Probing the Fidelity between Gas Phase and Solution Phase Protein Structures with Top-Down Mass Spectrometry

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Mass spectrometry (MS) has been used to characterize higher order structure and assembly states of solution phase proteins and protein complexes. Implicit in this application of MS is the assumption that elements of the structural details of the solution phase protein molecule are preserved in the gas phase molecule. Despite numerous studies that have been published, there is some skepticism among the biophysical/biochemical community that this application of MS has utility for understanding solution phase protein structures.

We have used electrospray ionization mass spectrometry (ESI-MS) not only for detecting noncovalent protein-ligand complexes [1], but also to define the sites of ligand-protein interactions. We are attempting to develop top-down protein MS for measuring the sites of noncovalent protein-ligand binding. We have discovered that electron capture dissociation (ECD) dissociates only covalent bonds of noncovalent protein-ligand complexes, i.e., the noncovalent ligand interaction is retained [2]. Therefore, an ESI-ECD-MS/MS strategy of native protein-ligand complexes may reveal the contact interface for protein-ligand interactions.

Moreover, because electrostatic interactions are significantly strengthened in the absence of solvent, these types of interactions can withstand energetic gas phase processes, such as collisionally activated dissociation (CAD). The sites of ligand binding can be probed by CAD-MS/MS for protein-metal and protein-nucleotide complexes [3]. Increasing the multiple charging (i.e., supercharging) of noncovalent proteins increases the efficiency of top-down MS [4]. Enhanced charging and dissociation of native protein complexes have been observed for a variety of supercharging reagents, including sulfolane.

We show that top-down MS is able to determine the sites of protein-ligand interactions, such as adenylate kinase-ATP, interactions of protein profilin and KabC (a naturally occurring toxin) with the hydrophobic pocket of the actin protein, and calcium ions with the EF-hands of calmodulin. These studies demonstrate that the structures of the solution phase complex have not been significantly perturbed upon transition to the gas phase.

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