ABSTRACT

The focus of this study was to examine whether solvent effects on the *de novo* peptide ACK16 are maintained in the gas phase upon ionization. Circular Dicroism (CD) was used to monitor the elipticity (helix propensity) as a function of mole fraction of trifluoroethanol (TFE), a well known stabilizing solvent. These measurements were compared with Ion Mobility measurements from an ESI / mobility cell / quadrupole mass spectrometer. In addition to varying TFE concentration, solutions were made with different pH levels to monitor the charge state distribution of ACK16, $ACK16^{3+}$ being the highest charge state. The peptides were sprayed from a nanospray set up using a a picotip needle. Arrival Time Distributions (ATD's) were measured to obtain the mobility of the ion which in turn determined the ion's collisional cross-section. The cross sections are compared to those from AMBER calculations in order to obtain a pictorial representation of the ion with the particular average cross section. The $ACK16^{3+}$ structure could be determined with confidence because only one conformation was found in the ATD. The corresponding cross section matched up within experimental error with that of the calculated structure. However, $ACK16^{2+}$ is more complicated because there is ambiguity in placement of the two positive charges on a peptide with three protonation sites. Also, complication is added because there are two Cystines in the peptide that are known to form disulfide bonds intramolecularly and intermolecularly. The experimental ATD's also showed two conformations. Analyzing the same peptide with the Cystines replaced with Alanine would perhaps clarify this issue.

Figure 1

De Novo Peptide ACK16

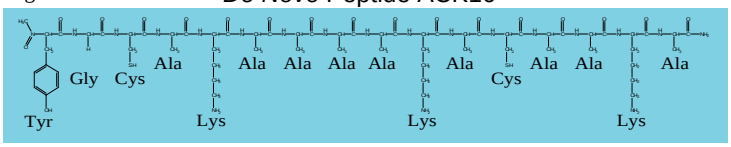
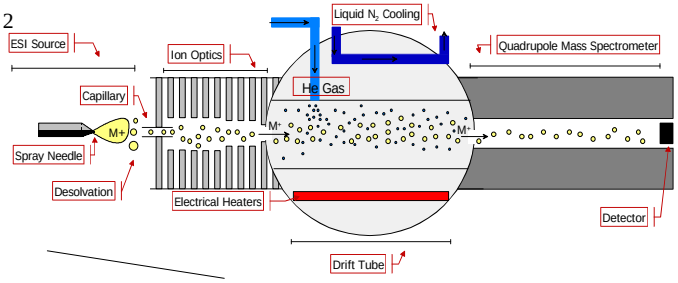


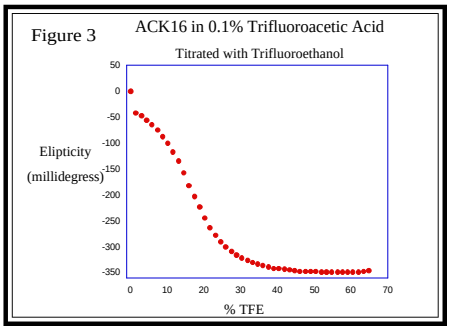
Figure 2



Desolvation

- The imposed electric field causes excess charge to accumulate in solution droplets released from spray needle
- Because of Coulombic repulsion, the sprayed drops break into smaller droplets
- Separation process occurs until the final charged droplets contain only one protonated peptide ion.

Figure 3



Background

De novo peptides such as ACK16 are strategic peptides with which to study secondary structure because they are specifically sequenced to obtain a specific structural motif. The α-helix forming propensity of ACK16 is examined under different solvent conditions. It is well known that Trifluoroethanol (TFE) stabilizes secondary structure. The figure to the left shows the amount of elipticity of the peptide versus percent TFE in water using Circular Dichroism (CD). It shows as the TFE concentration increases the amount of helical peptide also increases. We used these measurements to monitor the conformation of ACK16 in the gas phase, originating from electrospray of different solvent conditions using ion mobility experiments

Figure 4

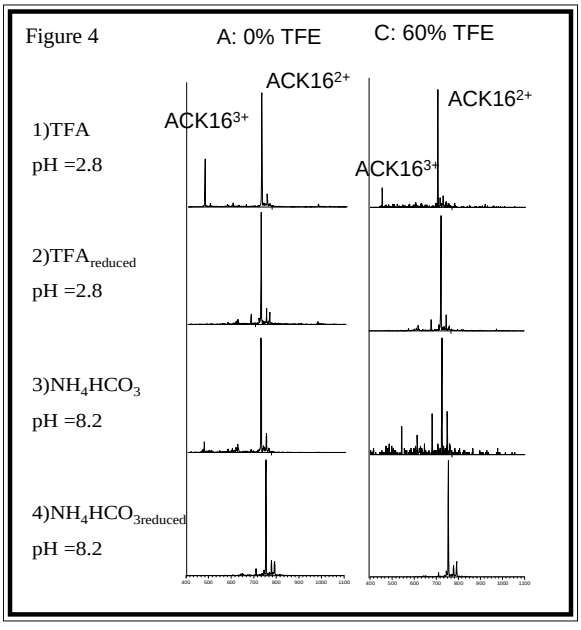
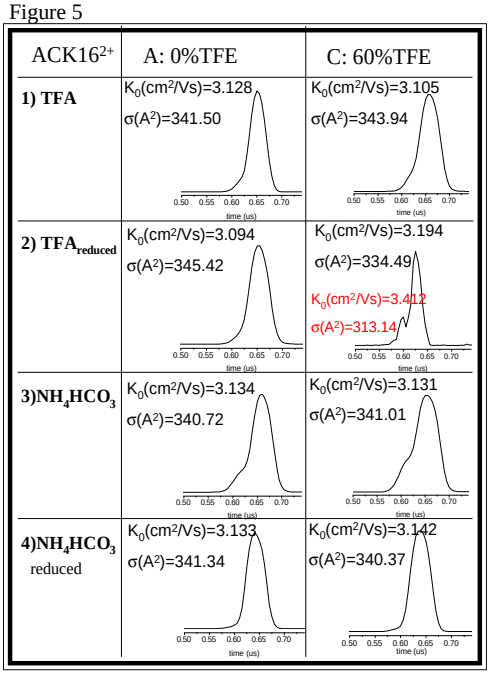


Figure 5



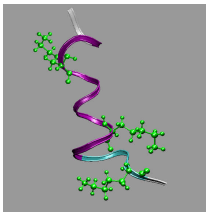
Summary of Experimental Results

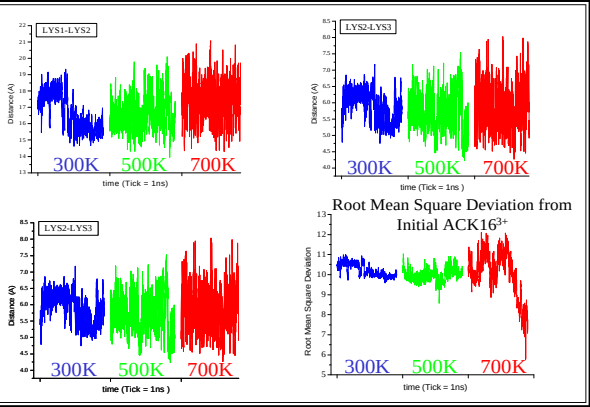
The mass spectra of ACK16 (figure 4) show that the non-reduced solutions have two charge states, $ACK16^{3+}$ at m/z 504 amu and $ACK16^{2+}$at m/z 754 amu. However the lower pH non-reduced solutions have a larger intensity for $ACK16^{3+}$ where each Lysine is protonated. Similarly, all of the Lysines are protonated in solution, however they are balanced by counter ions which gives the solution an overall neutral charge. The arrival time distributions (ATD) for $ACK16^{3+}$ (not shown) reveal a narrow distribution with an average mobility (K_0)= 4.521 (cm^2/Vs) and collisional cross section (σ) = 354.55 A^2. These results indicate a single conformation for $ACK16^{3+}$.

The results become much more complicated for the $ACK16^{2+}$ species, which is the dominant peak in each solution. The ATDs of this ion (figure 8) have much wider distributions that are indicative of two conformations existing for the same m/z. Fortunately, solution 2C reveals two peaks, verifying the presence of two conformations of $ACK16^{2+}$. Deciphering what these two peaks are is not trivial because there are two different possibilities in the primary sequence of ACK16. It is well known that dimers may form *via* disulfide bridges internally in the peptide, or as a dimer between peptides. High resolution mass scans were performed to assess any contribution to the dimer peak from an ion of m/z 753, however, they were inconclusive. As a final measure, solutions at each pH were examined with and without a reducing agent to eliminate disulfide bonds hoping to eliminate some of the ambiguity. In addition computational analysis was used to help determine the conformations observed in the gas phase ion mobility experiments.

Figure 6

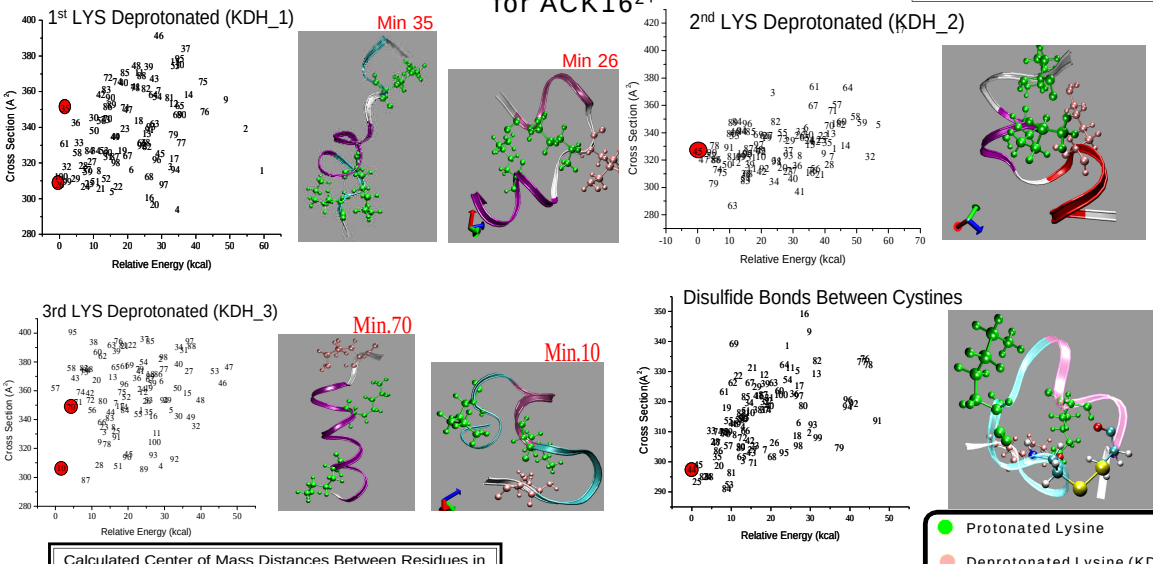
Calculated Center of Mass Molecular Dynamics Simulation Between Residues

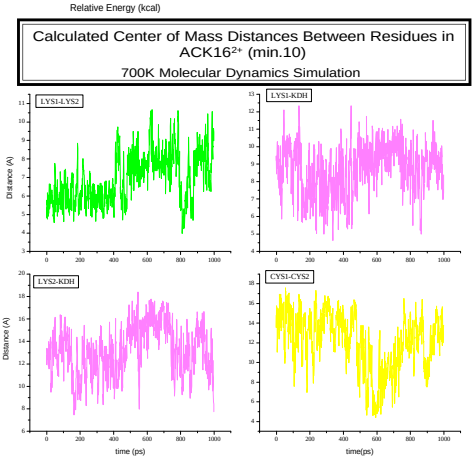




Cross Sectional Scatter Plots reveal an average low energy structure of 379.5A^2, which is consistent with the results observed in the experiment. The MD calculations at temperatures ranging from 300K to 700K show that $ACK16^{3+}$ does not deviate from the initial structure shown until about 700K where it seems to move more freely. This indicates that the helical structure in the gas phase is quite stable Tracking the movement of each lysine in the peptide chain confirms the stability of the helix (figure 6).

Cross Sectional Scatter Plots and Structures for $ACK16^{2+}$





Protonated Lysine

Deprotonated Lysine (KDH)

Sulfur (Cystine)

Conclusion

- $ACK16^{3+}$ has one conformation which most likely has an α-helical conformation
- It is clear that $ACK16^{2+}$ has at least two structures; however, it is not clear what the two conformations are.
- Is one of the conformations for $ACK16^{2+}$ due to a disulfide bond between the Cystines?

Future Work

- Perform experiments on AK16 where the Cystines are replace by Alanines
- Look at biologically relevant cases such as Cytochrome C, which has a structure that is highly dependent on solvent conditions