Using Mass Spectrometry to Study Copper-Protein Interactions: The Case of β-2-Microglobulin

Juma D. Bridgewater, Jihyeon Lim, Richard W. Vachet

Department of Chemistry, University of Massachusetts-Amherst

β-2-microglobulin (β2m) is a 12 kDa subunit of the class I major histocompatability (MHC) complex. Upon turnover of the MHC complex, β2m is normally transported to the kidneys and degraded, but in people undergoing dialysis because of kidney disease, β2m concentrations increase up to 60-fold from the normal level of ~ 0.1 μM. In these patients β2m eventually forms painful amyloid deposits in joint spaces. The exact cause of β2m amyloidosis *in vivo* is unclear, but a molecular role for Cu(II) has been implicated from *in vitro* studies and from a consideration of Cu(II) concentrations under dialysis conditions. Studying the molecular level interactions between Cu(II) and β2m by techniques such as X-ray crystallography, NMR, etc. is complicated by the protein's tendency to aggregate in the presence of this metal. Thus, we have begun to investigate a new method based on mass spectrometry (MS) to study Cu-β2m binding under low protein concentrations in which protein aggregation is slow.

The MS-based method that we are developing relies on metal-catalyzed oxidation (MCO) reactions to generate reactive oxygen species (ROS) that oxidize amino acids bound to Cu(II). After oxidation, MS is used to sequence the protein and identify the modified sites. Experiments with proteins having well-defined Cu(II) binding sites (e.g. Cu/Zn SOD, azurin) have allowed us to identify MCO reaction conditions that lead to oxidation of *only* the amino acids bound to Cu(II). The MCO reaction conditions necessary to achieve such site-specific oxidation include the presence of 100 mM ascorbate, as a reducing agent, and atmospheric O_2 and 1 mM of H_2O_2 or $S_2O_8^{2-}$ as oxidizing agents.

Using the MCO/MS method, we have been able to elucidate the Cu(II) binding site of β2m. Cu(II) binds to β2m via the N-terminal amine, a backbone amide nitrogen near the N-terminus, and His31. This binding site suggests that Cu(II) destabilizes the N-terminal β-strand of β2m, allowing the protein to partially unfold and aggregate. A variation to our MCO/MS method in which the reaction conditions are "detuned" allows us to gather further insight into the effect of Cu(II) on β2m destabilization by gathering insight into the secondary coordination structure of Cu(II).