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Interpreting the Collision Cross Sections of Native-Like Protein Ions: Insights from Cation-to-Anion Proton-Transfer Reactions

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Abstract

The effects of charge state on structures of native-like cations of serum albumin, streptavidin, avidin, and alcohol dehydrogenase were probed using cation-to-anion proton-transfer reactions (CAPTR), ion mobility, mass spectrometry, and complementary energy-dependent experiments. The CAPTR products all have collision cross section (Ω) values that are within 5.5% of the original precursor cations. The first CAPTR event for each precursor yields products that have smaller Ω values and frequently exhibit the greatest magnitude of change in Ω resulting from a single CAPTR event. To investigate how the structures of the precursors affect the structures of the products, ions were activated as a function of energy prior to CAPTR. In each case, the Ω of the activated precursors increase with increasing energy, but the Ω of the CAPTR products are smaller than the activated precursors. To investigate the stabilities of the CAPTR products, the products were activated immediately prior to ion mobility. These results show that additional structures with smaller or larger Ω can be populated and that the structures and stabilities of these ions depend most strongly on the identity of the protein and the charge state of the product, rather than the charge state of the precursor or the number of CAPTR events. Together, these results indicate that the excess charges initially present on native-like ions have a modest, but sometimes statistically significant, effect on their Ω values. Therefore, potential contributions from charge state should be considered when using experimental Ω values to elucidate structures in solution.

TOC image

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Supporting Information. The Supporting Information is available free of charge on the ACS Publications website.

Figures S1 to S7, Tables S1 to S2, Converting Drift Times to Ω and Consideration of Error, Comparisons with Ω Values Reported for Other Charge-Reduced, Native-Like Ions

Introduction

Native-like ions are generated using electrospray ionization of proteins, nucleic acids, lipids, and other biological molecules in aqueous solutions with biologically relevant pH and ionic strength.^{1–3} These ions can retain noncovalent interactions in the gas phase that were present in the original solution.^{1,4} The stoichiometry and relative abundance of these noncovalent complexes can be probed using mass spectrometry (MS).¹ Advances in ion mobility (IM) have enabled accurate measurements of collision cross sections (Ω) of ions of proteins and their noncovalent complexes.^{5–7} As a result, there is increasing interest in using IM-MS measurements in structural biology and biophysics.^{8,9} For example, IM-MS measurements have contributed to the fields of amyloid^{10,11} and serpinopathy¹² formation, viral capsid assembly,^{13,14} small heat shock protein subunit dynamics,¹⁵ and ATPase function.¹⁶

The charge states of native-like ions appear to be independent of the magnitude and polarity of their charge states in solution, and instead appear to depend on the ionization mechanism.^{17,18} Therefore, it is important to consider the effects of charge state (z) in the interpretation of experimental Ω values for biological molecules in the gas phase. The Ω values of many native-like ions of larger proteins and protein complexes can depend weakly on z .^{5,18,19} In contrast, stronger relationships between Ω and z have been reported for native-like ions of smaller proteins,^{20,21} prion and intrinsically disordered proteins,^{22,23} and proteins generated from solutions with “supercharging” reagents,²⁴ as well as protein ions from denaturing solutions.^{25–27} Thus, it is unclear from IM-MS measurements alone the extent to which the z of a native-like ion affects its structure, and whether low- z ions have “more native” structures. As a result, several approaches to report Ω with respect to z have been implemented that each implicitly assume different relationships between z and the structure of a native-like ion, including reporting a Ω for each z measured,^{5,18} reporting an average Ω ,²⁸ and extrapolating to the Ω of a “zero-charge” protein.²⁹ Those approaches will lead to different Ω values, which in turn will affect the extent of agreement with candidate structures for those ions.

Several studies have investigated the relationship between Ω and z for native-like protein cations by reducing the magnitude of z . Modest charge reduction caused by addition of a base to the solution prior to ionization or reacting the protein cations with a neutral with a higher gas-phase basicity revealed that the Ω of the charge-reduced ions are similar to those produced under typical native MS conditions and depend weakly on z .^{18,30,31} In contrast, there have been reports of significant compaction for lower- z products of native-like ions based on traveling-wave IM and more extensive charge reduction enabled by ion/ion reactions.^{32,33} Charge reduction in those experiments was the result of competitive proton-transfer and electron-transfer reactions:

These competitive reaction products complicate data interpretation because the m/z difference between the products often not resolved, and Reaction 1 results in the formation of radical cations that may undergo additional chemistry and concomitant changes to the identity and/or structure of the product.³⁴

Recently, we used cation-to-anion proton-transfer reactions (CAPTR), which yields a long series of charge-reduced product ions originating from quadrupole-selected precursor ions,³⁵ to reduce the charge states of cytochrome c^{36} and lysozyme³⁷ cations generated from native-like, aqueous conditions. The CAPTR products were then analyzed using an rf-confining drift cell,¹⁹ which enables absolute measurements of mobilities that are indistinguishable from those determined using electrostatic drift tubes.^{5,19,38} The lowest- z CAPTR products had Ω values that were up to 10.6% and 8.4% smaller than those for the highest- z precursors of native-like cytochrome c^{36} and lysozyme,³⁷ respectively. For comparison, CAPTR of the highest- z ions generated from denaturing conditions results in the formation of low- z products with Ω values that are roughly twofold smaller than those of the precursors.^{26,36,37} The objective of this study is to use CAPTR, IM-MS, and complementary energy-dependent experiments to determine the effect of z on the Ω and stabilities of native-like ions of large proteins and protein complexes, which is important for using Ω to restrain potential structures of those ions in the context of structural biology.

Methods

Samples and Ionization

All proteins were purchased from Sigma Aldrich and dissolved into the buffer, which was aqueous 200 mM ammonium acetate at pH 7. For serum albumin, streptavidin, and alcohol dehydrogenase, Micro Bio-Spin 6 columns (Bio-Rad, Hercules, CA) were used to exchange the buffer and remove nonvolatile salts from the original solution. Final electrospray solutions contained 5 to 20 μ M protein. Nanoelectrospray sample capillaries were prepared and loaded as described previously.³⁹ The inlet to the mass spectrometer was heated to 120 °C; under these conditions there is some convective heat transfer to the sample capillary.^{26,36}

Cation-to-Anion Proton-Transfer Reactions

Experiments were performed using a modified Waters Synapt G2 HDMS equipped with a glow-discharge ionization source⁴⁰ for anion generation (Figure 1). Briefly, perfluoro-1,3-dimethylcyclohexane (PDCH)⁴¹ vapor was introduced into nitrogen gas that was passed through a sharpened glow-discharge ionization needle. Anions were generated for 0.1 s, during which time the fragment [PDCH-F]⁻ at m/z 381 was quadrupole selected and accumulated in the trap cell.³⁵ Following anion accumulation, the instrument was switched into positive ion mode for 2 to 10 s for protein cation transmission. Example relative potentials for cation transmission are shown in Figure 1. To initiate CAPTR, quadrupole-selected precursor ions were trapped with the stored anions. Both unreacted precursor and CAPTR product ions were then analyzed using IM-MS.

Ion Mobility

IM separations were performed using an rf-confining drift cell,¹⁹ which replaced the traveling-wave IM cell of the original instrument. The drift velocity of ions in this cell is the product of their mobility (K) and the applied drift field,^{19,38} similar to electrostatic drift cells. IM experiments were performed in 2 mbar of helium, unless otherwise noted. K values for the CAPTR product ions were determined from drift times measured using a drift voltage of 212 V that were corrected for the transport time to the time-of-flight mass analyzer. Values of K were then converted to Ω using the Mason-Schamp equation:⁴²

$$\Omega = \frac{3ez}{16N} \left(\frac{2\pi}{\mu k_B T} \right)^{1/2} \frac{1}{K} \quad (\text{Equation 1})$$

where e is the elementary charge, z is charge state, N is the neutral gas number density, μ is reduced mass, k_B is Boltzmann's constant, and T is temperature. The method used to correct the drift times, a discussion of the errors associated with the resulting Ω values, and comparisons with field-dependent methods (Figures S1 to S3) are included in the Supporting Information.

Pre- and Post-CAPTR Activation

Selected experiments were performed in which ions were activated as they enter the atmospheric-pressure interface of the instrument (pre-CAPTR activation) or the drift cell (post-CAPTR activation). The relative potentials in those experiments are indicated in Figure 1. For post-CAPTR activation, the drift cell was modified by adding an additional aperture to the front of the rf-confining drift cell,¹⁹ establishing a short pressurized region with independent gas control that is analogous to the "helium cell" of the traveling-wave IM cell of a Synapt G2 HDMS instrument.⁷ During post-CAPTR experiments, 15 mL min⁻¹ of argon gas was introduced into this region. The flow rate of helium to the drift cell was adjusted so that the total pressure of argon and helium was 2 mbar.

Ω Calculations

Ω for serum albumin (PDB: 4F5S),⁴³ streptavidin (PDB: 4Y5D),⁴⁴ avidin (PDB: 1AVD),⁴⁵ and alcohol dehydrogenase (PDB: 5ENV)⁴⁶ were calculated using the projection approximation⁴⁷ and exact hard-sphere scattering⁴⁸ methods as implemented in EHSS2/k.⁴⁹ Prior to the Ω calculations, all noncovalent additions to the protein(s) in the deposited structures, including water molecules and metal ions, were deleted and Chimera⁵⁰ was used to complete side chains and add hydrogen atoms.

Results and Discussion

Ω values determined using IM measurements of native-like ions are used to restrain models for proteins and protein complexes in solution. Therefore, an accurate understanding of the effects of charge state on the structures of gas-phase protein ions is important for interpreting the results of native IM-MS experiments in the context of structural biology and

biophysics. Here, the effects of z on the Ω values of native-like ions were investigated using CAPTR, IM-MS, and energy-dependent experiments. Serum albumin (67 kDa, monomer), streptavidin (53 kDa, homotetramer), avidin (64 kDa, homotetramer), and alcohol dehydrogenase (147 kDa, homotetramer) were selected based on their use as model systems^{51–53} and as calibration standards^{5,19} for native IM-MS. Streptavidin and avidin are homologs, but avidin is glycosylated, and as a consequence, has a larger mass.^{54,55}

IM-MS was used to characterize the CAPTR products originating from two to four of the most abundant charge states of serum albumin, streptavidin, avidin, and alcohol dehydrogenase. For example, Figure 2 shows representative data for serum albumin. First, native-like ions were generated using electrospray ionization (Figure 2a). From those ions, the 17+ cations were quadrupole selected (Figure 2b) and subjected to CAPTR (Figure 2c). In CAPTR, multiply charged protein cations are reacted with [PDCH-F][–] anions to yield a long series of charge-reduced products via Reaction 2. CAPTR products will be referred to as “ $P \rightarrow C$ ”, where “ P ” is the charge state of the precursor and “ C ” is the charge state of the CAPTR product. Following CAPTR, product ions and residual precursor ions were injected into an rf-confining drift cell^{19,38} and separated on the basis of their mobility in helium gas (Figure 2d). The arrival-time distributions were then used to determine apparent Ω distributions, as described in the *Methods* and Supporting Information.

The Ω values determined using this approach are shown in Figure 3. These values are bracketed by those calculated using the PA and EHSS methods, which are respectively used as the lower and upper bounds of the plot. This bracketing is consistent with previous reports that these methods typically underestimate and overestimate the Ω values for native-like ions, respectively.²⁰ Significantly, the CAPTR products do not exhibit evidence for large-scale structural collapse with decreasing z , as reported for the CAPTR products of high- z protein ions generated from denaturing conditions.^{26,36,37} Note that all experimental Ω values reported in this work include the 95% confidence intervals, based on three replicate measurements and t -statistics. Visual analysis of the data in Figure 3 suggests that after the first few CAPTR events for a given protein, the Ω values for ions of a given C originating from precursors with different P are similar and the differences are likely not significant. The general trends in the Ω values for each protein as a function of C appears to be general to all precursors of a given protein, which creates a larger sample size and increases the significance of those trends.

The Ω values of the serum albumin precursor ions are 43.0 ± 1.1 , 42.1 ± 1.0 , and 41.7 ± 1.5 nm² for the 17+, 16+, and 15+ ions, respectively (Figure 3a). These values are 3.3 to 4.4% larger than those reported previously,¹⁸ indicating that relative to previous experiments the ions in these experiments may have partially unfolded prior to CAPTR. Following the first CAPTR event, the Ω of the products are 2% to 3% smaller than those of the corresponding precursor ions. The Ω of the most compact CAPTR product ions are 3% to 5.5% smaller than their respective precursor ion. Interestingly, the Ω values for all $P \rightarrow 14$ to 6 ions vary by only $\pm 1.2\%$. Although all CAPTR product ions are more compact than the corresponding precursor ion, most of the change in Ω occurs during the first one to three CAPTR events.

For streptavidin, the Ω values of the precursor ions are 34.3 ± 0.5 and 34.2 ± 0.4 nm² for the 15+ and 14+ ions, respectively (Figure 3b). The $P \rightarrow 13$ ions are the most compact CAPTR products, but the Ω values of those products are less than 2.5% smaller than the original precursor ions. The $15 \rightarrow 5$ and $14 \rightarrow 5$ ions have Ω of 35.0 ± 0.3 and 34.7 ± 0.2 nm², respectively, which were the largest of all the CAPTR products for each precursor. For avidin, the Ω values of the 17+ to 15+ precursor ions are 0.9 to 1.8% smaller than those reported previously,¹⁸ consistent with these ions retaining their native-like conformations. The Ω of 17+ avidin was 35.6 ± 0.6 nm². The most-compact product was formed following two CAPTR events (34.9 ± 1.2 nm²), but the Ω of the products of the subsequent CAPTR events increase slightly until the value of 36.7 ± 0.7 nm² determined for the $17 \rightarrow 5$ ions. Analogous trends were observed for the $16 \rightarrow C$ and $15 \rightarrow C$ ions, and the Ω values for all of the CAPTR products of avidin are within 4% of the precursor ions. The relationship between Ω and z for avidin is similar to that observed for streptavidin, which suggests that the presence of glycans has a minor effect on how avidin ions respond to changes in z .

For alcohol dehydrogenase, the Ω values of the precursor ions are 70.4 ± 2.3 , 69.1 ± 0.5 , 69.3 ± 1.8 , and 68.0 ± 0.6 nm² for the 27+ to 24+ ions, respectively (Figure 3d). These values differ by 0.2%, -1.6%, -1.3% and -3.3%, respectively, relative to those reported previously.¹⁸ The value for the 24+ precursor is notably small, suggesting that these ions may be more compact than those investigated previously.¹⁸ Relative to each precursor, the Ω values of the products of the first two to three CAPTR products decrease and are up to 3.6% smaller than the respective precursors. In contrast, the Ω values of the subsequent CAPTR events increase and have values that are up to 1.3% larger than the precursors. The Ω values for $P \rightarrow 11$ ions, which was the lowest C observed, ranged from 69.4 ± 1.3 to 68.8 ± 1.5 nm², which correspond to a -1.4% to +1.3% change in Ω relative to the respective precursor. Therefore, the differences in the Ω of the precursor ions are larger than the differences for the lowest- C products observed.

The CAPTR products of native-like serum albumin, streptavidin, avidin, and alcohol dehydrogenase all have Ω values that are within 5.5% of their original precursors. The first CAPTR event for each precursor yields products that have smaller Ω values and frequently exhibit the greatest magnitude of change in Ω resulting from a single CAPTR event. The Ω values of the products of the subsequent CAPTR events exhibit either small decreases or increases in Ω . The Ω values of the CAPTR products, relative to Ω value of the precursor, span from -5.5% to -2.9% for serum albumin, -2.3% to 2.1% for streptavidin, -2.9% to 4.0% for avidin, and -3.6% to 1.3% for alcohol dehydrogenase. The maximum decreases in Ω with decreasing charge are smaller than the decreases reported based on CAPTR and rf-confining drift cell analysis of lower-mass (8.6 to 14 kDa) protein ions^{26,36,37} and reported based on other charge-reduction approaches using ion/ion chemistry and traveling-wave IM of alcohol dehydrogenase³³ and pyruvate kinase (230 kDa).³² It should also be noted that some of the native-like precursor ions in this study had slightly larger Ω values than reported previously,¹⁸ which may be attributable to destabilization of the precursor ions concomitant with heating in the sample capillary³⁶ or during transport through the elevated-temperature, atmospheric-pressure interface.⁵⁶ The destabilization of the precursor ions under these conditions is supported by arrival-time distributions of 27+ alcohol dehydrogenase measured as a function of the bias voltage between the first two ion optics in the atmospheric-pressure

interface at ambient temperature and at 120 °C; ions under the latter conditions unfold at lower voltages (Figure S5). Therefore, it is possible that the small decreases observed for the initial CAPTR events are consistent with the refolding of partially disrupted structures. To investigate how the structures of the precursors affect the structures of the CAPTR products, we performed additional energy-dependent experiments that will be discussed in *Pre-CAPTR Activation*.

The origin of the small increase in Ω with decreasing z for some ions is not obvious. One possibility is that there is a systemic error in our measurements. Possible biases based on the strength of the electric field in the IM cell are discussed in the Supporting Information. In order to explain the subtle increase in Ω for very low charge states of cytochrome *c* formed by ion/ion proton-transfer reactions, Badman and co-workers proposed that the loss of protons disrupts some hydrogen bonds, resulting in structures that are more flexible and less compact.⁵⁷ The loss of some stabilizing interactions after charge reduction is also consistent with the enhanced ultraviolet photodissociation observed for the ion/ion proton transfer products of native-like ions, which the authors attribute to fraying in the terminal regions of the charge-reduced products relative to the native-like precursors.⁵⁸ Additionally, each CAPTR event is exothermic and the internal energy of CAPTR products may increase over the course of several reactions. For comparison, we estimated that CAPTR events are exothermic by at least 200 kJ/mol per reaction.³⁵ To investigate how activation of the products affect their structures, we performed additional energy-dependent experiments that will be discussed in *Post-CAPTR Activation*.

More generally, the results of these CAPTR experiments indicate that the excess charges on native-like ions with large masses have a relatively small (less than 5.5%) effect on their Ω . This finding is consistent with the conclusions drawn from modest charge-reduction using solution additives^{18,30} and ion/neutral reactions.³⁰ In contrast, two studies using traveling-wave IM to analyze ions with comparable extents of charge reduction reported significant compaction with decreasing z .^{32,33} Differences in the apparent effects of charge reduction may have contributions from differences in the charge reduction and/or ion mobility methods used in these studies. Results from previous charge-reduction experiments^{18,30,32,33} are compared further in Figure S4, Table S2, and the associated discussion in the Supporting Information.

Pre-CAPTR Activation

To probe the effects of the structures of the precursors on the structures of the CAPTR products, native-like ions were analyzed as a function of the bias voltage between the first two ion optics in the atmospheric-pressure interface. Increasing the bias voltage will increase the extent of collisional activation in the interface. These ions will be referred to as “ $P^* \rightarrow C$ ”, where “ P ” is the charge state of the ion that was isolated after activation and then subjected to CAPTR. Note that in our previous pre-CAPTR activation experiments, ions were activated as a function of their injection energy into the helium-filled trap cell where CAPTR is performed.²⁶ For these larger ions with more degrees of freedom, collisions with helium at the kinetic energies accessible on this instrument were inadequate to induce

changes in Ω . One disadvantage of the current approach is that activation, which can also result in loss of charge,⁵² is performed prior to quadrupole selection.

Figure 4a shows the Ω distributions of 15+ serum albumin as a function of the activation voltage. With increasing energy, the distribution increases monotonically to values centered near 50 nm². Figures 4b–d show the Ω distributions of the 15*→9, 15*→7, and 15*→6 ions as a function of the activation voltage. With increasing activation voltage, the Ω distributions indicate that the CAPTR products of the activated precursor ions are more compact than the activated precursor ions and similar in Ω to the minimally activated precursor ions. The pre-CAPTR activation results for the 15*→C ions of streptavidin, 17*→C ions of avidin, and 27*→C ions of alcohol dehydrogenase are shown in Figure S6. In each case, the Ω distributions of the activated precursors increase with increasing energy, but the Ω distributions indicate that the CAPTR products are significantly more compact than the activated precursors. Interestingly, there is no evidence for the formation of CAPTR products with Ω distributions that are centered at smaller Ω values than those of the minimally-activated precursor ions.

In most cases with adequate energy and time, slow heating of a protein ion (including activation under multiple collision conditions) results in the loss of a monomeric subunit that retains a disproportionately large fraction of the total charge relative to the masses of the two products.^{59,60} This asymmetric charge partitioning has been attributed to a dissociation mechanism in which the unfolding of a single subunit and charge migration to that subunit work in concert to reduce the Coulombic energy of the complex prior to dissociation.^{59,61,62} This model suggests that the increase in Ω for the activated precursors is the result of unfolding of a single protein.⁶³ Because the unfolded region will have a disproportionately large fraction of the total charge, this region may be the origin of a disproportionately large fraction of the protons transferred during these experiments. The results from these pre-CAPTR activation experiments therefore suggests that the CAPTR products fold to more compact structures that enable additional noncovalent interactions, consistent with their lower charge states and reduced Coulombic energy. This finding may have implications for the interpretation of results from surface-induced dissociation of native-like ions of protein complexes, which generally yields products with lower charge states and Ω than collisional activation.⁶⁴ For example, if unfolded intermediates are formed during surface-induced dissociation, they may refold as a consequence of their low z . The folding in these pre-CAPTR activation experiments appears to be analogous to that reported for the CAPTR products of unfolded ubiquitin,²⁶ cytochrome *c*,³⁶ and lysozyme³⁷ ions generated from denaturing conditions. In all these cases, the Ω of the lowest-*C* products are consistent with folding to structures similar in size to the corresponding native-like ions. However, beyond Ω , the degree to which refolded CAPTR product ions and native-like ions share similar structures remains uncertain.

Post-CAPTR Activation

To investigate the stabilities of CAPTR products, the products were activated as a function of their kinetic energy prior to IM. These ions will be referred to as “*P*→*C**”, where “*C*” is the charge state of the CAPTR product that was activated prior to IM. Note that in our

previous post-CAPTR activation experiments, ions were activated as a function of their injection energy into a helium-filled drift cell.^{26,37} As per the pre-CAPTR experiments, collisions with helium were inadequate to induce changes in Ω . Instead, ions were injected as a function of their kinetic energy into a small cell positioned at the entrance to the drift cell (see *Methods*) that was filled with argon to enable more efficient conversion from translational to internal degrees of freedom. One disadvantage of this approach is that the drift cell contains a mixture of helium and argon gases and has a net flow of gas along the longitudinal axis. Therefore, the IM results will be reported in terms of drift time instead of Ω .

Figure 5a shows the arrival-time distributions for 15 $\rightarrow$ 13* serum albumin as a function of the laboratory frame energy, which is the product of C and the voltage used to inject the ions into the argon-filled cell. At the lowest energy, the distribution is centered near 7.4 ms and unimodal, which is consistent with the peak shape for experiments performed without the additional cell. With increasing energies to ~ 780 eV, the distributions shift to smaller values centered near 7.2 ms that are consistent with the formation of more compact structures. Then with increasing energies to ~ 1500 eV, the distributions shift to larger values centered near 8.9 ms. That distribution persists with increasing energy, consistent with the formation of a quasi-equilibrium of structures in these experiments.⁶⁵ The results of the 16 $\rightarrow$ 13* ions are shown in Figure S7a and are nearly identical to those for the 15 $\rightarrow$ 13* ions.

Results for 16 $\rightarrow$ 11* avidin are shown in Figures 5b. At low energies, the arrival-time distributions are unimodal and centered near 7.3 ms. With energies of at least 750 eV, an additional feature near appears 8.3 ms. The relative intensity of the larger Ω feature increases with energy until both features exhibit similar intensities at the highest energies. These results are qualitatively similar those obtained from collision-induced unfolding of 11+ avidin that was generated from solution containing triethylammonium, which also yielded a bimodal arrival-time distribution at the highest energies.⁶⁶ The results for the 17 $\rightarrow$ 11* ions (Figure S7b) are nearly identical to those for the 16 $\rightarrow$ 11* ions. Results for 25 $\rightarrow$ 17* alcohol dehydrogenase are shown in Figures 5c. At low energies, the arrival-time distributions are unimodal and centered near 8.9 ms. With energies increasing to ~ 1800 eV, the distributions shift to smaller values centered near 8.2 ms that are consistent with the formation of much more compact structures. From 2000 eV to 2500 eV, the distributions become significantly broader until an additional feature centered near 9.7 ms appears and persists with increasing energy. The results for the 27 $\rightarrow$ 17* ions (Figure S7c) are nearly identical to those for the 25 $\rightarrow$ 17* ions.

The results in Figures 5 and S7 demonstrate that the structures of CAPTR products can depend on the extent of activation. Depending on the identity of the ion and the extent of activation, new structures with smaller or larger Ω can be formed. For $P\rightarrow 13^*$ serum albumin, $P\rightarrow 11^*$ avidin, and $P\rightarrow 17^*$ alcohol dehydrogenase and a given energy, the arrival-time distribution appears to be independent of P . This suggests that the structures and stabilities of these ions depend most strongly on the identity of the protein and C , rather than P or the number of CAPTR events. These results are consistent with the CAPTR products of native-like ions retaining elements of native-like structure, rather than the exothermicity of each CAPTR event fully annealing the structures. These results suggest that the structures of

native-like CAPTR products depend weakly on P , and are consistent with the all precursor ions having similar, native-like structures or that CAPTR products isomerize to similar structures. These data support the hypothesis that the structures of native-like ions generated from ESI of 200 mM aqueous ammonium acetate conditions are all similar and depend weakly on z . Consequentially, both the error of Ω measurements and the range of Ω values measured should be considered when using ion mobility measurements to restrain structural models.

Conclusions

Although Ω values can be determined for each z in native IM-MS measurements, many methods for reporting values^{28,29} or restraining/filtering structural models^{10,67} reduce those measurements to a single value and in turn assume (explicitly or implicitly) specific relationships between Ω and z . We probed this relationship for native-like serum albumin, streptavidin, avidin, and alcohol dehydrogenase using CAPTR, IM-MS, and complementary energy-dependent experiments. The CAPTR products all have Ω values that are within 5.5% of their original precursors and provide no evidence for large-scale structural collapses resulting from charge reduction (Figure 3). The first CAPTR event for each precursor yields products that have smaller Ω values and frequently exhibit the greatest magnitude of change in Ω resulting from a single CAPTR event. The Ω values of the products of the subsequent CAPTR events exhibit either small decreases or increases in Ω . The maximum decrease in Ω , relative to the original precursor, is -5.5% for serum albumin, -2.3% for streptavidin, -2.9% for avidin, and -3.6% for alcohol dehydrogenase. These maximum decreases in Ω with decreasing charge are smaller than those reported based on CAPTR and rf-confining drift cell analysis of lower-mass (8.6 to 14 kDa) protein ions^{26,36,37} and based on alternative charge-reduction approaches and traveling-wave IM of native-like alcohol dehydrogenase³³ and pyruvate kinase (230 kDa).³²

Native IM-MS experiments use a wide range of activation conditions,⁶⁸ from minimal (for maximizing structural retention), to moderate (for increasing transmission and determining mass), to high (for collision-induced unfolding and determining oligomeric state). To investigate the effects of precursor activation on the structures of CAPTR products, ions were activated in the atmospheric-pressure interface as a function of energy prior to CAPTR (Figures 4 and S6). In each case, the center of the Ω distributions of the activated precursors increase with increasing energy, but those for the CAPTR products are significantly more compact than those for the activated precursors. To investigate the stabilities of the CAPTR products, selected products were activated as a function of energy immediately prior to IM (Figures 5 and S7). These results show that new structures with smaller or larger Ω values can be formed with increasing extents of activation; this indicates that these CAPTR products had not fully annealed to lower-energy, gas-phase structures prior to this activation. Because CAPTR³⁵ and other charge-reduction techniques^{69,70} can increase the resolution of interfering species in congested native mass spectra, the retention of structure after CAPTR opens up new avenues for characterizing the structures of components in challenging samples.

The ions in these experiments were generated from aqueous solutions containing 200 mM ammonium acetate at pH 7, which is one of the most common sample solutions for native IM-MS experiments. Therefore, the outcomes of this research support the hypothesis that elements of solution structure are retained in typical native IM-MS experiments and the use of Ω values for characterizing the structures of proteins and protein complexes in solution. These results indicate that the excess charges initially present on native-like ions can have a modest effect on their Ω values, *e.g.*, values for $P \rightarrow 11$ avidin ions are indistinguishable from those for the 17+ to 15+ precursor ions (Figure 3c). However, larger effects are also possible, *e.g.*, all $P \rightarrow 5$ avidin ions have statistically larger Ω values than the 17+ to 15+ precursor ions. Lower- z ions typically adopt smaller ranges of Ω values, which is appealing for structure elucidation. Unfortunately, limiting analysis to a subset of ions will inherently bias results and may systematically exclude subpopulations of extended structures from the ensemble that was present in solution, which will preferentially adopt higher charge states during ionization. Analysis of lower- z ions may also obfuscate when structures were altered at some point during analysis (Figure 5). This finding indicates that potential contributions from charge should be considered when using experimental Ω values to restrain or filter models of candidate structures in solution; simply using the precision of the measurements may considerably underestimate the true uncertainty. Together, these results indicate that advancing the molecular understanding of the structures that give rise to small difference in Ω values will be important for increasing the accuracy of models generated using restraints from native IM-MS experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Instrument diagram of Waters Synapt G2 HDMS mass spectrometer, modified for ion/ion reactions and absolute mobility measurements.¹⁹ (b) Typical potentials used for cation transmission (*black*). For pre-CAPTR activation experiments, the bias between the first two ion optics in the atmospheric-pressure interface was increased (*red*). For post-CAPTR activation experiments, ions were injected as a function of energy into a region immediately prior to the rf-confining drift cell was pressured with argon gas (*cyan*), as described in *Methods*.

Figure 2.

(a) Mass spectrum of native-like serum albumin. Additional peaks centered around m/z 6,000 correspond to non-specific dimers of serum albumin. (b) Quadrupole-selected 17+ serum albumin. (c) CAPTR of the selected ions results in a series of charge-reduced products ($17 \rightarrow C$). Intensities are plotted logarithmically. (d) Plot of the IM-MS data for the $17 \rightarrow C$ ions. Colors in this panel are proportional to a nested logarithm function of the intensity, *i.e.* $\log_{10}(\log_{10}(\text{intensity}))$, to aid in visualization of the data.

Figure 3.

Ω values of the $P \rightarrow C$ ions of (a) serum albumin, (b) streptavidin, (c) avidin and (d) alcohol dehydrogenase, where “ P ” is the charge state of the precursor and “ C ” is the charge state of the CAPTR product. The bars span the 95% confidence interval for each value, and the upper and lower limits of each panel correspond to the Ω values calculated using the PA and EHSS methods, as described in *Methods* and summarized in Table S1. The different colors indicate ions from different P .

Figure 4.

Ω distributions of $15^+ \rightarrow C$ ions of serum album that were activated prior to CAPTR, where the “*” indicates the collisionally activated ion. Data is shown as a function of the voltage bias between the first two optics in the atmospheric-pressure interface (Figure 1b). Note that these distributions were determined using a lower drift voltage than used to acquire the data summarized in Figure 3, which may bias these apparent Ω distributions to slightly larger values as discussed in the Supporting Information. Results from pre-CAPTR activation of 15^+ streptavidin, 17^+ avidin, and 27^+ alcohol dehydrogenase are shown in Figure S6.

Figure 5. Post-CAPTR activation of (a) 15→13* serum albumin, (b) 16→11* avidin, and (c) 25→17* alcohol dehydrogenase, where “*” indicates the collisionally activated ion. The data show the arrival-time distributions plotted as colors, against the laboratory frame energy used to inject the CAPTR products into the argon-filled cell. Results for 16→13* serum albumin, 17→11* avidin, and 27→17* alcohol dehydrogenase are shown in Figure S7.