Communication

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Extreme Stability of an Unsolvated α-Helix

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Isolated α-helices are marginally stable in solution. For example, designed alanine-based peptides (which are among the most strongly helical peptides in aqueous solution) consist of an ensemble of partially helical structures that are around 50% helical at room temperature and almost completely melted at 70 °C.[1,2] The stability of the helical conformation depends on the solvent.[3−5] However, the role of the solvent in stabilizing the helix is poorly understood. Here we show that unsolvated Ac-$A_{15}K+H^+$ remains almost completely helical to at least 450 °C (725 K) where the peptide is almost entirely dissociated.

Unsolvated Ac-$A_{15}K+H^+$ peptides have previously been shown to be almost completely helical at room temperature, while unsolvated Ac-$KA_{15}+H^+$ has a globular conformation (a compact, random-looking, three-dimensional structure).[6,7] This structural difference is attributed to protonation at the lysine side chain leading to helix-stabilizing interactions in the former and helix-destabilizing interactions in the latter. At higher temperatures, both conformations are expected to unfold (or melt) into random coils. Recent simulations suggest that the helix−coil transition for neutral polyalanine peptides is a first-order phase transition *in vacuo*.[8−10] Melting temperatures of around 500 K (225 °C) were predicted, but there was no experimental evidence to support such high transition temperatures. The high-temperature ion mobility measurements reported here were performed to test these predictions and determine the melting temperatures for some alanine-based peptides *in vacuo*. The mobility provides a measure of the average collision cross section of the ion, which in turn depends on its structure. Helical, globular, and random coil conformations can be easily resolved using this approach. The helix has a larger cross section than the globule, and the unfolded random coil has a larger cross section than the helix.

The ion mobility measurements were performed using a new high-temperature drift tube which will be described in detail elsewhere. The apparatus consists of an electrospray source, the drift tube, a quadrupole mass spectrometer, and a detector. The 30.8 cm long drift tube is divided into six sections that are individually heated by thermocoax heaters. The temperature of each section is regulated by a microprocessor-based controller to better than ±1 °C. The helium buffer gas pressure was 3−5 torr and the drift voltage was 280 V. The peptides were synthesized on an Applied Biosystems Model 433A peptide synthesizer using *FastMoc* chemistry. After cleavage with a cocktail of 95% trifluoroacetic acid (TFA) and 5% water, the peptides were precipitated, centrifuged, and then lyophilized. The electrospray solutions were prepared by dissolving 2 mg of unpurified peptide in 1 mL of TFA and 0.1 mL of water. Drift time distributions were recorded by admitting a short pulse of peptide ions into the drift tube and recording their arrival times at the detector.

The drift time distributions for Ac-$A_{15}K+H^+$ and Ac-$KA_{15}+H^+$ show only a single narrow feature throughout the temperature range examined. The average drift times (which are around 5−10 ms) are converted into collision cross sections using standard methods.[11]

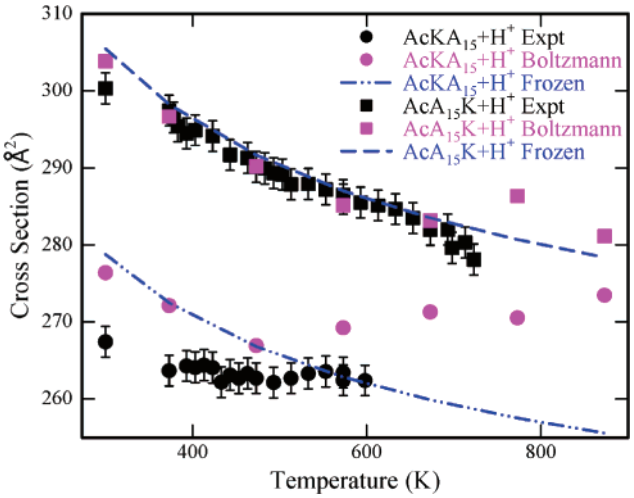

Figure 1. Measured and calculated collision cross sections for Ac-$A_{15}K+H^+$ and Ac-$KA_{15}+H^+$. The solid black points are the measured values. The purple points are Boltzmann-weighted average calculated cross sections derived from MD simulation results. The dashed blue lines show the calculated temperature dependence of the collision cross sections for a rigid α-helix (top) and rigid globule (bottom).

The cross sections are plotted against temperature in Figure 1. The upper series of points is for Ac-$A_{15}K+H^+$ (which adopts an α-helical conformation at room temperature) and the lower series is for Ac-$KA_{15}+H^+$ (which adopts a globular conformation at room temperature). The measured cross sections for Ac-$A_{15}K+H^+$ (black squares) systematically decrease with increasing temperature, while those for Ac-$KA_{15}+H^+$ (black circles) remain nearly constant as the temperature is raised. The signals disappear because of fragmentation at around 600 K for Ac-$KA_{15}+H^+$ and at around 725 K for Ac-$A_{15}K+H^+$. The dominant dissociation pathway for the Ac-$KA_{15}+H^+$ peptide is loss of a water molecule followed by loss of alanine units to give the b_n^+, b_{n-1}^+, b_{n-2}^+, b_{n-3}^+, etc. product ions (using the standard notation[12]). For the Ac-$A_{15}K+H^+$ peptide the dominant dissociation pathway is formation of a charged lysine fragment (the y_1^+-H_2O ion at m/z 129). In both cases, dissociation appears to originate from the C-terminus. Similar fragmentation processes have been observed in collision-induced dissociation studies.[13,14]

To interpret the results in Figure 1, it is necessary to account for the temperature effects on the collision cross sections. There are two contributions: changes in the conformation with temperature and changes in the cross sections with temperature. As the temperature is raised, collisions between the peptide ion and the buffer gas become harder (the helium rides further up the repulsive part of the intermolecular potential). In addition, the long-range attractive interactions between the ion and buffer gas become less important. Thus the cross sections for the same structure systematically decrease with increasing temperature.

To model the temperature dependence of the cross sections, molecular dynamics (MD) simulations were performed for Ac-

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$A_{15}K+H^+$ and Ac-$KA_{15}+H^+$ at seven different temperatures: 300, 373, 473, 573, 673, 773, and 873 K. The simulations were performed with the MACSIMUS suite of programs[15] using CHARMM potentials[16] (21.3 parameter set). Forty 240 ps MD simulations were performed at each temperature for Ac-$A_{15}K+H^+$ starting from an α-helical structure. Sixty simulated annealing runs were performed for each temperature for Ac-$KA_{15}+H^+$ using a fully extended starting structure. The simulation time was 915 or 1200 ps per run depending on the cooling schedule (two different cooling schedules were employed: a linear step down and a step down with temperature spikes[17]). Cross section calculations were performed using the trajectory method,[18] which uses model potentials to treat the interactions between the peptide ion and buffer gas. Average cross sections were calculated by averaging over 50 snapshots taken from the last 35 ps of each simulation. The calculated cross sections are expected to be within 2% of the measured value if the conformation is correct.

The dashed blue lines in Figure 1 show the cross sections calculated as a function of temperature using the lowest energy conformations found in the 300 K simulations (a helix for Ac-$A_{15}K+H^+$ and a globule for Ac-$KA_{15}+H^+$). These results show the behavior of the cross sections assuming that the structure remains "frozen" as the temperature is raised. The purple points in Figure 1 show the Boltzmann-weighted average cross sections from all the simulations performed at each temperature. For Ac-$A_{15}K+H^+$, the average cross sections and the cross sections for the frozen conformation (a helix) remain in reasonable agreement up to 673 K. The helix is the dominant conformation found in the MD simulations for Ac-$A_{15}K+H^+$ from 300 to 673 K. At 773 K and above, the Boltzmann-weighted cross sections are larger than the cross sections for the frozen conformation, indicating significant disruption of the helix. Inspection of the MD trajectories shows that the structure fluctuates between helical, compact, and unfolded (random coil-like) conformations at 773 K. Furthermore, the helical conformation (when it exists at 773 K) is often frayed, particularly at the N-terminus. For Ac-$KA_{15}+H^+$, the Boltzmann-weighted cross sections and the cross sections calculated for the frozen conformation (a globule) remain in reasonable agreement up to only 473 K. Above 473 K the average cross sections become systematically larger than the cross sections for the frozen conformation. The deviation appears to result from a nonspecific expansion of the globular conformation. This expansion cancels out the expected decrease in the cross section with increasing temperature, so that the cross section for the globule is predicted to be almost temperature independent.

The measured cross sections for the Ac-$KA_{15}+H^+$ peptides are significantly smaller than the calculated values at room temperature. This discrepancy, which has been observed in previous studies,[19] occurs because MD is not effective at locating compact low-energy conformations (even with simulated annealing). An evolutionary algorithm-based method can locate compact low-energy conformations, but at considerable computational expense.[20] The measured cross sections for the Ac-$KA_{15}+H^+$ peptide show little dependence on temperature, as predicted by the MD simulations (though the measured and calculated cross sections do not match exactly). The Ac-$KA_{15}+H^+$ globule survives to around 600 K, beyond which the signal becomes too small to measure because of dissociation.

The measured cross sections for the Ac-$A_{15}K+H^+$ peptide are in excellent agreement with the calculated cross sections up to 673 K. Above 673 K the measured cross sections appear to diverge slightly from the calculated ones. Beyond around 725 K measurements are no longer possible because the signal disappears due to fragmentation. These results indicate that the Ac-$A_{15}K+H^+$ peptide remains a helix up to at least 725 K. Thus the Ac-$A_{15}K+H^+$ helix and the Ac-$KA_{15}+H^+$ globule do not melt into a random coil structure as the temperature is raised, they both remain folded to the point where they are sufficiently energized to dissociate.

The results presented above show that the unsolvated Ac-$A_{15}K+H^+$ helix is remarkably resilient, surviving to at least 725 K without melting. The fact that the helix survives to the point where the peptide dissociates indicates that the combined contributions of the non-covalent interactions that stabilize the helical conformation exceed the energy of a covalent bond. In addition to the network of helical hydrogen bonds, the Ac-$A_{15}K+H^+$ helix is stabilized by the C-terminus lysine through helix capping interactions and by the interaction between the charge and the helix macrodipole. The globular Ac-$KA_{15}+H^+$ peptide fragments at a significantly lower temperature than helical Ac-$A_{15}K+H^+$. This suggests that the helical conformation may stabilize the Ac-$A_{15}K+H^+$ peptide and delay its fragmentation. The dissociation of the helical Ac-$A_{15}K+H^+$ peptide appears to be quite closely correlated with disruption of the helix in the MD simulations. Presumably, uncoupling the lysine side chain from the C-terminus is a key precursor step to dissociation. The dominant fragment ion is a lysine fragment (the y_1^+-H_2O ion), and the production of this fragment obviously requires the lysine side chain to be uncoupled. The observation that both peptides retain their conformations close to the point where they dissociate is consistent with studies of the collision-induced dissociation of proteins,[21] where it appears that the solution conformation can be at least partly transferred to the gas phase and reflected in the fragmentation patterns.

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