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Ion Mobility-Mass Spectrometry Differentiates Protein Quaternary Structures formed in Solution and in Electrospray Droplets

Linjie Han and Brandon T. Ruotolo*

Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109-1055, United States

Abstract

Electrospray ionization coupled to mass spectrometry is a key technology for determining the stoichiometries of multiprotein complexes. Despite highly accurate results for many assemblies, challenging samples can generate signals for artifact protein-protein binding born of the crowding forces present within drying electrospray droplets. Here, for the first time, we study the formation of preferred protein quaternary structures within such rapidly-evaporating nanodroplets. We use ion mobility and tandem mass spectrometry to investigate glutamate dehydrogenase dodecamers and serum amyloid P decamers as a function of protein concentration, along with control experiments using carefully chosen protein analogs, to both establish the formation mechanisms operative and assign the bimodal conformer populations observed. Further, we identify an unprecedented symmetric collision induced dissociation pathway which we link directly to the quaternary structures of the precursor ions selected.

TOC image

Introduction

Electrospray ionization (ESI) coupled to mass spectrometry (MS) has emerged as a key technology for elucidating the stoichiometry of protein complexes from small dimers to megadalton-scale macromolecular machines.¹ MS offers unparalleled accuracy and resolution for assessing the composition and copy number of polydisperse protein

*Corresponding Author: bruotolo@umich.edu, Phone: 734-615-0198.

Supporting Information

Tables of experimental and model CCS values for both GDH and SAP, concentration dependant IM-MS data, and denatured protein MS spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

complexes that exist simultaneously in multiple oligomeric states, either as part of their native function or as part of a disease-associated aggregation pathway.² The accuracy of this method comes primarily from the ESI ion formation process itself, where bulk solution is aerosolized into droplets which then deplete in size to the point where one biological unit is partitioned into a single ion-producing droplet. In principle, if protein concentration is kept low, and most droplets remain empty (having a Poisson distribution), then the probability that the vast majority of ion-producing droplets created carry only a single solution-phase relevant complex is high. Thus, the MS signals recorded under these conditions match the expected, biologically-relevant constructs. If, however, ESI-MS experiments are designed such that protein concentrations in bulk solution are high, then the chances of generating droplets that carry more than one protein or complex in an ion-producing droplet are also high.³ Proteins trapped within such droplets are forced to bind within rapidly evaporating ESI droplets, despite the fact that no similar assemblies exist in solution. Similar crowding forces⁴ can be found within protein-containing nanoparticles used for targeted drug delivery,⁵ microdroplet microreactors used for high-throughput biological reactions in microfluidic devices,⁶ and within the sub-cellular environment.⁷ The nano-scale version of ESI, nESI,⁸ coupled with innovative data analysis strategies and experimental designs, has driven MS measurements performed under native conditions to their current position of prominence in the determination of protein stoichiometries. Despite these advances, nESI-MS protein stoichiometry measurements are still influenced by droplet-related artifacts that necessitate further study, particularly as the crucial role of weakly-associated multiprotein complexes is increasingly being recognized.⁹

The relatively recent coupling of ion-mobility with mass spectrometry (IM-MS) has significantly enhanced the capabilities of MS by enabling the separation of protein ions in time according to their charge and orientationally-averaged size on the millisecond timescale.¹⁰ IM-MS is capable of rapidly analyzing the structure and connectivity of multiprotein complexes in the context of mixtures that would be indecipherable by most classical structural biology methods,^{11,12} and as a result, the conformations of ESI-artifact protein complexes have come under experimental scrutiny. Many protein complex ions that conform to the broad description of ESI artifacts given above have been analyzed and compared with specific assemblies that exist in bulk solution by IM-MS.² Surprisingly, most of these results indicate that the absolute sizes, and size ranges, occupied by ESI-artifact ions conform broadly to those inhabited by functional complexes. Recent reports, however, have pointed to ESI droplet surface charge and surface tension effects as specific mechanisms that may give rise to aberrant protein complex conformational forms, rather than aberrant protein binding stoichiometries.^{13,14}

In this report, we have collected the first data to probe the ability of the crowding forces within nESI droplets to produce artifact protein quaternary structures within the resulting gas-phase ions. We begin by identifying the structures present within the model systems of glutamate dehydrogenase (GDH) dodecamers and serum amyloid P (SAP) decamers, both of which display bimodal profiles in IM measurements. Such an analysis is necessary to correctly deduce the origins of these ions. We then record the relative abundances of these structures produced as a function of protein concentration, use detailed control experiments and tandem MS datasets to verify these assignments, and link the structures identified to

formation mechanisms within either bulk solution or nESI droplets. Furthermore, we observe that elongated GDH dodecameric conformers exhibit a unique collision induced dissociation (CID) pathway resulting in symmetric fissioning of the intact complex into equally-charged hexamers. This pathway is rationalized based exclusively on the structure of the precursor GDH dodecamer ions selected, which we find to be the preferred structure of the assembly within crowded nESI droplets.

Materials and Methods

Protein preparation

Glutamate dehydrogenase, purified from bovine liver (bovGDH), and Glutamate dehydrogenase, purified from *Proteus* spp. (bacGDH) were purchased from Sigma (St. Louis, MO, USA). Serum amyloid P component, purified from human serum (SAP), and recombinant human C-reactive protein, purified from *Escherichia coli* (CRP) were purchased from Calbiochem (San Diego, CA, USA). Standards used to construct collision cross-section (CCS) calibration curves, including cytochrome c (equine heart), avidin (egg white), concanavalin A (jack bean), alcohol dehydrogenase (*Saccharomyces cerevisiae*) and glutamate dehydrogenase (bovine liver), as well as small molecules used to perform SAP-ligand binding experiment, including calcium acetate and deoxyadenosine monophosphate (dAMP) were all purchased from Sigma (St. Louis, MO, USA). Protein samples were buffer exchanged into 100 mM ammonium acetate at pH 7 (bovGDH, bacGDH and CCS calibrants) and pH 8 (SAP and CRP) using Micro Bio-Spin 6 columns (Bio-Rad, Hercules, CA) and prepared to a final concentration of 5 μ M to 80 μ M for bovGDH and 5 μ M to 30 μ M for SAP to perform concentration dependent analysis, and 5 μ M for CCS calibrants.

Ion Mobility-Mass Spectrometry

Sample aliquots (~7 μ L) was analyzed using a quadrupole-ion mobility-time-of-flight mass spectrometry (Q-IM-ToF MS) instrument (Synapt G2 HDMS, Waters, Milford MA, USA).^{15,16} Protein ions were generated using an nESI source in the positive mode, with the capillary typically held at 1.5 kV (for bovGDH and bacGDH) and 1.8 kV (for SAP and CRP). The sample cone was operated at 20 V to avoid any in-source activation. Instrument settings were optimized to allow transmission of intact protein complexes and to preserve non-covalent interactions.¹⁷ The trap traveling-wave ion guide was pressurized to 3.6×10^{-2} mbar of argon gas. The traveling-wave ion mobility separator was operated at a pressure of ~3.5 mbar, and employed a series of DC voltage waves (15 V wave height traveling at 150 m/s) to generate ion mobility separation with optimal resolution. The ToF-MS was operated over the *m/z* range of 800–15000 (for bovGDH and bacGDH) and 800–9000 (for SAP and CRP) and at a pressure of 1.8×10^{-6} mbar.

Collision Induced Dissociation

Tandem-MS (Quad selection) mode was performed, in which selected ions were activated in the ion trap traveling-wave ion guide prior to the ion mobility separator by increasing the trap collision voltage (Trap CE, as indicated in the instrument control software) which acts as a bias voltage between the quadrupole and ion trap traveling-wave ion guide to accelerate ions to increased kinetic energies. Notably, trap collision voltage of 200 V, which is the

maximum accessible voltage in the ion trap, was not sufficient to profoundly dissociate 52⁺, 55⁺ and 57⁺ charge state of boVGDH dodecamer precursor ions, therefore the transfer collision voltage (Transfer CE, as indicated in the instrument control software) was used to further activate ions in the ion transfer traveling-wave ion guide which sits after the ion mobility cell. Besides, the energy-dependent CID profile was constructed over a range of trap collision voltages.

Ligand binding and CRP interaction

The SAP complex with Ca²⁺ and dAMP was formed by addition of calcium acetate to SAP, followed, after thorough mixing, by dAMP to final concentrations of 4 mM salt/ligand and 5 μM SAP. The mixture was incubated at pH 8 for 18 hours at room temperature. To study the interactions between SAP and CRP, mixtures were incubated for 2 hours at a ratio of 1:1 in the absence of calcium acetate. Excess ligands or other impurities were removed before analysis with a single Micro Bio-Spin buffer-exchange step.

Data analysis

Mass spectra were calibrated externally using a solution of cesium iodide (100 mg/mL) and processed with Masslynx V4.1 software (Waters, Milford MA, USA). Collision cross-section (CCS) measurements were made using known CCS values of cytochrome c monomer, avidin tetramer, concanavalin A tetramer, alcohol dehydrogenase tetramer, and glutamine dehydrogenase hexamers as calibrants using the method described previously.^{18,19} It is noteworthy that masses and mobilities of the boVGDH and bacGDH dodecameric ions we studied are not bracketed by the current calibrant database, may result in enhanced CCS errors (3–4%).¹⁸ These errors do not impede our ability to correctly assign the structures presented in Figure 1.

Protein-protein docking

The protein-protein docking was performed by using HEX 6.3 software (<http://hex.loria.fr/>), which is a fast Fourier transform (FFT)-based protein docking server powered by graphics processors. Hex 6.3 necessitates the ‘ligand’ and the ‘receptor’ as input in PDB format, thus the crystal structures of SAP (PDB ID: 1SAC) and CRP (PDB ID: 1GNH) were downloaded from the protein data bank (<http://www.rcsb.org/pdb>). It should be noted that the purpose for protein docking herein is to establish the models for SAP/SAP (via ‘ABAB’ mode) and CRP/CRP (via ‘BAAB’, ‘BABA’ and ‘ABBA’ modes) stacked decamer that might arise from the nonspecific interaction as nESI artifacts, therefore no electrostatic correlation and energy minimization aimed to simulate biologically-relevant complex structure were employed. Instead, center-to-center distance between the two pentamers was the major concern, using the existing crystal structure of SAP ‘ABBA’ decamer (PDB ID: 1LGN) as a reference. Especially when constructing SAP ‘BAAB’ model via carbohydrate-carbohydrate interaction, since carbohydrate was not visible in the crystal structure, the oligosaccharide structure at Asn32 was modeled using the coordinates of oligosaccharide chain at Fc region in the crystal structure of human IgG1 b12 (PDB ID: 1HZH) while the pentamers were rotated 36° relative to each other. This achieves the SAP ‘BAAB’ best-fit structure according to the previous X-ray and neutron scattering analysis, which leads to the center-to-center separation of 3.4–3.5 nm between the two pentamers, larger than that without carbohydrate

interaction (2.7 nm). While we note that this approach to modeling the carbohydrate interface in SAP has limitations in the context of the molecular-level details and affinities of protein-carbohydrate interactions observed, the approach captures all of the coarse-grained aspects of the complexes observed by our IM-MS experiments. Visualization of the docked complex has been done by using PyMol (<http://pymol.sourceforge.net/>) molecular graphic program.

Theoretical CCS determination

CCSs were calculated for the docked and crystal structures using the projection approximation (PA) method implemented in DriftScope V2.1 (Waters, Milford MA, USA). The PA CCS typically underestimates experimental CCS by 14%. For this reason, a scaled PA (eq. 1), based on an empirically determined scaling factor which accounts for scattering phenomena, any missing atoms and truncations carried out to the full-length protein for a high-resolution crystal structure of the complex, is used here to correlate experimental CCS with model structures. The use of such scaling factors, therefore, entails all of the above as primary assumptions in using such values in the analysis of experimental data.

$$CCS_{calc} = 1.14 \times CCS_{PA} \left(\frac{M_{exp}}{M_{pdb}} \right)^{\frac{2}{3}} \quad (1)$$

While we note that there are other means of computing theoretical CCS values for model structures,^{20,21} none of these procedures have proven more accurate or precise than scaled PA values when comparing X-ray derived models to experiment for large proteins and complexes.²

Results and Discussion

Fig. 1a–d shows data for GDH, a homohexameric enzyme that plays a pivotal role in metabolism, ubiquitous in most organisms. Mammalian GDH is a stacked dimer of trimers, with each subunit composed of three domains: the Glu binding domain, the NAD binding domain, and the antenna domain, which is not found in bacterial and fungal GDHs.²² Fig. 1a shows IM-MS data for bovine GDH (bovGDH) sprayed from 100 mM ammonium acetate buffered solution (pH = 7.0) at a protein concentration of 20 μ M, which reveals the presence of both the intact hexamer (337 kDa) and dodecamer (675 kDa). Analysis of the IM profiles captured for dodecameric bovGDH reveal a bimodal distribution of structures, which have collision cross section (CCS) values of 21,800 $\text{\AA}^2 \pm 0.7\%$ and 23,900 $\text{\AA}^2 \pm 1\%$ (Fig. 1b). We also note a significant shift in average charge state between these two GDH dodecamers, resulting in the larger structure accumulating 6% more charge during nESI than the smaller conformer (blue and red respectively, inset Fig. 1a), in a manner consistent with both their recorded CCS values and the general mechanisms of ESI ion formation.²³ Despite the dominance of the GDH hexamer, there are structural and functional precedents for GDH dodecamers *in vitro* and *in vivo*. Hexamer-hexamer packing architectures are readily obtained from X-ray data, and suggest a role for the dodecamer in regulating NAD cofactor binding to the enzyme.²² In addition, electron microscopy²⁴ and additional X-ray data²²

indicates that dodecamers can further polymerize to form long helical filaments. Furthermore, higher-order structures are suggested to play an essential role in the crowded environment of the mitochondria through the formation of enzyme homooligomers, although the connection between such higher-order assemblies and enzymatic activity remains a subject of debate.²² A unit cell packing analysis of available X-ray data reveals two main boVG DH dodecamer forms: one having an extended quaternary structure (estimated CCS of 23,300 Å²), where the hexamer-hexamer contact area is small and made only by the antenna regions, and the other a bent quaternary structure (estimated CCS of 22,800 Å²) which bears substantially larger contact area between hexamers including both the NAD-binding and antenna regions²² (Fig. 1b, Table S1). Measured CCS values for the dodecamers display excellent agreement with the two forms described above (Table S1). To further verify this assignment, we made IM-MS measurements on bacterial GDH (baGDH, hexamer, ca. 290 kDa), which shares a 92% fold similarity with boVG DH but lacks the antenna region (Table S2). All baGDH dodecamer ions observed exhibit a monomodal IM profile (Fig. 1c and 1d), confirming the importance of the boVG DH antenna in driving the formation of the distinct dodecamer conformations observed in Fig. 1a. The average CCS obtained for baGDH dodecamer ions is in close agreement with theoretical values based on a baGDH model derived from X-ray packing data (Table S1), and we note that GDH self-polymerization is uncommon in bacterial cells.^{22,24}

Similar to GDH dodecamers, IM-MS data for SAP decamers, in the absence of Ca²⁺ (pH = 8.0) at 10 μM protein concentration, displays a bimodal IM distribution (Fig. 1e–h) as reported previously.²⁵ As a member of the pentraxin family of proteins, SAP adopts a planar, disc-like configuration composed of 5 identical subunits, and is named for its universal association with amyloid deposits *in vivo*, protecting such disease-associated structures from proteolysis.²⁶ Inspection of the IM-MS data shown in Fig. 1e reveals two IM peak populations for SAP decamers (256 kDa), separated by a 9% overall difference in IM drift time, but a monomodal population for pentamers (128 kDa). Averaged CCS values for these two SAP decamer populations (10,900 ± 1.9% and 11,300 Å² ± 1.3%) confirm that the size difference observed here is considerably smaller than that observed for GDH dodecamers, resulting in only minor differences in the average charge state recorded for the decameric ions.

SAP pentamers possess two non-equivalent binding faces, defined here as A and B. Face A is an α-helix interface glycosylated at Asn-32 (indicated in yellow on the model structures, Fig. 1e), while the B face contains β-sheet and Ca²⁺ binding sites.²⁶ SAP decamers can be constructed from either A-A, A-B, and B-B type interactions, all of which have been reported in the literature and are highly dependent upon solution conditions.²⁷ Previous data have shown that Ca²⁺-free, alkaline solution conditions promote A-A decamers, characterized by a loosely-packed interface dominated by oligosaccharide interactions. Using the well-characterized oligosaccharide interactions found within the F_c fragment of IgG, which have been shown to be similar to those found within SAP decamers,²⁶ we constructed docked models of the SAP A-A decamer (Table S3), and found good agreement with the larger features from the IM-MS experiment (theoretical CCS = 11,200 Å²). X-ray derived models for B-A and B-B type SAP decamers are tightly-packed (theoretical CCS for both = 10,600 Å²) and are thus indistinguishable both from each other and from the smaller

SAP decamer feature observed experimentally. To verify the critical nature of A face glycosylation in the observation of bimodal SAP decamer IM profiles, data for C-reactive protein (CRP), which lacks any sites of glycosylation but shares 96% fold similarity with SAP (Table S2), was collected. CRP decamers exhibit a single decamer IM profile (Fig. 1g, h), supporting our SAP structure assignments. Models for all possible CRP decamer configurations have indistinguishable CCS values (Table S3), in agreement with our monomodal IM-MS data.

For IM resolved boVGdH dodecamers and SAP decamers, we used data recorded as a function of protein concentration in solution, and measured the relative abundances of each form observed to study the influence of protein crowding and drying forces within nESI droplets in the formation of protein ion quaternary structure. While we note that the ultimate protein concentrations achieved in our experiments (30–80 μM) is lower than those expected to achieve classical macromolecular crowding,⁴ effective concentrations are much greater in drying ESI droplets. In both cases, we observe a clear preference for one protein quaternary structure over the other, indicating a role for such forces in driving protein topology formation. In the case of boVGdH dodecamers (Fig. 1i) the more-extended quaternary structure is preferred at higher protein concentrations. In contrast, smaller SAP decamers are favored at higher protein concentrations (Fig. 1j). That two out of the three possible SAP quaternary structures populate the smaller CCS observed is consistent with our observations that the short drift time feature for SAP decamers increases in intensity to a greater extent than the specific A-A decamer at high concentrations. Similarly, larger boVGdH dodecamers may be preferred as these structures rely upon contact only in the antenna region of boVGdH, which extends into solution and increases the likelihood of complex formation.

In order to further characterize the two dodecamer forms of boVGdH, and verify the structure assignments made above, we acquired CID data for the ions observed as a function of both precursor charge state and collision energy. While the two structural forms of boVGdH are indistinguishable by mass, IM-MS analysis reveals that they have different average charge states and, as such, isolation of different boVGdH charge states for CID provides an effective means of interrogating the dissociation properties of each conformer population separately. We observe that low charge state boVGdH dodecamers dissociate following collisional activation through the standard asymmetric pathway (Fig. 2a).²⁸ However, as precursor ions of increasing charge are selected, product ions that correspond to a rarely-observed symmetric CID pathway are revealed. This trend contrasts sharply with our observations for bacGDH dodecamers, which undergo asymmetric CID uniformly across all charge states observed (Fig. 2b), supporting a mechanism where the precursor ion structure, rather than its charge, is the driving force behind the symmetric CID data acquired for boVGdH dodecamers. By making detailed CID measurements as a function of activation energy for high charge state boVGdH ions (Fig. 2c,d) we are able to verify that the larger boVGdH conformer undergoes hexamer loss at low CID energies, while at higher energies, monomers are ejected from activated hexamer product ions (Fig. 2e). To further validate the role of glycan interactions within the A-A interface in driving the formation of the loosely-packed SAP decamers observed in our experiments, we first analyzed mixed SAP/CRP decamers and measured their CCS values (Fig. 3a). Upon mixing SAP and CRP (1:1) in the absence of Ca^{2+} , abundant signals are observed corresponding to mixed decamers (242,709

± 7 Da) and these ions exhibit monomodal IM profiles due to a lack of a glycosylated binding face for CRP (Fig. 3c). In addition, we incubated SAP in the presence of physiological levels of Ca^{2+} with deoxyadenosine monophosphate (dAMP), which is known to favor SAP B-B decamer formation through Ca ion bridging and nucleotide base stacking interactions.²⁶ We observe a trimodal bound population of SAP complexes under these conditions, corresponding to apo-SAP decamers, along with those half-occupied, and fully-occupied with dAMP. SAP complexes bound to dAMP exhibit CCS distributions that strongly favor smaller structures (Fig. 3c), in line with the expected enrichment of B-B type decamers through ligand binding and further supporting our structural assignment described above.

Conclusions

The data presented herein address key challenges associated with the interpretation of ESI-MS data for multiprotein complexes, as well as broader issues related to the basic biophysics and the plasticity of protein quaternary structure. First, the origins of the bimodal IM profiles observed for both boVGHD dodecamers and SAP decamers can be linked directly to experimentally-verified structural models. While all the conformers observed for the complexes studied here likely pre-exist in solution, and previous reports have analyzed bimodal IM profiles for proteins and complexes,^{13,25} no previous study has linked so clearly the relative abundances of specific protein structures to the crowding forces associated within rapidly drying nanodroplets. Furthermore, we verify our protein conformer assignments by studying key structural analogs, performing ligand binding experiments, and conducting detailed CID measurements. In addition, we identify a previously unknown symmetric CID pathway, unique among previous reports by virtue of its origins in the boVGHD structural populations observed through our IM-MS data. Surprisingly, ca. 1000 Å² of contact area between hexameric boVGHD units differentiate the structures observed and drive the stark contrast in CID product ions generated in our dataset. Such CID results are especially interesting in light of the growing use of surface induced dissociation (SID) for the analysis of protein complex ions.²⁹ In these experiments, ions collide with a surface to generate product ions that largely reflect protein complex sub-structure, similar to the hexamers produced by the extended boVGHD conformers observed here. Further experiments will be necessary to assess the connectivity between the mechanistic details revealed here and those operative during SID in order to further enable such tandem MS measurements.

It is interesting to consider the similarities between the ESI droplets studied here, and other nano-encapsulation environments encountered in both targeted drug delivery and high-throughput microfluidics.^{5–7} Clearly, the results discussed in this report strongly indicate that such environments can have a significant impact on protein quaternary structures. Although there are obvious and important differences between ESI droplets and living cells, one can also extend some of the discussion above to the growing body of evidence indicating the importance of protein-protein crowding forces in defining the native structural ensemble of functional proteins *in vivo*. As defined previously,⁴ crowding forces influence the immediate environment of macromolecules, potentially altering the thermodynamics of protein folding or assembly through the exclusion of free volume. In nESI crowding is

linked with drying,³⁰ or the rapid removal of water, and both likely provide driving forces for protein association in our experiments. Typical experiments aimed at assessing such crowding forces *in vitro* using model systems typically employ protein concentrations in the mM range and above.⁴ While our experiments do not achieve such concentrations in bulk solution, they far exceed them during the drying steps of ESI,¹³ as the probability of trapping multiple biological units within ESI droplets scales non-linearly with concentration.³ Results shown Fig. 1i,j indicate that concentration dependant changes in IM arrival time distributions can be used to detect such protein quaternary structure effects.

The influence of rapidly-removed solvent on protein quaternary structure,³⁰ collectively termed ‘drying forces’ above, is unique to our dataset in the context of those experiments seeking to measure the influence of intra-molecular crowding on biomolecular structure. Previous reports have evaluated the influence of protein concentration on the formation of artifact or ‘accidental’ complexes in ESI, and such lines of inquiry have led to the assumption that such assemblies would both possess lower association strengths and more polydisperse structures when compared with functional complexes.³¹ The complexes studied here clearly do not fall into such a paradigm, but nor should they be necessarily taken as evidence of a general mechanism of protein complex ion formation. Future experiments and next-generation molecular modeling efforts,³² aimed at delineating the influence of solvent removal and protein crowding within ESI droplets will undoubtedly further enable the use of IM-MS and other gas-phase tools for detailed structural biology measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

IM-MS data for boVGDH (a, b) and bacGDH (c, d), recorded under identical instrument conditions, reveals ion signals for both hexamer and dodecamer. Bimodal and monomodal drift time distributions are observed for the 54⁺ charge state of boVGDH dodecamer (b) and 49⁺ bacGDH dodecamer (d), respectively. Similarly, SAP (e, f) and CRP (g, h) ions analyzed under the same instrument parameters exhibit both pentamer and decamer signal, with two structural forms and a single conformational state observed for SAP (f, 33⁺) and CRP (h, 31⁺) decameric ions, respectively. When the relative abundance of the isoforms observed in (b) and (f) are measured as a function of protein concentration, the larger boVGDH dodecamer (i) and the smaller SAP decamer (j) are strongly preferred at higher concentrations.

Figure 2.

Tandem MS data for bovine GDH dodecamer 52⁺, 55⁺ and 57⁺ charge state precursors at a trap collision voltage of 200 V and transfer collision voltage of 150 V (a) is compared with that of 47⁺, 49⁺ and 51⁺ for bacterial GDH dodecamer at a trap collision voltage of 180 V (b).

Symmetric hexamer dissociation is observed for the larger of the two bovine GDH conformers observed (squares). Plot of precursor and product ion intensity for the larger bovine GDH dodecamer form versus trap collision voltage indicates that hexamer formation is a low-energy product ion, which then goes on to produce monomer product ions at higher energies (c). This plot is derived from the stacked CID MS spectra of the large conformer observed for bovine GDH 63⁺ dodecamer ions (d). A schematic representation illustrates the unique CID pathway of the extended bovine GDH structure (e).

Figure 3.

MS and IM-MS data for a solution containing both SAP and CRP proteins, mixed in a 1:1 ratio in the absence of added Ca^{2+} (a). MS signals for SAP/CRP decamers (purple) dominate CRP decamers observed at low relative abundance (orange). MS and IM-MS data for SAP decamers following an 18h incubation in the presence of calcium acetate and dAMP (b). Three different SAP decamer populations are observed, corresponding to: apo (red), half-occupied (5 ligands bound, green) and fully-occupied (10 ligands bound, blue) dAMP bound states. Isolated CCS distributions are shown for the 32+ charge states of each species observed in (a) and (b), using a color code that matches the annotations found in these panels (c). Gaussian fits to the CCS distributions are indicated by dashed lines, with CCS values indicated for each centroid.