Alternate Dissociation Pathways Identified in Charge-Reduced Protein Complex Ions

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Tandem mass spectrometry (MS) of large protein complexes has proven to be capable of assessing the stoichiometry, connectivity, and structural details of multi-protein assemblies [1]. While the utility of tandem MS is without question, a deeper understanding of the still controversially discussed mechanism of protein complex dissociation will undoubtedly drive the technology into new areas of enhanced utility and information content.

We present here the systematic analysis of the charge state dependent decay of the non covalently associated complex of human transthyretin (TTR), generated by collision induced dissociation (CID). A crown ether-based charge reduction approach was applied to generate intact transthyretin tetramers with charge states ranging from 15+ to 7+. These nine charge states were subsequently analyzed by means of tandem MS and ion mobility spectrometry. Three different charge-dependent mechanistic regimes were identified: 1) Ions with a charge above 12+ followed the commonly observed CID dissociation pathway resulting in highly charged, unfolded monomers and compact, charge-stripped trimers [2]. 2) Tetramers with charge states between 11+ and 9+ still dissociated into monomer and trimers, but the expelled monomers were carrying fewer charges and retained their compact, native-like conformation. 3) 9+ and 8+ ions primarily yielded C-terminal peptide fragments which were cleaved from intact and fully folded TTR tetramers. Ions with a charge below 8+ were virtually indestructible in the instrument and neither showed signs of dissociation nor unfolding.

Taken together, the results presented highlight the potential of charge state modulation as a method for directing the course of gas phase dissociation and unfolding of protein complexes. Particularly noteworthy is the fact that under certain conditions the remaining stripped complex, as well as the departing subunit, retain compact, native-like structures. This has implications for various gas phase-spectroscopic and structural biology experiments in which maintaining conformations close to the native state is of paramount importance.

[1] J.L.P. Benesch, B.T. Ruotolo, D.A. Simmons, C.V. Robinson *Chem. Rev.* **2007**, 107, (8), 3544-3567.

[2] J.L.P. Benesch *J. Am. Soc. Mass Spectrom.* **2009**, 20, (3), 341-348.