SUPPORTING INFORMATION

Host-Guest Chemistry in the Gas Phase: Selected Fragmentations of CB[6]-Peptide Complexes at Lysine Residues and Its Utility to Probe the Structures of Small Proteins

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EXPERIMENTAL

Chemicals and Reagents. All model peptides (A_nKA_{9-n}, n = 0, 1, 3, 5, 7, 9), LiK, and LeK are purchased from Peptron (Daejeon, Korea). The peptide LVP is purchased from Anygen (Gwangju, Korea). Ubiquitin and formic acid are purchased from sigma-aldrich. All solvents (water and acetonitrile) are HPLC grade and purchased from J. T. Baker (Phillipsburg, NJ). CB[6] is prepared by the reaction of glycoluril and formaldehyde based on a literature procedure.[1] CB[6] stock solution is prepared by dissolving CB[6] in 80% formic acid. Then, sample solutions are prepared in a solvent consisting of 50/50 water and acetonitrile by volume. The final concentrations of CB[6] and the guest peptide are adjusted to 50 μM and 10 μM, respectively.

Electrospray Ionization Mass Spectrometers. Model peptide experiments are performed on a Waters Synapt G2 HDMS traveling wave ion mobility orthogonal acceleration time-of-flight in positive ion mode. Source temperature of 100 °C, capillary voltage of 3 kV, desolvation temperature of 250 °C, and cone voltage of 40 V are set as parameters for electrospray ionization (ESI). Helium gas is introduced to the Helium Cell at a 170 mL/min flow rate. Nitrogen drift gas is introduced to the TWIMS stacked ring ion guide (SRIG) at a 70 mL/min flow rate, which corresponds to 2.69 Torr. The optimized traveling wave (T-wave) height and velocity are 21 V and 300 m/s, respectively. For each sample, 100 spectra are obtained and averaged for analysis. The drift times of analyte ions are determined from the location of the ion mobility peak maxima extracted using MassLynx (version 4.1) software (Waters corp. Milford, MA). A Thermo Scientific LTQ Velos dual ion trap mass spectrometer is utilized in positive ion mode for the experiments of ubiquitin. Electrospray voltage of 3.5 kV and capillary temperature of 215 °C are set as parameters for ESI. Each spectrum is averaged from 100 scanned spectra obtained using enhanced scan mode for analysis. The nomenclature for the parent and fragment ions is adopted from Roepstorff and Fohlman.[2] The star symbol (*) left superscript for the fragment ion refers to the presence of CB[6] in the fragment. For example, a y_n^{2+} fragment ion complex with CB[6] is referred to as $^*y_n^{2+}$.

Computational Modeling. The mechanisms and energetics of the observed reactions are evaluated with doubly protonated CB[6] complex of tetra-peptide, AAKA, based on optimized structures and their corresponding energies for reactants and products using density functional theory (DFT) calculations. More than 3000 possible molecular conformations are investigated and their corresponding potential energy maps are determined at Amber force field using HyperChem 8.0 (Hypercube, INC., Gainesville, FL). The partial charge of the molecule is assigned at PM3 level before searching the conformations. Then, the lowest-energy structures are determined using DFT with a number of candidate low energy structures from the molecular dynamic calculations. The DFT calculations are performed using Gaussian 09 (Gaussian Inc., Wallingford, CT)[3] utilizing the Becke three-parameter functional (B3)[4] combined with the correlation functional of Lee, Yang, and Parr (LYP).[5] The 6-31G basis set is used for DFT optimizations then single point calculation are carried out using the 6-31G(d) basis set without zero-point correction.[6]

Collision Cross Sections. The collision cross sections (Ω_D) of doubly charged CB[6] and CB[6] complex of 5IPA are evaluated using calibration method adopted from Thalassinos *et al.*[7] Hexaglycine and Gly-Arg-Gly-Asp-Ser are used to create a calibration curve with previously published Ω_D values.[8] The effective drift time of the calibrant is corrected for mass independent and mass dependent time. The effected drift time was plotted against the corrected published collision cross-section. The plot is used to fit a power trend. The equation from the fitting result is used to estimate collision cross-sections of CB[6] and its complex molecules. For the calculations of theoretical collision cross section of ions, project approximation (PA) method, which is based on a Lennard-Jones description of the interaction potential was used.[9] Three to five lowest energy structures from DFT calculations are used to obtain an average Ω_D.

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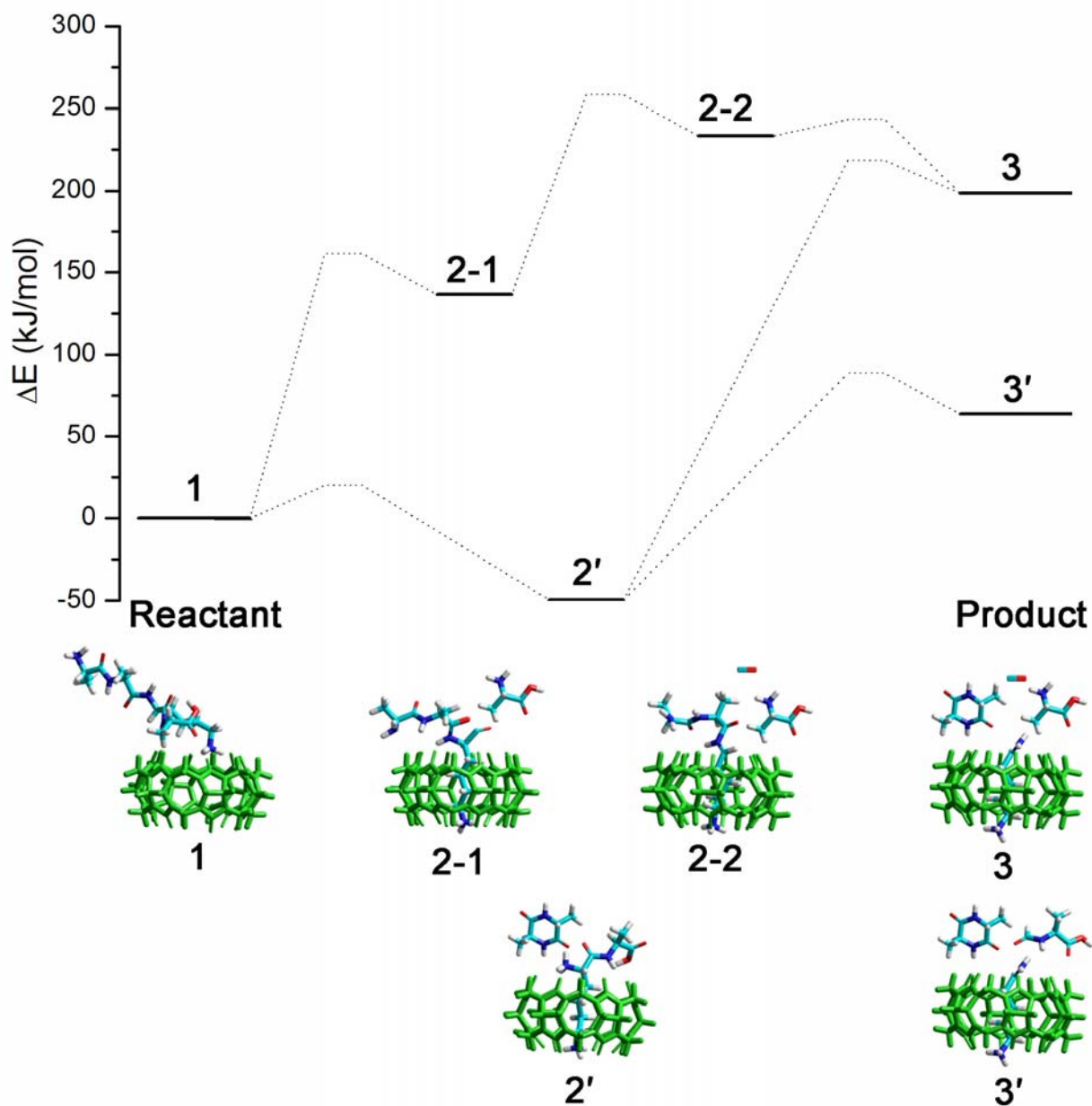
Figure S1. Reaction coordinate diagrams showing relative energies in kJ/mol for the internal iminium fragment to yield CB[6]-5IPA from doubly charged CB[6] complex of AAKA at the B3LYP/6-31G(d) level. Barrier heights are not known. Optimized geometries are obtained at the B3LYP/6-31G level. The geometries for reactants, products, and intermediates are shown below the diagram. The reaction mechanism of each numbered step is shown in Scheme 2.
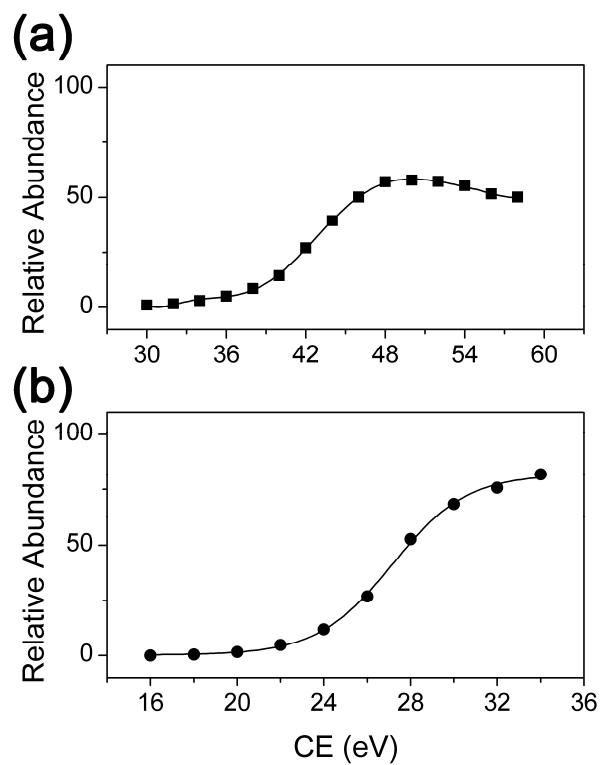
Figure S2. Plots of fraction of CB[6]-5IPA as a function of collision energy for CB[6] complexes of (a) LiK and (b) LeK.
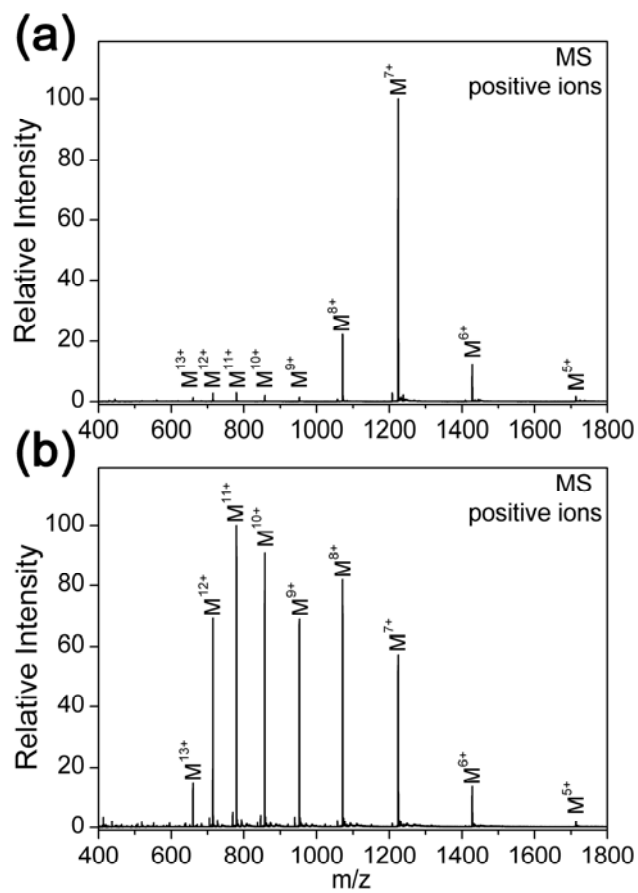
Figure S3. ESI-MS spectra of ubiquitin in (a) water and (b) 50/50 water/acetonitrile.