducts.

Figure 4.

Average collision cross section in N_2 ($\text{\AA}^2$) for the fully protonated molecular ions and ions with two Na^+ , Ca^{2+} , or La^{3+} adducts for all charge states formed for (a) ubiquitin and (b) cytochrome *c* from aqueous solutions with and without the respective chloride salts. The average collision cross section of fully protonated molecular ions formed from denaturing solutions for these charge states are also shown for comparison.

Figure 5.

Drift profiles for fully deprotonated molecular ions and ions with various extents of metal ion adduction for (a) γ -lactalbumin 7- and (c) ubiquitin 5- formed from a purely aqueous solution (open squares) and aqueous solutions with 1.0 mM NaCl (solid line), $CaCl_2$ (dashed line), or $LaCl_3$ (solid squares).

Figure 6.

(a) Partial ESI positive ion mass spectrum from an aqueous solution containing 10 μM α -lactalbumin and 100 μM CaCl_2 ; (b) drift profiles in N_2 of α -lactalbumin 7⁺ with no adducts (bottom) and one Ca^{2+} adduct (top).