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Factors that Influence Helical Preferences for Singly-Charged Gas-Phase Peptide Ions: The Effects of Multiple Potential Charge-Carrying Sites

Janel R. McLean^{†,%}, John A. McLean^{†,#}, Zhaoxiang Wu[†], Christopher Becker[†], Lisa M Pérez[‡], C. Nick Pace[§], J. Martin Scholtz[§], and David H. Russell^{†,*}

[†] Texas A & M University, Department of Chemistry, Laboratory for Biological Mass Spectrometry, College Station, TX 77843

[‡] Texas A & M University, Laboratory for Molecular Simulation, College Station, TX 77843

[§] Texas A & M University, Department of Molecular & Cellular Medicine, College Station, TX 77843

Abstract

Ion mobility-mass spectrometry is used to investigate the structure(s) of a series of model peptide $[M + H]^+$ ions to better understand how intrinsic properties affect structure in low dielectric environments. The influence of peptide length, amino acid sequence and composition on gas-phase structure is examined for a series of model peptides that have been previously studied in solution. Collision cross-sections for the $[M + H]^+$ ions of Ac-(AAKAA)_n-NH₂ ($n = 3 - 6$) and Ac-Y(AEAACA)_n-NH₂ ($n = 2 - 5$) are reported and correlated with candidate structures generated obtained using molecular modeling techniques. The $[M + H]^+$ ions of the AAKAA peptide series each exhibit a single, dominant ion mobility arrival time distribution (ATD) which correlates to partial helical structures, whereas the $[M + H]^+$ ions of the AEAACA ion series are composed of ATDs which correlate to charge-solvated globules (*i.e.* the charge is coordinated or solvated by polar peptide functional groups). These data raise numerous questions concerning intrinsic properties (amino acid sequence and composition as well as charge location) that dictate gas-phase peptide ion structure, which may reflect trends for peptide ion structure in low dielectric environments, such as transmembrane segments.

Keywords

Anhydrous peptide structure; Ion mobility-mass spectrometry; Molecular dynamics simulations; Helical content

Introduction

Helices are the most common secondary structural element of transmembrane proteins,¹ thus the forces that dictate formation and stability of helices are fundamental to understanding membrane protein folding. On the other hand, much of our understanding of protein folding is based on experiments performed in aqueous solutions and we have very limited understanding of the effects of low dielectric environments and/or non-polar solvents on secondary structure.

russell@mail.chem.tamu.edu.

[%]Current address: Howard Hughes Medical Institute and Vanderbilt University School of Medicine, Department of Cell and Developmental Biology, Nashville, TN 37232.

[#]Current address: Vanderbilt University, Department of Chemistry and Vanderbilt Institute for Chemical Biology, Nashville, TN 37235.

^{2,3} The potential importance of solvent-free peptide structure studies is underscored by Pauling's prediction of helical peptide folding motifs in the absence of solvent.⁴ Over the past decade new experimental tools that permit detailed studies of solvent-free peptides/proteins have been developed. For example, combining ion mobility (IM) spectrometry, a gas-phase electrophoretic separation technique, with modern mass spectrometry (MS) and molecular dynamics (MD) simulations has produced powerful biophysical tools to study solvent-free or stepwise-solvated peptide and protein ion structure.^{5–10} Although IM spectrometry has been extensively used for fundamental ion chemistry for many years, only recently has its utility for biological studies been realized.^{11,12} The importance of IM-MS as a biophysical tool is the ability to correlate empirical collision cross-sections and accurate mass-to-charge measurements to candidate structures derived using MD and molecular orbital calculations.^{12–17} More recently, spectroscopic techniques, *i.e.*, infrared-ultraviolet double resonance spectroscopy, have been used to acquire conformation-specific data for gas-phase peptide ions. These new experimental tools combined with MD simulations and density functional theory (DFT) calculations provide new approaches to the study of secondary structure of gas-phase ions.^{18–20} Lastly, recent work by Bowers and coworkers²¹ and coworkers suggest that such studies may have direct relevance to human diseases and provide complimentary information to that derived from solution-phase studies.²²

Previous studies have shown that most singly-charged gas-phase peptide ions adopt compact, globular conformations dictated by their intrinsic gas-phase packing efficiencies, defining an average globular peptide mobility-mass correlation;^{23–26} however, some peptide ions exhibit ordered structure in the gas phase,^{11,27} yielding collision cross-sections that are either larger or smaller than those predicted by the globular peptide ion mobility-mass correlation.^{6,7,10,14,28} That is, for a given m/z value helical structures yield larger collision cross-sections, whereas intramolecular interactions such as salt-bridges or solvation of charge sites by the peptide backbone amide groups can yield smaller than predicted collision cross-sections.^{10,29,30}

Jarrold and coworkers used IM-MS and molecular modeling to study gas-phase polyaniline ions,^{13–15,28,31} which exhibit high helical content in aqueous solution;^{32–34} however, they found that polyaniline $[M + H]^+$ ions adopt extended (presumably helical) conformations only if the charge is localized near the C-terminus (*i.e.* by blocking the N-terminus by acetylation and introducing a basic, charge carrying residue near the C-terminus), which stabilizes the helix owing to favorable interactions of the positive charge with the helix macrodipole.^{14,28} Previous work clearly demonstrates correlation between peptide ion structure assignments based on IM/computational experiments^{9,14,27,28,31} and other techniques¹⁶, including circular dichroism (CD),⁷ gas-phase hydrogen/deuterium exchange,³⁵ and IR-UV double resonance spectroscopy.^{18–20}

The present work builds upon these earlier studies and is aimed at understanding how amino acid sequence affects the structure(s) of solvent-free peptide ions that contain multiple potential charge-carrying residues. The solution-phase conformation of the peptides Ac-(AAKAA)_nY-NH₂, $n = 3 - 6$, and Ac-Y(AEAAKA)_nF-NH₂, $n = 2 - 5$, has been well-characterized by CD.^{36,37} Although both series exhibit increasing helical content with increasing peptide length, the E and K side chains of the AEAAKA series (i, i+3 spacing) are significantly less helix-stabilizing (by *ca.* 40%) than the optimal spacing (i, i+4),^{33,38} owing to the spatial constraints of the helix which prevent strong side-chain interactions (*i.e.* glutamic acid-lysine H-bonding and/or ion-pairing) in the i, i+3 spacing. Here, we characterize the gas-phase conformation of these peptides using IM-MS, molecular modeling and chemical derivatization.

Experimental Methods

Sample preparation

The model helical peptides, Ac-(AAKAA)_nY-NH₂ ($n = 3 - 6$) and Ac-Y(AEAAKA)_nF-NH₂ ($n = 2 - 5$), were synthesized, lyophilized, and stored at $-20\text{ }^{\circ}\text{C}$.^{36,37} Peptide samples were prepared for matrix-assisted laser desorption ionization (MALDI) using the dried droplet method by diluting the peptides (5 mg/ml in H₂O) 1:1 with α -cyano-4-hydroxycinnamic acid (20 mg/ml in methanol) resulting in a 250:1 matrix-to-analyte ratio. We prefer MALDI because this ionization method yields almost exclusively singly-charged ions, which minimizes structural changes owing to Coulombic repulsion associated with high charge state ions.^{12,39} Although sample preparation for MALDI requires addition of a large excess of an organic matrix, our previous studies suggest that helical solution-phase structure is not altered by the presence of the matrix.^{7,40}

Ion mobility-mass spectrometry

The MALDI-IM-MS apparatus used in these studies was constructed in collaboration with Ionwerks, Inc. (Houston, TX) and based on instrumentation previously developed by our laboratory.^{10,41} MALDI was performed using a frequency-tripled solid state Nd:YLF laser (349 nm, Crystal laser, Reno, NV) operated at a frequency of 300 Hz.⁴² Singly-charged ions were directed into a 15 cm-long drift cell maintained at approximately 2.5 Torr He (measured with a capacitance manometer (Inficon, Balzers, Liechtenstein)), resulting in IM separation field strengths of $20 - 50\text{ V cm}^{-1}\text{ Torr}^{-1}$. All measurements were performed at ambient temperature (*ca.* 297 K). Under normal operation, the stainless steel MALDI target is held at ground potential, and the effects of collisional-heating of the ions were investigated by applying a positive potential to the target to obtain an ion kinetic energy of $10 - 50\text{ eV}$.

The mass resolution of the TOF mass spectrometer used for these studies was *ca.* 2500, which is sufficient to resolve the isotopic cluster for these peptides. The ATDs were generated by integrating two-dimensional IM-MS spectra across a range of m/z values that correspond to the isotopic cluster for the peptide of interest. Two-dimensional IM-MS data were acquired and analyzed using custom software (Ionwerks, Inc.).

Collision cross-section calculations

The principles of IM separations are discussed in detail elsewhere.⁴³ Briefly, ions are injected into a drift cell containing a neutral buffer gas and migrate under the influence of a weak electrostatic field. Ion-neutral collision cross-sections (Ω) are calculated according to the following equation:

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_B T)^{1/2}} \left(\frac{1}{m_i} + \frac{1}{m_B} \right)^{1/2} \frac{t_d E}{L} \frac{760}{P} \frac{T}{273.15} \frac{1}{N_0} \quad [1]$$

where Ω is related to charge of the ion (q , where $q = ze$ and z = number of charges, e = elementary charge), mass of the ion (m_i), mass of the buffer gas (m_B), temperature (T), Boltzmann's constant (k_B), the transit time of the ion packet in a drift cell (t_d) of length L , the electric field strength (E) and the gas number density at STP (N_0). All IM-MS spectra were acquired under "low-field" conditions^{43,44} at five IM voltages to accurately estimate the mass-dependent drift time correction, t_o ($t_{\text{measured}} - t_o = t_d$), which represents the time the ion resides in parts of instrument outside of the IM drift cell.

Helical content is defined by Eq. 2:

$$\text{Helical content (\%)} = \left(\frac{\Omega_{\text{obs}} - \Omega_{\text{glob}}}{\Omega_{\text{helix}} - \Omega_{\text{glob}}} \right) \times 100 \quad [2]$$

Ω_{obs} is the collision cross-section determined at the maximum of the peak profile. Ω_{glob} is the predicted collision cross-section for charge-solvated globules (derived from the MALDI ion mobility-mass correlation of 964 $[M + H]^+$ peptide ions^{24,26,45}) and Ω_{helix} is the calculated collision cross-section for a rigid α -helix. Conversion of the measured collision cross-sections to helical content (Eq. 2) normalizes the dataset for the mass-dependent relative difference between globular and helical collision cross-sections, allowing us to examine structural trends as a function of peptide length.

IM peak deconvolution

IM peak broadening is modeled using classical diffusion and flux equations.^{43,46} Peak profiles were modeled for an ion population composed of a *single* collision cross-section using Monte Carlo simulations of ion electrodynamics developed by Raznikov.⁴⁷ Predicted peak width values were used in conjunction with peak deconvolution techniques to estimate the minimum number of collision cross-sections that might be present in a single experimental IM peak.³⁵ The number of peaks fitted to the experimental data were constrained in width by the Monte Carlo simulations described above and this process was iterated until the correlation value (R^2) was >0.98 (ORIGIN v7.5).

Molecular Modeling

Candidate peptide structures were generated using simulated annealing as previously described.⁶ *In silico* models were generated using Insight II v2000.2 and simulated annealing was performed using Cerius² v4.9 (Accelrys, San Diego, CA). Initially, simulations were started from two conformations: α -helical and fully extended. Because the gas-phase basicity of lysine is approximately 16 kcal/mol higher in energy than the carboxamide C-terminus,⁴⁸ only lysine residues were considered as charge carrying sites in the simulations. Protons were covalently attached to the lysine side chain of both peptide conformations; this resulted in $2n$ starting conformations (n = the number of lysines). The final structure from each annealing cycle was minimized, generating 300 structures per trajectory.⁴⁹ In total, $600n$ ($1200 - 3600$ for $n = 2 - 6$ respectively) candidate structures were generated for each species in this first tier of modeling. A second tier of simulated annealing was performed starting from the lowest energy conformations of the first tier simulations. All molecular modeling images were generated using Insight II. The collision cross-sections of all models were calculated using the trajectory method in MOBCAL.⁵⁰

Results and Discussion

The peptides Ac-(AAKAA)_{*n*}-Y-NH₂ and Ac-Y(AEAAKA)_{*n*}-F-NH₂, abbreviated **AAKAA** and **AEAAKA**, are used to examine the effects of peptide length (*i.e.* total number of residues) and primary sequence (*i.e.* basic (K) and acidic (E) residues) on gas-phase ion structure. These same peptides AAKAA and AEAAKA were originally designed to study the effect of peptide length on solution-phase helical content,^{36,37} and to more closely approximate helical propensities of proteins in solution, the N- and C-termini were modified (N-acetylation and C-amidation) to minimize unfavorable charge-helix macrodipole interactions.⁵¹ Comparison of structural preferences for well-characterized model peptides provides a unique opportunity to investigate the forces that dictate solution-phase and gas-phase peptide ion structures. Gas-phase studies of helical structure have revealed a similar preference for charge location in anhydrous helices.^{14,28} For example, localization of a positive charge at the N-terminus

destabilizes helical structure whereas positive charge localized on the C-terminus stabilizes helical structure owing to favorable interaction with the helix macrodipole. Thus N-terminal modifications, which reduce proton-affinity (*i.e.* positive charge localization) of AAKAA and AEAAKA, are advantageous for gas-phase studies of helical preferences.

Experimental arrival time distributions (ATDs) for AAKAA and AEAAKA $[M + H]^+$ ions (Figure 1) are plotted as abundance of $[M + H]^+$ versus IM arrival time (t_d , upper axes) and collision cross-section (Ω , lower axes), and these data are summarized in Table 1. The dashed vertical lines in the figure represent the expected value (t_d and collision cross-section) for an ion population composed exclusively of charge-solvated, globular structures, and the solid vertical lines represent the collision cross-section for an ion population composed exclusively of rigid α -helical conformations.^{24,26,45} Note that the absolute differences in collision cross-section between globular and helical structures increases as peptide length increases because helix propagation impacts the collision cross-section more per amino acid due to the aspect ratio of a helix (approximated by a cylinder) compared to a globule (approximated by a sphere).⁵² The shaded peaks represent calculated peak profiles obtained by using simulations developed by Raznikov and co-workers, and the calculated peak profiles are arbitrarily centered at the experimental peak maxima. The simulated peak widths approximate the experimental profile assuming the ion population is composed of a single structure or an ensemble of closely related structure(s), *i.e.*, these peak profiles represent the expected peak profile assuming peak broadening arises exclusively from ion diffusion.⁴⁷ Clearly, the experimental ATDs are broader than predicted, thus it appears likely that the ion population is composed of a number of similar structures, which remain unresolved or are rapidly interconverting under our experimental conditions.

The ATDs for both AAKAA and AEAAKA $[M + H]^+$ ions (Figure 1A) are intermediate between globules and helices, with the AAKAA series showing a much higher preference for extended structures, whereas the ATDs for AEAAKA $[M + H]^+$ ions (Figure 1B) more closely approximate that for globular structures. The AEAAKA $[M + H]^+$ ion of $n = 2$, which yields an ATD that spans the entire range of collision cross-section values for globular and helical structures, represents an extreme example. It appears that this ion exhibits both globular and partial helical structures while the larger peptide ions of this series prefer a more globular conformation. For example, the ATD for AEAAKA $n = 3 - 5$ $[M + H]^+$ ions are skewed toward the expected value for globular structures, and the peak profiles are not as broad as that observed for the $n = 2$ ions. Clearly, this trend contradicts results from solution phase studies which indicate that helical content increases as a function peptide chain length.^{37,38}

Candidate structures for AAKAA and AEAAKA $[M + H]^+$ ions obtained from molecular modeling are shown in Figure 2. Note that the calculated collision cross-sections for these structures closely match the measured collision cross-sections (see Table 1). All of the structures for AAKAA $[M + H]^+$ ions contain helical regions, but the helices are disrupted by intramolecular charge solvation. For example, the regions of the structures contained in Figure 2C(i) show how the protonated ϵ -amino group of lysine is solvated by backbone amide groups. These interactions are similar to those previously proposed for bradykinin analogs containing an N-terminal arginine. That is, for $[M + H]^+$ ions of bradykinin fragments 1-5, 1-6, 1-7 and 1-8 the charge is localized on the arginine side chain, and the charge site is solvated by the amide backbone.³⁵ On the other hand, the structures for the AEAAKA series are best described as charge-solvated globules with the charge site buried within the peptide ion (Figure 2B and 2C(ii)). Thus, it appears that intramolecular charge solvation is the major driving force involved in determining the structure of these gas-phase ions. This hypothesis is supported by preliminary results from IM-MS studies of AEAAKA peptide ions coordinated to alkali metals or derivatized by methylation of the glutamic acid groups and acetylation of the lysine side chains (see Figure 4 and discussion below). For example, the $[M + Na]^+$ ions for both the

AAKAA and AEAACA series show a much higher helix preferences than do the $[M + H]^+$ ions, and the methyl ester and acetylated AAKAA and AEAACA $[M + H]^+$ ions also show an increase in helical preference (Figure 4 and data not shown).

We postulate that structural preferences for the $[M + H]^+$ ions are a result of intramolecular charge solvation of the charge-carrying E/K side chains which stabilize globular conformations relative to helical ones. The structures contained in Figure 2 focus on a single type of interaction, charge solvation of the protonated ϵ -amino group of lysine; however, ion-pairing (salt-bridge type interactions) between the E and K groups also accounts for significant proportion of the globular structural population. This conclusion is consistent with Jarrold's previous results which showed that insertion of an E/K pair into Ac-A₃G₁₂K in the i, i+3 spacing decreased helix abundance with respect to globular structures;⁵³ the authors suggested that the main reason for decreased helical content was competition for backbone H-bonds by side-chains, resulting in destabilization of the helix. Likewise, this conclusion is also supported by the IR-UV double resonance spectral features for Ac-Phe-(Ala)₁₀-LysH⁺ versus that for Ac-Lys(H⁺)-Phe-(Ala)₁₀, which are helical and globular, respectively.²⁰ In our earlier studies of bradykinin analogs, *i.e.*, bradykinin fragments 1-5, 1-6, 1-7 and 1-9 (RPPGFSPFR), we proposed that the protonated guanadinium group is solvated by backbone carbonyl groups to yield at least two distinct conformations;⁵⁵ however, in the case of AEAACA species, other stabilizing interactions are possible. For example, formation of proton bridges between the ϵ -amino groups of the K side-chains (R-NH₂---H⁺---NH₂-R), which are subject to rather large entropic effects owing to the required linear bond arrangement of the H-bond donor-bridging proton-H-bond acceptor,⁵⁴ and/or salt bridges of the type R-NH₂H⁺---CO₂⁻---NH₂H⁺-R provide significant stabilization of gas-phase peptide ions.⁵⁵ Our MD simulations were setup to model non-covalent interactions within the peptide ion or with an alkali metal ion, but other types of possible ionic interactions (*e.g.* salt bridges) were not modeled due to the complexity, size and number of the AAKAA and AEAACA peptides. Thus, salt-bridge interactions were not revealed by our MD simulations; however, higher level calculations suggest that these interactions can stabilize structure by 3–10 kcal mol⁻¹.⁵⁵ Lower helical content of the larger members of both ion series suggests that the effects of intramolecular solvation become more pronounced as peptide length and the number of E/K pairs increases. For example, helical preferences for AAKAA do not change dramatically for $n = 3 - 6$, whereas a sharp decrease in helical content is observed for AEAACA $n = 2 - 5$ (Figure 3). Note the differences in these same trends for the solution phase studies; appropriately spaced E⁻/K⁺ pairs stabilize helices by ion pairing; however, gas-phase E⁰/K⁰ pairs in $[M + H]^+$ ions stabilize globules.⁵⁶

Plots of gas-phase helical content (obtained by using Eq. 2) for $[M + H]^+$ ions and corresponding solution-phase helical values for AAKAA and AEAACA peptides are shown in Figure 3. Converting the measured collision cross-sections to helical content normalizes the dataset for relative differences in globular and helical collision cross-sections, and allows examination of structural trends as a function of peptide length. Note that the helical content for AAKAA $[M + H]^+$ ions remains relatively high, ranging from 50% to 60% for $n = 3 - 6$, but the helical content decreases rapidly with peptide length for AEAACA $[M + H]^+$ ions, with only the shortest peptide ion, $n = 2$, exhibiting appreciable helical content; however, even for the $n = 2$ peptide it does not appear that extended structures comprise a dominant fraction of the ion population. The rapid decrease in helical content with increasing peptide length in the AEAACA series again suggests that it is the *combination* of E/K residues (salt-bridges and/or other intramolecular interactions) which destabilizes gas-phase helices.

We tested our hypothesis regarding intramolecular interactions, *i.e.*, salt-bridges and/or charge-solvation of protonated ϵ -amino group of the lysine side chain by amide groups by examining the effects of chemical derivatization of the polar side chains on both ion ATD and collision cross-sections. To underscore our point, Figure 4A contains the ATD for AEAACA $n = 2$ $[M$

+ H]⁺ ions. The experimental peak has been fitted with a series of ten Gaussian peak profiles, each having a peak width broadened only by longitudinal diffusion (see Experimental Methods for details). Thus it appears that the ion population could be composed 10 or more different structural sub-populations. The MD simulations suggest that the principle difference among these structural sub-populations is charge location, *i.e.* how the polar functional groups of the molecule interact with the charge site. If this supposition is correct, then chemical derivatization of the charge-carrying groups should yield a different structural population (collision cross-section). Thus, acetylation of the ϵ -amino groups of lysine and/or methylation of the carboxylic acid groups of glutamate reduces the energetic stabilization afforded by charge-peptide intramolecular interactions. Figure 4B-D contains ATDs for AEAACA ($n = 2$) peptide ions that have been derivatized by addition of a single CH₃ group to the E side chains and the C-terminus (Figure 4B) or an acetyl group to the K side chains (Figure 4C). The collision cross-section for the modified AEAACA ion is larger than that obtained for the unmodified [M + H]⁺ ion, and the ATD for the derivatized ions are significantly narrower than that for the unmodified ions. Clearly, the derivatized ions adopt more extended gas-phase structures, presumably a helix. Note that the ATD for the [M + Na]⁺ ion is quite different from that of the [M + H]⁺ ion and it appears to be primarily helical. Although the [M + H]⁺ ion of the methyl ester derivative (Figure 4B) exhibits a collision cross-section that is slightly larger than that predicted for a rigid α -helix (solid line in Figure 4), we attribute this to the precision of our collision cross-section calculations which are typically $\pm 4\%$ for the trajectory method. Although we see significant changes in the collision cross-sections upon derivatization, it is equally important to note that the ATDs for the modified AEAACA ions are narrower than those for non-derivatized [M + H]⁺ ions (Figure 4A), illustrating that intramolecular interactions play a key role in determining gas-phase structure. In the absence of charge-solvating side chains, the modified peptide ions rely primarily on the backbone for charge solvation, which limits accessible low-energy conformations and results in narrower ATDs.

We also examined acetyl and methyl ester derivatives for AEAACA $n = 3 - 5$ ions and in each case we observe narrower ATDs and changes in the relative abundance of globular versus helical structures.⁵⁸ These are the first studies reported where the effects of derivatization or modification of polar side chains on ion structure has been examined by IM-MS. On the basis of these results we are hopeful that new insights concerning such effects can be derived from such studies.

It is also important to note that the [M + Na]⁺ ion of AEAACA $n = 2$ exhibits a narrow ATD corresponding to extended, helical structure, a result that suggests that coordination of charge by the [M + H]⁺ and [M + Na]⁺ species must be quite different (Figure 4D). Jarrold and co-workers previously showed that coordination of metal ions predominately occurs at the C-terminus of gas-phase helices,^{59,60} *i.e.* gas-phase helical structure is stabilized by charge-helix macrodipole interactions; however, for the AEAACA series this effect is highly dependent upon peptide length. While the [M + Na]⁺ species of the shortest AEAACA peptide exhibits an ATD predominately composed of a population indicative of an extended helix (Figure 4D), the helical content of the longer AEAACA [M + Na]⁺ ions dramatically increase with increasing peptide length (data not shown), illustrating that salt-bridge and/or other intramolecular interactions can overcome alkali metal stabilization of gas-phase helical conformations as has been previously reported for alkali adducts of alanine-rich peptides.^{59,61} We are currently investigating these effects further, and we are also exploring similar effects for negatively charged peptide ions, *i.e.*, [M - H]⁻ as well as derivatives that place the negative charge on the N-terminus.

In general we find that ATDs for helical peptides are significantly narrower (Figure 4D vs. 4A) than that for their globular counterparts, and we attribute this to structural heterogeneity of the ion population. That is, the globular ion population is composed of many, quite similar

structures, whereas the helical ion population is very structurally restricted. To estimate the minimum number of subpopulations that could remain unresolved in a peak profile, peak deconvolution analyses were performed for AAKAA $n = 6$ with peak widths constrained to that determined by Monte Carlo simulations (Figure 6, see Experimental Methods for details).⁴⁷ It appears that the experimental ATD represents an ion population composed of many different structural elements. As noted above, the ion population probably corresponds to globular and helical species; however, the 'bridging' region between the two populations could indicate that some fraction of the ion population is interconverting on the timescale of the experiment.⁶² That is, do the observed multi-modal ATDs represent a distribution of stable ion structures, or are some ions still exploring the potential energy surface in search of the most stable conformation? Low temperature (80–300 K) IM studies are currently underway to better understand the nature of these conformers.

Alternatively, questions related to thermodynamic stability and interconverting structural forms can be addressed by using ion injection (collisional heating) experiments.⁶³ These studies were performed by incrementally increasing the potential between the sample plate and the entrance to the drift cell, similar to studies reported previously.⁶³ Under these experimental conditions, we estimate that the ions experience *ca.* 10–100 low energy collisions (E/p estimated to range from 50 – 100 V cm⁻¹ torr⁻¹) prior to entering the drift cell where they are thermalized by collisions with the buffer gas. If the ions comprising a particular population are easily converted to another structure (*i.e.* helix → globule or the reverse), then we would expect to observe changes in the relative peak area as a function of the ion injection voltage. Representative data from injected ion studies for both AAKAA ($n = 3$ and 6) and AEAACA ($n = 3$ and 4) are contained in Figure 5. The ATDs for the shorter AAKAA $[M + H]^+$ ions do not change significantly, whereas the ATD for $n = 6$ changes quite dramatically. In this case, the relative abundance of the helical conformation is diminished, suggesting that helical ions are being converted to globules. Jarrold's lab showed that polyalanine ions form extremely stable helices using high temperature IM-MS and based on these results, we propose that changes in gas-phase structure from helix to globule, as we observed for AAKAA $n = 6$, indicate that a charge-solvated globule is the more stable conformation.⁶⁴ The ATD for the 100 V potential (Figure 5B, top panel and Figure 6) is bimodal with significant bridging between the globule and partial helix populations, suggesting that the ions are interconverting on the timescale of the experiment. Candidate structures for AAKAA $n = 6$ (Figure 2A) suggest partial helix conformations that are best described as a kinked helix, with the charge located on the C-terminal K, which is partially stabilized through H-bonding with backbone carbonyls^{14,28}. Thus, it is possible that heating the ion mobilizes the proton in AAKAA $n = 6$ ion due to its proximity to other potential charge site locations on the peptide, and the mobile proton results in structural changes as a consequence of disruption of the charge-macro-dipole interaction.

For the AEAACA species, collisional heating of the ions does not change either the ATD or the abundance of individual peaks, thus it appears that these ions do not undergo structural conversion under the rather mild heating conditions employed here (see data for $n = 3$ and 4, Figure 5C and 5D). In our previous studies of bradykinin fragments we found that our ability to separate individual conformers had a strong dependence on E/p (electric field/pressure ratio), which determines the effective ion temperature; at high E/p ratio the two component ATDs merged into a single, broad ATD, indicative of structural interconversion.³⁵ In this case, both the small size of the molecule, number of ion-molecule interactions and the strength of the interactions are contributing factors. Likewise, for the AAKAA species the ion-molecule interactions (most likely proton bridges or solvation of the protonated ϵ -amino group of lysine by the backbone amides) are also probably weaker than the salt-bridge interactions between the E/K groups in the case of AEAACA $[M + H]^+$ ions. For these ions we do not access effective ion temperatures that are sufficiently high to elicit structural change (*i.e.* break multiple non-

bonding E/K or peptide-proton interactions) supporting the idea that intramolecular charge solvation stabilizes globules in the AEAAKA peptide ions.

Conclusions

Our IM-MS, chemical derivatization, and molecular modeling studies of the AAKAA and AEAAKA peptides series support the following conclusions: 1) $[M + H]^+$ ions of AAKAA have a higher helical preference than $[M + H]^+$ ions of AEAAKA; 2) increasing peptide length and the number of E and or K residues significantly reduces helical content for both peptide series, suggesting that other types of intramolecular interactions are favored over helix-stabilizing ones as the number of potential interaction sites increases; 3) $[M + Na]^+$ ions of both AAKAA and AEAAKA exhibit higher helical preference than their $[M + H]^+$ ion counterparts; 4) derivatization of E (methylation) and K (acetylation) increases helical content owing to reduction in H-bonding, H^+ bridges and salt-bridges; 5) results from ion heating (collisional activation) results in interconversion of helix and globule structures for AAKAA $n = 6$; however, structural changes are not observed for the AEAAKA ions where intramolecular interactions stabilize ion structure.

In comparison to solution phase results, our gas-phase studies show a clear difference in structural preference for the AAKAA and AEAAKA model peptides in the low dielectric environment of the vacuum. Although solution-phase helicity increases as a function of peptide length for both model peptide systems, the opposite trend is observed for gas-phase studies. This result implies that the gas-phase appears to stabilize shorter helices over longer ones in a sequence dependent manner. It appears that a complex interplay between intramolecular charge solvation and helix-macrodipole effects ultimately determine the preference for gas-phase structure. Here, we focus our attention on intramolecular interactions (H^+ bridging) between the lysine residues; however, more detailed studies employing DFT calculations on the effects of salt-bridges (between E and K residues) are currently underway. The potential importance of salt-bridge interactions are implicated by the effects of derivatization (methylation) of the glutamic acids side chains on the arrival time distributions for the AEAAKA $[M + H]^+$ ions.

The results presented here serve as further demonstrate of the utility of IM-MS to probe solvent-free properties of polypeptide ions with multiple basic residues and the forces which dictate structure in low dielectric environments. Although the relevance of gas-phase data to biological systems is still an open issue, it is interesting to note that gas-phase, low dielectric environments more closely approximate the estimated dielectric of the protein interior ($E = 2-20$)^{65,66} or cell membrane ($E = 2$) than does aqueous solution ($E = 80$).⁶⁷ Studies are underway to examine the effects of step-wise solvation, metal coordination and charge state (comparisons of positive and negative ions as well as the numbers of charge sites) on the structure of the AEAAKA and AAKAA peptide series in an effort to better understand how the intrinsic chemical properties of polypeptides affect their ultimate structure and function in proteins.

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Abbreviations

ATD	arrival time distribution
DFT	density functional theory
E	dielectric constant

IM-MS	ion mobility-mass spectrometry
$[M + H]^+$	protonated molecule
$[M + Na]^+$	sodium-coordinated molecule
Ω	collision cross-section
MD	molecular dynamics
m/z	mass-to-charge ratio
CD	circular dichroism
MALDI	matrix-assisted laser desorption ionization

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Figure 1.

Ion mobility arrival time distribution (ATD, t_d , upper x-axis) and collision cross-section (Ω , lower x-axis) profiles for $[M + H]^+$ ions of (A) Ac-(AAKAA) $_n$ Y-NH $_2$ ($n = 3, 4, 5$, and 6) and (B) Ac-Y(AEAAKA) $_n$ F-NH $_2$ ($n = 2, 3, 4$, and 5). The dashed vertical lines represent the predicted collision cross-sections for globular peptide mobility-mass correlation (a best-fit to a dataset of collision cross-sections (see text)) and the solid vertical lines represent the α -helical ion mobility-mass correlation (calculated collision cross-sections for α -helices of the same amino acid sequence). The shaded profiles are simulated IM profiles for a single collision cross-section, assuming peak broadening is solely due to longitudinal diffusion.⁴²

Figure 2.

The lowest energy structures generated using molecular dynamics simulations for $[M + H]^+$ ions of (A) for Ac-(AAKAA) $_n$ Y-NH $_2$ ($n = 3, 4, 5$, and 6) and (B) Ac-Y(AEAAKA) $_n$ F-NH $_2$ ($n = 2, 3, 4$, and 5). (C) Enlarged view of two charge solvation networks within the modeled structures of (i) AAKAA $n = 3$ and (ii) AEAAKA $n = 3$. “N” and “C” indicate the N- and C-termini, respectively. The protonated lysine side chains and all atoms H-bonded to the proton are shown in cylinder representation and dashed green lines represent H-bonds.

Figure 3.

Plot of gas- (Eq. 2, [—]) and solution-phase (---) helical content versus number of basic amino acid residues for the AAKAA (■) and AEAACA (▲). Error bars represent $\pm 1\sigma$ for 10 measurements. Solution-phase data taken from Scholtz *et al.* and adapted from Rohl *et al.* using AGADIR.^{36,37,57}

Figure 4.

Arrival time distributions plotted in terms of collision cross-section for $[M + H]^+$ ions of (A) unmodified, (B) methylester-derivatized, (C) acetylated, and (D) sodium-coordinated AEAACA, $n = 2$. Panel A contains peak fitting data with peak widths constrained by peak broadening owing solely to longitudinal diffusion (shaded). The solid line is the measured ATD (parent profile). The theoretical subpopulations are filled under the parent profile and the dotted line is the composite fit (in most cases beneath the solid line). The residuals from the deconvolution analysis (not shown) were $R^2 > 0.99$. Inset in B, C and D is a predicted peak profile for the indicated collision cross-section broadened only by longitudinal diffusion (shaded). The dashed vertical line represent the globular mobility-mass correlation and the solid vertical line represent the helical mobility-mass correlation as described in Figure 1.

Figure 5. Arrival time distributions for $[M + H]^+$ ions of (A) Ac-(AAKAA)₃Y-NH₂, (B) Ac-(AAKAA)₆Y-NH₂, (C) Ac-Y(AEAACA)₃F-NH₂ and (D) Ac-Y(AEAACA)₄F-NH₂ are shown for 0, 50, and 100 V lab frame ion injection energies. The dashed vertical lines represent the globular mobility-mass correlation and the solid vertical lines represent the helical mobility-mass correlation as described in Figure 1.

Figure 6.

Peak deconvolution analysis constrained using peak widths derived from Monte Carlo simulations for AAKAA $n = 6$ $[M + H]^+$ ion (1700V, 100V injection potential). The solid line is the measured IM profile (parent profile). The theoretical subpopulations are shown under the parent profile and the dotted line is the composite fit. The residuals from the deconvolution analysis are shown in the bottom panel ($R^2 > 0.99$).

Table 1

Average mass-to-charge ratios (m/z), experimental and model collision cross-sections (Ω) and reduced mobilities (K_o) for $[M + H]^+$ ions for Ac-(AAKAA) $_n$ Y-NH $_2$ ($n = 3, 4, 5$, and 6) and Ac-Y(AEAAKA) $_n$ F-NH $_2$ ($n = 2, 3, 4$, and 5) where n = the number of peptide repeats. Observed collision cross-sections (Ω_{obs}) and reduced mobilities are reported as the average $\pm 1\sigma$ for m measurements. Model collision cross-sections (Ω_{mod}) correspond to the calculated collision cross-sections of the representative models shown in Figure 2 from MOBCAL.⁵⁰

	n	species	m/z	Ω_{obs} ($\text{\AA}^2$)	Ω_{mod} ($\text{\AA}^2$)	K_o	m
Ac-(AAKAA) $_n$ Y-NH $_2$	3	$[M + H]^+$	1460.72	362 ± 5	365	1.486 ± 0.020	10
	4	$[M + H]^+$	1873.21	446 ± 6	446	1.207 ± 0.018	10
	5	$[M + H]^+$	2285.70	522 ± 4	520	1.030 ± 0.009	10
	6	$[M + H]^+$	2698.19	609 ± 20	600	0.908 ± 0.005	27
Ac-Y(AEAAKA) $_n$ F-NH $_2$	2	$[M + H]^+$	1453.64	339 ± 12	344	1.590 ± 0.056	10
	3	$[M + H]^+$	1995.24	393 ± 3	400	1.370 ± 0.009	10
	4	$[M + H]^+$	2536.85	456 ± 6	472	1.181 ± 0.016	10
	5	$[M + H]^+$	3078.45	521 ± 3	545	1.032 ± 0.006	10