Threshold collision-induced dissociation measurements of protonated peptides

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The "pathways in competition" model has significantly improved our understanding of peptide fragmentation reactions and allows prediction of activation barriers for generation of key sequence ions for small model peptides. In this study, threshold collision-induced dissociation (TCID) on a guided ion beam instrument and variable time ion trap CID experiments were used to study the fragmentation of a group of protonated tripeptides. Our focus was on formation of b_2^+ and y_1^+, and the influence of peptide sequence on the relative threshold energies for generation of these product ions. In addition, density functional theory (DFT) calculations were used to predict structures and energies for relevant minima, including reaction intermediates, post-reaction complexes and proton-bound dimers, and transition states.

Tripeptides (GGG, GAG, and GGA) were either purchased or synthesized using Wang resin and conventional Fmoc chemistry. Protonated ions were generated using electrospray ionization. Ion trap CID was performed using a ThermoFinnigan LCQ-Deca mass spectrometer with He as the bath/collision gas. A guided ion beam tandem mass spectrometer was used to measure kinetic-energy-dependent cross sections for CID of the same peptides. Density functional theory calculations (B3LYP/6-31+G(d,p) level) were performed using the Gaussian 03 program.

Ion trap CID of the peptides generates nearly exclusively b_2^+. Initial experiments with AGG, GAG and GGA (using variable energy and variable time ion trap CID experiments) suggest that energy required to produce b_2^+ is sensitive to the position of the A residue, with an observed trend GGA > AGG > GAG. These qualitative conclusions were then examined using the guided ion beam instrument where a more diverse group of product ions, including a_3^+, y_2^+, a_2^+, and a_1^+, were generated from the respective peptides. Thresholds for generation of the various product ions have been measured for GGG, GAG, and GGA. In GGG, the threshold for generation of b_2^+ is lower by ~ 1 eV (center of mass frame) compared to other products such as a_3^+, y_2^+, and y_1^+, consistent with the lower energy (multiple collision) ion trap CID experiments. For GAG, the b_2^+ threshold shifts down another 0.3 eV, a_2^+ shifts down ~1 eV, and other products retain similar energy profiles. For GGA, the b_2^+ threshold is comparable to that for GGG, but now the y_1^+ threshold is very similar and much lower than for GGG and GAG.

The trends with respect to how easily the b_2^+ ion is generated, both in the ion trap and guided ion beam experiments, are consistent with the relative energies for transition states and products predicted by DFT calculations. For GGG, the transition state for generation of b_2^+ is 30-50 kJ/mol lower than those for the b_3^+ and y_2^+ pathways. This supports the observation of b_2^+ as the dominant fragment in the low energy ion trap CID experiments, and the relative thresholds for the respective products in the guided ion beam experiments. Predictions of transition state and product energies for the other peptides examined are also consistent with both the ion trap and guided ion beam measurements.