Examining the Structure-Function Relationship of Enzymes using Temperature-Controlled Nanoelectrospray Mass Spectrometry

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Tools for investigating the structural dynamics of enzymes are of great importance to researchers in academia and industry. Most solution-based techniques cannot readily provide detailed information for how the quaternary structure of a protein changes with temperature, thus limiting our understanding of their structure-function relationship. By coupling temperature-controlled nanoelectrospray ionization (TC-nESI) with mass spectrometry (MS), it is possible to measure such changes. In this work, the effects of temperature on the structure of beta-galactosidase (tetramer), jack bean urease (hexamer), and alcohol oxidase (octamer) were examined by TC-nESI. These melting data were compared with enzyme kinetics data to obtain a better understanding how of temperature-induced changes in their structure affect their function.

During the TC-nESI experiments of beta-galactosidase, the tetramer dissociated into monomer and dimer species with increasing temperature. The dimers and monomers appear at similar temperatures, suggesting that the tetramer has two dissociation pathways. Two other prominent charge state series were detected in the spectrum, which had calculated masses of 32.9 and 83.6 kDa. The sum of these masses matches the monomer mass, indicating they are fragments. This fragmentation was also found to occur slowly at 35 ℃. However, it increases rapidly with temperature. These data would explain why beta-galactosidase has been observed to lose activity over time, and rapidly with increasing temperature.

Alcohol oxidase gave rise to multiple dissociation products with increasing temperature. Monomeric species with and without FAD were detected during these experiments. The monomers without FAD appeared at lower temperatures than those with FAD, indicating that if the cofactor is not present, the complex is more readily dissociated. At higher temperatures, the alcohol oxidase complex dissociated into dimers and trimers. Previous studies on this enzyme show that this enzyme reaches its maximum activity before the dissociation of the complex, and rapidly losses activity as the complex dissociates.

When jack bean urease was examined using TC-nESI, several dissociation and aggregation products were observed. The hexamer gained high charge states, the ion mobility (IM) analysis of which indicated they are extended conformations. Monomers and trimers of different conformational states were also observed as the hexamer dissociated. Aggregates with masses corresponding to nonamers and octadecamers we also detected during these experiments. Interestingly, the temperature at which the octadecamers were at their most abundant also corresponds to the temperature at which this enzyme reaches its maximum activity. This suggests that aggregation could play a role in the activity of this enzyme.