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FRONTIERS ARTICLE

Gas-phase protein assemblies: Unfolding landscapes and preserving native-like structures using noncovalent adducts

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ABSTRACT

Mass spectrometry is a rapidly emerging technology for characterising the native structures of protein complexes. One challenge in interpreting results from mass spectrometry experiments is that the structures of protein complexes in the gas phase may differ from those in solution. As such, there is great interest in using small molecules to stabilise the structure of large proteins and their complexes in the gas-phase. Here, we investigate the stabilisation properties of trisH$^+$, a cationic non-volatile electrospray buffer component, by experimentally characterising the unfolding and dissociation of three gas-phase tetrameric protein complexes. We find that trisH$^+$ preferentially stabilises the compact native-like state of the complexes studied here. We put these results in context, and look beyond the water-soluble complexes studied here to discuss the mechanistic implications of this work on the stabilisation of membrane protein complexes during electrospray ionisation.

1. Introduction

Most cellular functions involve assemblies of proteins and other biological molecules that are often large, heterogeneous and dynamic. Such complexes may be formed transiently or be stable over the lifetime of the cell. As a consequence of these properties, assemblies can pose significant challenges for established structural biology tools that aim to determine their 3D architecture. To gain structural information on these assemblies, new approaches are required that often involve combining datasets derived from multiple techniques to generate hybrid structural models [1]. Mass spectrometry (MS) in combination with ion mobility (IM) is one such combination that is increasingly being applied to develop course-grain architectural models of assemblies.

It is now well established that MS can yield insights into the composition and stoichiometry of heterogeneous multiprotein assemblies at low micromolar concentrations [2–7]. When combined with IM it becomes possible to separate species not only according to their mass-to-charge ratio (m/z) but also according to their ability to traverse an ion guide containing inert gas under the influence of a weak electric field [8–17]. IM experiments yield a collision cross section (CCS) which when combined with modelling can enable a series of candidate structures to be proposed. As such, IM–MS is well positioned to reveal the topology of interacting subunits and consequently to contribute to the characterisation of structurally important biological assemblies [18].

One challenge in using IM–MS datasets for building architectural models of multiprotein complexes is that the structures of protein complexes in the gas phase can differ from those in solution. The processes of electrospray ionisation (ESI), desolvation, transport and analysis can occur over a range of time scales and energies. As a consequence, biological molecules and assemblies can rearrange at the local residue level, unfold to more elongated conformations, and even refold to compact, yet non-native conformations [19–21]. There is substantial evidence however, that gas-phase assemblies can maintain many aspects of their solution architectures under the appropriate conditions [12,19,22,23]. The stability of such 'native-like' structures may be attributable to kinetic trapping in the absence of bulk solvent, which mediates many structural rearrangements in solution [19,24]. A detailed understanding of the relationship between the native structures of proteins and their assemblies in solution and their structural evolutions in the gas phase remains elusive, but would enable both broader and more accurate application of IM–MS to structural biology.

Abbreviations: MS, mass spectrometry; IM, ion mobility; CCS, collision cross section; ESI, electrospray ionisation; nESI, nano-electrospray ionisation; CID, collision induced dissociation; CIU, collision induced unfolding; TTR, tetrameric transthyretin; Tris, 2-amino-2-hydroxymethyl-propane-1,3-diol; TIV, trap injection voltage; TMV, transition midpoint voltage.

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Increasing the internal energy of protein complex ions, through high-energy collisions with neutrals, results in collision-induced unfolding (CIU) of the complex and the eventual expulsion of a highly charged subunit and the formation of a residual, stripped complex through collision induced dissociation (CID) [20,25,26]. IM has been shown to be very sensitive to conformational differences between compact, native-like structures and CIU products formed prior to subunit ejection [27,28]. Unfolding has also been observed when protein ions are trapped for extended periods in the gas phase [29]. The CIU products of transthyretin homotetramers (TTR, 55 kDa) adopt a number of distinct and long-lived elongated conformations which exist prior to dissociation of the subunits [27,28]. The limited data available currently suggests that these families of elongated structures are populated in a largely sequential manner. The CCS measured for the largest CIU product observed for TTR is comparable to that predicted for a fully-extended monomer docked onto a folded trimer, suggesting that monomer-based, localised unfolding dominates the CIU observed [27].

IM and MS experiments have been used recently to test the ability of a number of small molecules to stabilise the structures of proteins in the absences of bulk solvent. For example, imidizole can be added to solution prior to ESI or during the ESI desolvation process, and can result in protein–ligand complex ion intensities that better reflect their binding ratios in solution through enhanced evaporative cooling [30,31]. Further, a number of anions have been tested for their ability to bind during ESI and stabilise protein [32] and multiprotein [33] systems *in vacuo*. These stabilised proteins and assemblies have been studied by IM and MS to provide not only the analytical ability to discern those anions that, when bound to the protein, stabilise its gas-phase structure, but also in order to provide mechanistic insights into the stabilisation process itself. Specifically, this Letter has revealed that while anions can stabilise gas-phase protein structure through tight, localised interactions with basic sites on the protein surface, [32,34,35] a far-more pervasive mechanism of structural stabilisation is generated through dissociation of multiple bound anions from the protein as neutrals [36]. Furthermore, the relative protein binding affinities of anions observed by MS in the gas-phase are often correlated with their known binding affinities in solution, allowing for CID and CIU results to be interpreted in light of Hofmeister-type protein stabilisation in solution [32]. For cation solution additives, analogous CIU and CID experiments to those described above were used to probe the effects of adding additional buffer salts to nano-ESI (nESI) solutions of the 800 kDa GroEL complex. The addition of $trisH^+$ acetate to the ESI solution resulted in gas-phase, noncovalent adducts that appeared to prevent the collapse of the internal cavity of GroEL [37]. Interestingly, for ions formed from these ESI solutions, additional energy was required to initiate unfolding. These results imply that interactions between protein complexes and involatile buffer species have a stabilising role. However, the precise effects on the structure by these adducts were difficult to establish as no distinct CIU conformations were resolved for the GroEL assembly [37].

Here, we report new data that both expands on our current knowledge of protein structural stability in the absence of bulk solvent and also serves as a general point of reference for the emerging field of gas-phase structural biology. In order to monitor the effects of unfolding and dissociation as a function of internal energy deposition and buffer composition we selected three tetrameric complexes with established X-ray structures and masses ranging from 64 to 232 kDa. By carefully manipulating collision energy, we use IM–MS to probe both the extent and pathways of protein unfolding within the three protein tetramers. We observe that the unfolding pathways are independent of the ESI buffer composition. We also find that the addition of tris-based salt preferentially stabilises the gas-phase conformation that is most consistent with the X-ray structures of these complexes. The relative stabilities of the subsequent conformations formed in the gas phase upon CIU are unaffected by the initial presence of adducts. This observation suggests that the CIU profiles of protein complexes depend predominantly on the intrinsic properties of the gas-phase assembly, rather than the composition of buffer components bound to the protein.

The preferential stabilisation of native-like forms of the assembly suggests that even minimal solvation by these noncovalent adducts provides an environment that is particularly informative for gas-phase structural biology. We close by putting the results presented here in context with other datasets measuring the role small molecules play in stabilising protein structure in the absence of bulk solvent and proffer some general mechanistic conclusions regarding protein structural stability *in vacuo*.

2. Methods

2.1. Sample preparation

Avidin (A9275), concanavalin A (C2010) and pyruvate kinase (P9136) were purchased from Sigma Aldrich (Dorset, UK). Samples in 200 mM ammonium acetate (Sigma Aldrich) were used for analysis, with a final protein concentration of 2–10 μM. These were purified by size-exclusion chromatography (BioRad micro biospin 6 columns) where necessary. Tris (2-amino-2-hydroxymethyl-propane-1,3-diol) acetic acid (Sigma Aldrich) was dissolved in 200 mM ammonium acetate. Solutions were adjusted to a pH of 6.9 and added to the existing solutions for analysis to give final $trisH^+$ acetate concentrations. The dilution factor of each analysis solution was the same.

2.2. IM–MS

Experiments were performed on a Synapt HDMS IM–MS instrument [38,39] (Waters, Manchester, UK) using a nESI source and instrumental parameters optimised for the transmission of large noncovalent complexes [39,40]. Similar instrumental parameters were used for all complexes, although the wave height in the travelling-wave IM cell was varied from 7 to 12 V to optimise the IM separations. For CIU experiments, ions were accelerated using a trap injection voltage prior to entering a 10 cm stacked ring ion trap containing 0.05 mBar of argon that is positioned between the quadrupole mass filter and the IM cell. Data were analysed using MassLynx V4.1 and DriftScope V2.1. IM drift times were calibrated using methods described elsewhere [39] and calibrant ions (chosen from ubiquitin, myoglobin, cytochrome c, avidin, concanavalin A, pyruvate kinase, GroEL, Sigma Aldrich), having CCS values measured directly using a drift tube without the use of travelling waves [41–43] and methods described elsewhere.

2.3. CCS calculations

Crystal structures of avidin (1VYO) [44], concanavalin A (1AZD) [45] and pyruvate kinase (1F3W) [46] were obtained from the Protein Data Bank. Extended monomers were generated for each complex using PYMOL (DeLano Scientific) setting all the φ and ψ angles to 180° and docked onto a folded complex missing one of its monomers using Hex 5.0 [47]. Projection approximation CCSs [48] for these structures were calculated using DriftScope V2.1 CCS Calc [49], a gas radius of 1 Å, and a CCS tolerance of 0.5%.