J|A|C|S
ARTICLES
Published on Web 02/13/2004

π-Helix Preference in Unsolvated Peptides

Rajagopalan Sudha, Motoya Kohtani, Gary A. Breaux, and Martin F. Jarrold*

Contribution from the Chemistry Department, Indiana University, 800 East Kirkwood Avenue, Bloomington, Indiana 47405-7102

Received August 26, 2003; E-mail: mfj@indiana.edu

Abstract: Ion mobility measurements have been used to examine helix formations in the gas phase for a series of alanine/glycine-based peptides that incorporate a glutamic acid (E) and lysine (K) at various positions along the backbone. Incorporation of an EK pair lowers the percent helix for all positions (presumably because hydrogen bonding between the backbone and the E and K side chains stabilize the nonhelical globular conformations). The largest percent helix is found when the EK pair is in an *i*,*i*+5 arrangement, which suggests that the preferred helical conformation for these peptides is a π-helix. This conclusion is supported by comparison of cross sections deduced from the ion-mobility measurements to average cross sections calculated for conformations obtained from molecular dynamics simulations. The glutamic acid and lysine may form an ion pair that is stabilized by interactions with the helix macrodipole.

Introduction

The α-helix is by far the most common helical motif in proteins. The more tightly wrapped 3_{10}-helix with *i*,*i*+3 hydrogen bonds (compared to *i*,*i*+4 in the α-helix) also occurs frequently, particularly at the ends of α-helices. On the other hand, the more loosely wrapped π-helix (with *i*,*i*+5 hydrogen bonds) is rare.[1−3] It has been suggested that π-helices only form when stabilized by very specific interactions (for example, side chain−metal ion interactions have been tailored to stabilize an isolated π-helix in solution[4,5]). There have been several reports of π-helical conformations in molecular dynamics simulations of peptides.[6−10] However, Feig et al. have recently suggested that these are the result of force field artifacts.[11] In this paper, we report experimental studies of helix formation in a series of alanine/glycine-based peptides (AG peptides) in vacuo. Our results suggest that the unsolvated AG peptides prefer a π-helical conformation, despite the absence of specific stabilizing interactions.

Studies of unsolvated peptides and proteins provide information about their intrinsic intramolecular interactions and the role they play in defining secondary and higher order structure. A wide variety of environments are important in biological systems, ranging from aqueous solution to the hydrophobic interior of membranes. Studies of unsolvated peptides are the natural starting point for efforts aimed at understanding how the environment influences the structure and properties of proteins. Recent studies have demonstrated that the helix-forming tendencies of at least some of the naturally occurring amino acids are significantly different in aqueous solution and in the gas phase. While glycine is usually thought of as a helix-breaker in solution, in the gas phase (and presumably also in transmembrane helices which can contain up to 20% glycine[12]) it appears to be near helix-neutral. Unsolvated glycine-rich peptides, such as Ac-$A_3G_{12}K+H^+$, can have a significant helix content in vacuo.[13−16] It also appears that amino acids with bulky nonpolar side chains (like valine) have a stronger tendency to form helices in vacuo than in solution because the bulky side chain destabilizes the globular state (a compact, random-looking three-dimensional structure that competes with the helix).[17,18] In the gas phase, peptide ions form a compact globule instead of the random coil that is ubiquitous for small peptides in solution. The random coil is stabilized by interactions with the solvent. In vacuo, van der Waals interactions and electrostatic interactions (particularly the self-solvation of the protonation site) drive the collapse of the random coil into a globule.

In this study, we use ion mobility mass spectrometry to examine the conformations of unsolvated peptide ions generated

(1) Low, B. W.; Baybutt, R. B. *J. Am. Chem. Soc.* **1952**, *74*, 5806−5807.
(2) Fodje, M. N.; Al-Karadaghi, S. *Protein Eng.* **2002**, *15*, 353−358.
(3) Weaver, T. M. *Protein Sci.* **2000**, *9*, 201−206.
(4) Morgan, D. M.; Lynn, D. G.; Miller-Auer, H.; Meredith, S. C. *Biochemistry* **2001**, *40*, 14020−14029.
(5) Boyington, J. C.; Gaffney, B. J.; Amzel, L. M.; Doctor, K. S.; Mavrophilipos, D. V.; Mavrophilipos, Z. V.; Colom, A.; Yuan, S. M. *Ann. N. Y. Acad. Sci.* **1994**, *744*, 10−13.
(6) Lee, K.; Benson, D. R.; Kuczera, K. *Biochemistry* **2000**, *39*, 13737−13747.
(7) Kovacs, H.; Mark, A. E.; Johansson, J.; van Gunsteren, W. F. *J. Mol. Biol.* **1995**, *247*, 808−822.
(8) Shirley, W. A.; Brooks, C. L., III. *Proteins: Struct., Funct., Genet.* **1997**, *28*, 59−71.
(9) Gibbs, N.; Sessions, R. B.; Williams, P. B.; Dempsey, C. E. *Biophys. J.* **1997**, *72*, 2490−2495.
(10) Duneau, J.-P.; Crouzy, S.; Chapron, Y.; Genest, M. *Theor. Chem. Acc.* **1999**, *101*, 87−91.
(11) Feig, M.; MacKerell, A. D.; Brooks, C. L. *J. Phys. Chem. B* **2003**, *107*, 2831−2836.
(12) Li, S.-C.; Deber, C. M. *FEBS Lett.* **1992**, *311*, 217−220.
(13) Kaleta, D. T.; Jarrold, M. F. *J. Phys. Chem. B* **2001**, *105*, 4436−4440.
(14) Kinnear, B. S.; Hartings, M. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2001**, *123*, 5660−5667.
(15) Kinnear, B. S.; Hartings, M. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2002**, *124*, 4422−4431.
(16) Breaux, G. A.; Jarrold, M. F. *J. Am. Chem. Soc.* **2003**, *125*, 10740−10747.
(17) Kinnear, B. S.; Jarrold, M. F. *J. Am. Chem. Soc.* **2001**, *123*, 7907−7908.
(18) Kinnear, B. S.; Kaleta, D. T.; Kohtani, M.; Jarrold, M. F. *J. Am. Chem. Soc.* **2000**, *122*, 9243−9256.

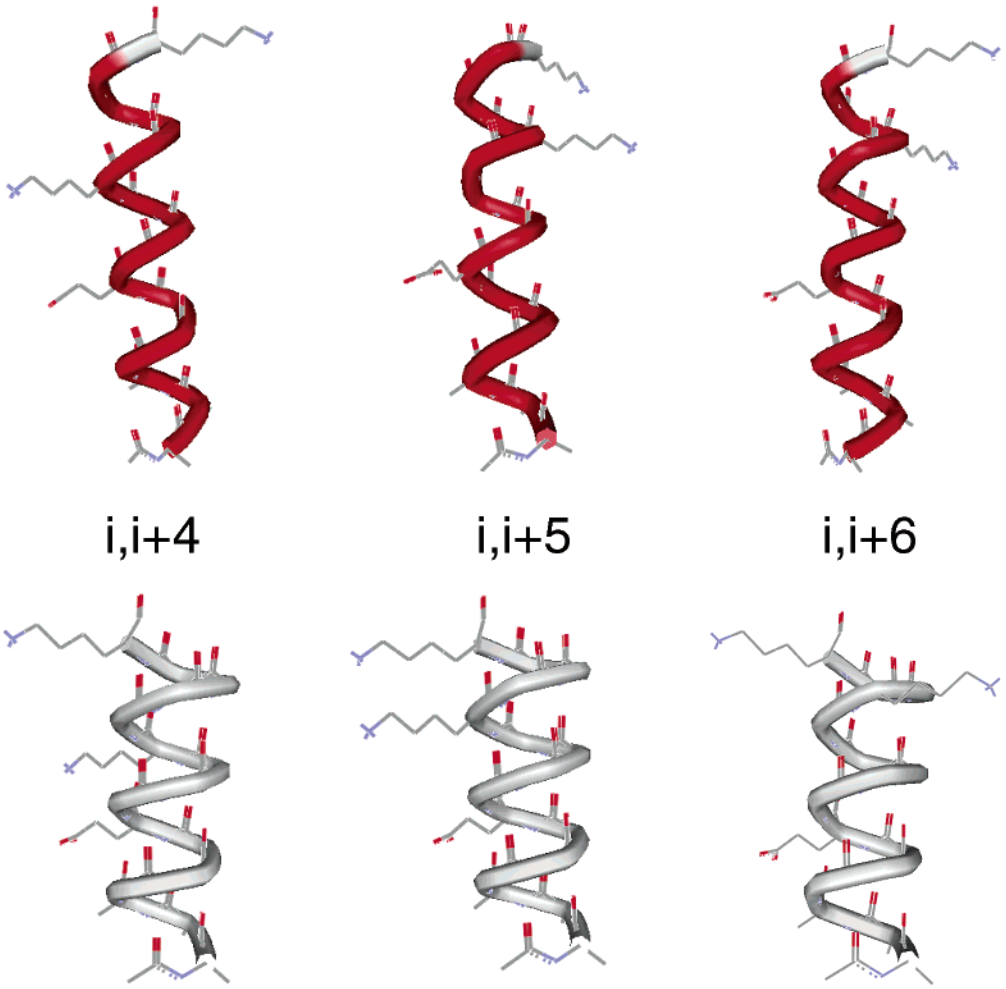

Figure 1. Side-chain orientations for *i*,*i*+4, *i*,*i*+5, and *i*,*i*+6 positions of the E and K residues in an ideal α-helix (top) and π-helix (bottom). Aliphatic hydrogens are not shown for clarity.

by electrospray. The mobility of an ion (how rapidly it travels through an inert buffer gas under the influence of a weak electric field) depends on the ion's average collision cross section, which in turn depends on its conformation. Ions with open structures have more collisions with the buffer gas and travel more slowly than ions with compact structures.[19−21] Conformations are assigned by comparing average cross sections calculated for trial structures (often derived from molecular dynamics simulations) to cross sections deduced from the measurements. Two main conformations have emerged as being important for peptides ions of the size range examined here: helices and globules. Helices have the larger cross sections and are easily resolved from the more compact globules.[22−26]

The AG peptides studied in this work incorporate E (glutamic acid) and K (lysine) at various positions along the backbone. We chose the sequence Ac-A_3G_{12}K for these studies because it has recently been shown to be partially helical.[16] Both helical and globular conformations are present at low temperatures, so the amount of helix can be compared for the peptides with E and K residues in different positions along the backbone. In all the peptides studied, the N terminus is protected with an acetyl group and a lysine or arginine residue is placed at the C terminus to help stabilize the helix through electrostatic interactions with the helix dipole (see below). Two of the glycine residues in the Ac-A_3G_{12}K peptide were replaced with E and K residues. The E was placed as the 7th residue in the 16 residue peptide, and the K was placed at different relative positions to the E residue from *i*+2 to *i*+6.

The orientation of the side chains of the E and K residues with relative positions *i*,*i*+4, *i*,*i*+5, and *i*,*i*+6 in an ideal α-helical conformation and an ideal π-helical conformation are shown in Figure 1. The side chains of the E and K residues placed at the *i*,*i*+4 positions are oriented on the same face of the α-helix and the π-helix. For the *i*,*i*+5 positions, the side chains are pointed in opposite directions in the α-helix while they are in close proximity in the π-helix. The side chains are on opposite faces for the *i*,*i*+6 positions for both the α-helix and the π-helix. Placing the residues in *i*,*i*+4 positions is a commonly employed method to study the side chain interactions in an α-helix. The *i*,*i*+2 and *i*,*i*+5 are often used as controls in these studies because side-chain interactions are not

(19) Hagen, D. F. *Anal. Chem.* **1979**, *51*, 870−874.
(20) Von Helden, G.; Hsu, M. T.; Kemper, P. R.; Bowers, M. T. *J. Chem. Phys.* **1991**, *95*, 3835−3837.
(21) Clemmer, D. E.; Jarrold, M. F. *J. Mass Spectrom.* **1997**, *32*, 577−592.
(22) Hudgins, R. R.; Ratner, M. A.; Jarrold, M. F. *J. Am. Chem. Soc.* **1998**, *120*, 12974−12975.
(23) Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1999**, *121*, 3494−3501.
(24) Hudgins, R. R.; Jarrold, M. F. *J. Phys. Chem. B* **2000**, *104*, 2154−2158.
(25) Hartings, M. R.; Kinnear, B. S.; Jarrold, M. F. *J. Am. Chem. Soc.* **2003**, *125*, 3941−3947.
(26) Counterman, A. E.; Clemmer, D. E. *J. Am. Chem. Soc.* **2001**, *123*, 1490−1498.

favorable for these positions for an α-helix. α-Helix stabilization through many interactions such as salt bridge, hydrogen bonding, and hydrophobic interactions,[27] as well as through linkers,[28] has been examined using this design strategy.

In aqueous solution, the E and K residues positioned at *i*,*i*+4 in an α-helix can form a hydrogen-bonded ion pair. This type of interaction has been used to stabilize α-helices and coiled-coils.[29−34] It is not clear that the charges on the EK ion pair will remain separated in the absence of a solvent. However, neutral E and K may still interact, albeit less strongly, through hydrogen bonds.

From the preceding discussion, favorable side-chain interactions in an *i*,*i*+4 orientation should stabilize an α-helix and a π-helix, while favorable side-chain interactions in an *i*,*i*+5 orientation will stabilize a π-helix (but not an α-helix). So the relative abundances of the helical conformations with different E and K orientations will provide information about which type of helix is preferred.

Materials and Methods

The peptides were synthesized using *FastMoc* chemistry on an Applied Biosystems 433A peptide synthesizer. After synthesis, they were cleaved from the HMP resin using a 95% trifluoroacetic acid (TFA) and 5% water (v/v) mixture, precipitated using cold diethyl ether, and lyophilized. The peptides were used without further purification. Solutions of 1 mg of peptide in a mixture of 1 mL of TFA and 0.1 mL of water were electrosprayed.

Mobility measurements were performed using a home-built ion mobility mass spectrometer, which is described fully in a previous paper.[14] Electrosprayed peptide ions enter a differentially pumped region through a heated capillary (380−400 K) which helps to desolvate the ions and reduce the abundance of multimers. The ions are focused into the drift tube using electrostatic lenses. The drift tube is 30.5 cm long and filled with 2.5−5.0 mbar of helium gas. The drift-tube temperature was varied between 173 and 400 K in these studies. The drift voltage was 280 V. Experiments were performed with an injection energy of 500 eV. Collisions with the buffer gas cause a transient heating of the ions as they enter the drift tube, but once the kinetic energy is thermalized, their internal degrees of freedom are rapidly equilibrated to the buffer-gas temperature. Because of this rapid collisional heating and cooling cycle the conformations probed in these studies are due to unsolvated peptide ions. There should not be a memory of the solution-phase structure. After the ions exit the drift tube, they are passed through a quadrupole mass spectrometer and are then detected with a collision dynode and two stacked microchannel plates. The time taken by the ions to travel across the drift tube (the drift time) is determined using an electrostatic shutter to permit 100 μs packets of ions to enter the drift tube and recording the arrival-time distribution at the detector using a multichannel scaler that is synchronized with the electrostatic shutter. Peaks in the drift-time distributions are assigned by comparing their collision cross sections (which are proportional to their drift times)[35] to average collision cross sections calculated for conformations derived from molecular dynamics (MD) simulations.

The MD simulations were performed using the MACSIMUS suite of programs[36] with the CHARMM 21.3 parameter set and a dielectric constant of 1.0. The 960 ps simulations were started from either fully extended or fully helical (α and π) conformations. Simulations were performed with a variety of basic sites protonated and acidic sites unprotonated as described in more detail below. Average collision cross sections were calculated using the empirically corrected exact hard spheres scattering model[24] from 50 snapshots taken from the last 35 ps of the simulations. If the structures sampled in the MD simulations match those present in the experiment, the measured and calculated cross sections are expected to agree within 2%. All MD simulations terminated at room temperature, and the cross-section calculations were performed at room temperature.

Table 1. Percent Helix Content (See Text) for Peptides **1**−**10** at 213 K

peptide name	peptide sequence	% helix at 213 K
3A12G (**1**)	Ac-AAAGGGGGGGGGGGK	30
E7K13 (**2**)	Ac-AAAGGG**E**GGGGG**K**GGK	0
E7K12 (**3**)	Ac-AAAGGG**E**GGGG**K**GGGK	24
E7K11 (**4**)	Ac-AAAGGG**E**GGG**K**GGGGK	12
E7K10 (**5**)	Ac-AAAGGG**E**GG**K**GGGGGK	5
E7K9 (**6**)	Ac-AAAGGG**E**G**K**GGGGGGK	0
K7E11 (**7**)	Ac-AAAGGG**K**GGG**E**GGGGK	0
E7 (**8**)	Ac-AAAGGG**E**GGGGGGGGK	14
globule (**9**)	Ac-KAAAGGG**E**GGG**K**GGGG	0
E7K11R (**10**)	Ac-AAAGGG**E**GGG**K**GGGGR	15

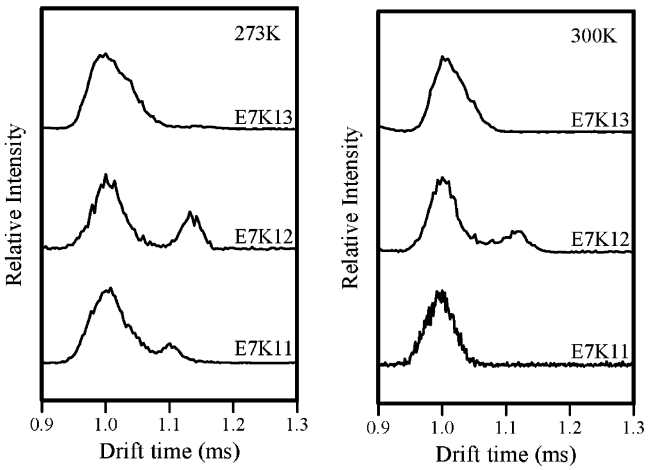

Figure 2. Drift-time distributions measured at 213 and at 300 K for peptides **2**−**4**.

Results

The specific peptides studied in this work are listed and labeled in Table 1. Peptides **2**−**7**, which have the general sequence $AcA_3G_3E(G)_nK(G)_mK$ ($n + m = 7$), are identical except for the relative positions of the E and K residues. In all cases, drift-time distributions were recorded for the singly protonated peptide ions that are completely desolvated. Either one or two main peaks are present in the drift-time distributions for the peptides listed in Table 1.

Figure 2 shows the drift-time distributions measured at 213 K and room temperature for peptides **2**−**4**. The peaks in the drift-time distributions at around 1 ms are assigned to globular conformations (see below). These are observed for all three peptides. The peaks at around 1.1 ms are assigned to a helix (see

(27) Andrew C. D.; Penel, S.; Jones, G. R.; Doig, A. J. *Proteins: Struct., Funct., Genet.* **2001**, *45*, 449−455.
(28) Schafmeister C. E.; Po, J.; Verdine G. L. *J. Am. Chem. Soc*. **2000**, *122*, 5891−5892.
(29) Marqusee, S.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8898−8902.
(30) Lyu, P. C.; Gans, P. J.; Kallenbach, N. R. *J. Mol. Biol.* **1992**, *223*, 343−350.
(31) Scholtz, J. M.; Qian, H.; Robbins, V. H.; Baldwin, R. L. *Biochemistry* **1993**, *32*, 9668−9676.
(32) Huyghues-Despointes, B. M. P.; Baldwin, R. L. *Biochemistry* **1997**, *36*, 1965−1970.
(33) Shi, Z.; Olson, C. A.; Bell, A. J., Jr.; Kallenbach, N. R. *Biopolymers* **2001**, *60*, 366−380.
(34) Litowski, J. R.; Hodges, R. S. *J. Biol. Chem.* **2002**, *277*, 37272−37279.
(35) Mason, E. A.; McDaniel, E. W. *Transport Properties of Ions in Gases*; Wiley: New York, 1988.
(36) Kolafa, J. http://www.icpf.cas.cz/jiri/macsimus/default.htm.

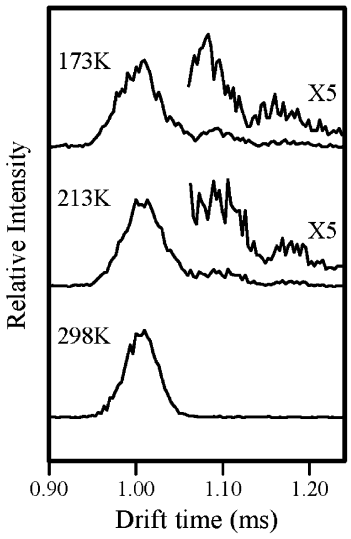

Figure 3. Drift-time distributions measured for peptide **10** at 173 and 213 K and room temperature.

below), and these peaks are observed for peptides **3** and **4** at 213 K but not for **2**. Both sets of peaks are broader than expected for a single conformation, indicating that a distribution of slightly different globular and helical conformations contribute to the experimental measurements. At room temperature the helix peak at around 1.1 ms is observed only for peptide **3** (E7K12) where the EK pair is registered in an $i,i+5$ arrangement. The helix peak was also observed for peptides **4** (E7K11), **5** (E7K10), **8** (E7), and **10** (E7K11R) at 213 K but not at room temperature. The helix for the unsubstituted peptide **1** also melts below room temperature. Peptide **3** (E7K12) is the only peptide studied here with a helix peak at room temperature. As the temperature is raised further, the helix for peptide **3** also melts. At 393 K, the drift time distributions for all the peptides consist of a single narrow peak. The room-temperature cross sections of the globular conformations of peptides **2**−**7** and **9** are all similar and lie between 238 and 244 Å^2.

Table 1 shows the helix content measured for peptides **1**−**10** at a drift-tube temperature of 213 K, where the conversion between the globular and helical structures is slow compared to the experimental time scale. The globule is present for all peptides while the feature assigned to the helix is only present for some of them. The percent helix in Table 1 is the area of the peak assigned to helical conformation divided by the area of both peaks. For peptides **2** (with the EK pair at $i,i+6$) and **6** ($i,i+2$) no helix is observed. These peptides have the EK pair arranged so that any interactions between them are helix (α and π) disrupting. The helix content for peptides **5** ($i,i+3$), **4** ($i,i+4$), and **3** ($i,i+5$) is 5, 12, and 24%, respectively. Thus, the highest amount of helix is observed when the EK pair is in an $i,i+5$ arrangement. If the K residue of the EK pair is removed (peptide **8**), the helix content decreases significantly compared to that for peptide **3**. Note that no helix is observed for peptide **7**, where the EK pair is $i,i+4$, but their positions are reversed. No helix is also observed for peptide **9**, where the C-terminus lysine is moved to the N terminus. Protonation at the N terminus is expected to destabilize the helical state through unfavorable electrostatic interactions with the helix dipole (see below).

Figure 3 shows the drift-time distributions measured for peptide **10**. Peptide **10** is similar to peptide **4** but has the C-terminus lysine substituted by a more basic arginine residue. No helix peak is observed for this peptide at room temperature. At 213 K, the helix content is similar to that for peptide **4** (E7K11), which also has the EK pair in the $i,i+4$ positions. A small third peak at a higher drift time (around 1.17 ms) appears around 213 K. This small third peak is only observed for peptides **4** and **10.** For peptide **4**, the third peak appears below 193 K.

MD simulations were performed in order to obtain cross sections for trial conformations to compare with the measured cross sections. In the gas phase, protonation at the amide carbonyl group is expected to be competitive to that of a lysine side chain and the proton may become mobile (rapidly jumping from one site to another) when the internal energy of the ion is high. At room temperature and below, however, if one site is significantly more basic than the others, the proton is expected to be localized. The peptides studied here contain residues with both basic and acidic side chains, and there is the possibility of ion-pair and salt-bridge formation. Studies of ion-pair and salt-bridge formation in small peptides have shown that they are often energetically competitive with the neutral forms. Since it is not possible to predict which forms will be energetically favored (and this may well be a function of the conformation), simulations were performed with a variety of basic sites protonated and acidic sites deprotonated: (i) both lysine residues protonated and the glutamic acid residue deprotonated; (ii) a neutral EK pair with the C-terminus K protonated; (iii) the C-terminus K and E neutral and the middle K protonated; and (iv) the C terminus deprotonated and the two K residues protonated. When the EK pair are neutral, they do not interact strongly enough in the simulations (they became uncoupled and uncorrelated). Since the experimental results clearly show evidence of strong interactions between the residues in the EK pair, the failure to observe these interactions in the simulations when the E and K residues are neutral either means that the E and K residues must form a more strongly interacting ion pair (in both the simulations and the experiments) or that there is a deficiency in the force field and the interaction between a neutral EK pair is underestimated. We discuss ion-pair formation in more detail below (there is some evidence in support of this). In the following we focus mainly on the simulations where the E and K are in the ion-pair form and interact, because these seem most relevant to our experimental results.

Two main types of low-energy conformation were found in the simulations: helices and globules. The relative energies of the lowest energy forms of these two conformations are within 20 kJ mol^{-1} for peptides **2**−**7**. In view of the uncertainties associated with force field calculations (see below for a discussion), we regard these conformations to be virtually isoenergetic and attempt to draw no conclusions from the energy differences. Figure 4 shows some representative snapshots of the low energy conformations found in the simulations for peptide **3** (E7K12), peptide **4** (E7K11), and peptide **9** (globule). For comparison, Figure 4a shows an ideal α-helix for peptide **4** (E7K11) and Figure 4b shows an ideal π-helix for peptide **3** (E7K12). The calculated cross sections for the ideal π-helix and ideal α-helix are 255 and 300 Å^2, respectively. A representative snapshot from the lowest energy globule found in the simulations for peptide **9** (the peptide that has the K at the N terminus) is shown in Figure 4c. The average cross section for this globular conformation (taken from the last 35 ps of the simulation) is

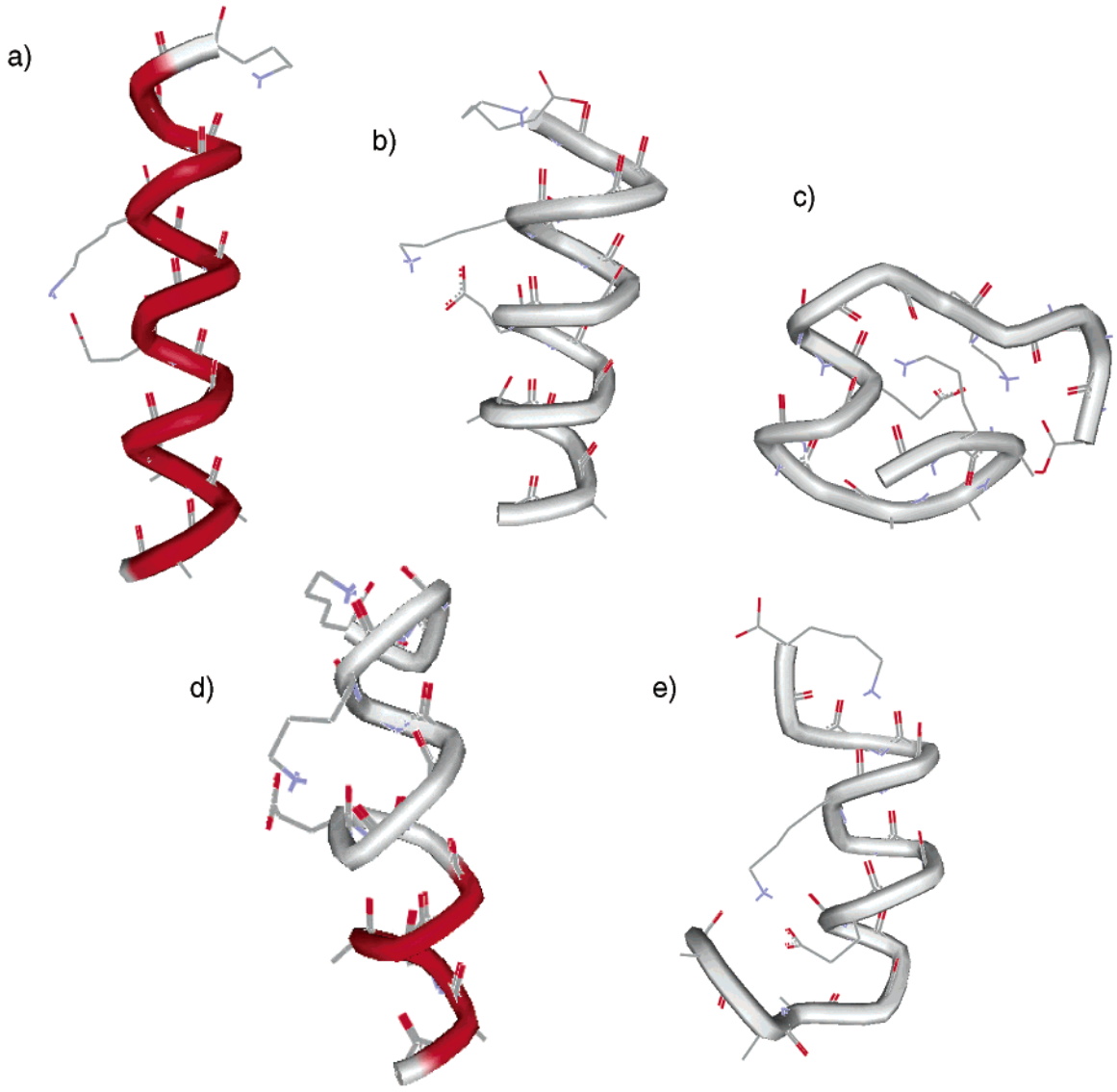

Figure 4. Representative structures for peptides **3**, **4**, and **9**: (a) ideal α-helix for peptide **3** (calculated cross section, 300 Å^2); (b) ideal π-helix (255 Å^2); (c) snapshot from the lowest energy simulation for peptide **9** (globule) (244 Å^2); (d) snapshot from the lowest energy simulation for peptide **3** (E7K12) (274 Å^2); (e) snapshot from the lowest energy simulation for peptide **4** (calculated cross section) (265 Å^2).

247 Å^2. This is slightly larger than the measured cross section for the peak assigned to the globule (241 Å^2). The discrepancy (which is attributed to MD not effectively locating compact low-energy globular conformations) is discussed below. For peptides **2**−**7** the measured cross sections for the globule were 238−244 Å^2, and the average cross sections from the lowest energy simulations yielding globular conformations were 244−251 Å^2.

A snapshot of the lowest energy conformation found in MD simulations for peptide **3** (E7K12) is shown in Figure 4d. This conformation appears to be π-helical in the middle and α-helical at the ends. The calculated average cross section (274 Å^2) lies between the values calculated for ideal π- and α-helices (255 and 300 Å^2), and it is in good agreement with the value measured for the helix peak for this peptide at room temperature (273 Å^2). A snapshot of the lowest energy conformation found in MD simulations for peptide **4** (E7K11) is shown in Figure 4e. This conformation appears to be π-helix with a distorted N terminus. The calculated average cross section for this conformation is 265 Å^2. The calculated cross section for peptide **4** (265 Å^2) is smaller than that for peptide **3** (274 Å^2) because of the distorted N terminus as evident in Figure 4e. It is obvious from Figure 2 that the helix peak for peptide **4** has a cross section that is slightly smaller than that for peptide **3**. The helix peak for peptide **4** melts below room temperature; however, the cross section extrapolated to room temperature from the low-temperature measurements is 267 Å^2. This is in reasonably good agreement with the average cross section calculated from the simulations for this peptide. While we only discuss the lowest energy conformations found in the simulations here, there are many slightly higher energy conformations (with slightly different structures) found in the simulations that also match the measured cross sections. The widths of the peaks in the drift-time distributions indicate that more than one conformation contributes to the experimental measurements.

The extrapolated cross section for the small low-temperature peak observed in the drift-time distribution of peptide **4** is 286 Å^2. This peak was only observed at low temperatures for peptides **4** and **10** which have the EK pair in an *i*,*i*+4

arrangement. It is plausible that this peak corresponds to an α-helical structure: the measured cross section is close to that for the ideal (E7K11) α-helix (300 Å^2), and it is only observed for peptides with an *i*,*i*+4 arrangement of the EK pair.

Discussion

As indicated above, the two main peaks identified in the drift-time distributions for the peptides in Table 1 are assigned to globular and helical conformations. These assignments are based on prior studies (for example, the two peaks present for unsubstituted peptide **1** were previously assigned to a helix and globule in ref 16) and on a comparison of measured and calculated cross sections performed as part of this work. The globular conformation (a compact random-looking three-dimensional structure) is the only one that has a cross section small enough to match the cross section for the peak at short drift times in the drift-time distributions. MD simulations have trouble finding compact low-energy globular conformations, and the low-energy globular conformations derived from MD simulations often have cross sections that are slightly larger than the measured quantities. In the present case, the average cross section from the lowest energy simulation for peptide **9** (the peptide that has the K at the N terminus) is 247 Å^2 and the measured cross section is 241 Å^2. We have recently shown that a genetic algorithm can find compact low-energy globular conformations that have cross sections that closely match the experimental values.[37] However, these calculations are computationally expensive, and they were not performed here because the assignment to globular conformations is not in question.

The larger cross sections for the second main feature in the drift-time distributions indicates an extended structure (or a family of closely related extended structures). Peptides **2**−**7** are identical except for the relative positions of E and K residues. The relative intensity of the peak with the higher drift time varied from 0 to 24% for these peptides. The variations in the abundance of this peak suggests an extended structure which allows favorable interactions between the E and K residues at some relative positions such as *i*,*i*+5 better than for others, such as *i*,*i*+6 and *i*,*i*+2. The peak with the higher drift time disappears completely when the terminal K residue is moved from C terminus to N terminus (peptide **9**), suggesting that the extended structure is stabilized by a positive charge at the C terminus. Switching the position of a K residue between the C terminus and the N terminus should not make much difference to the stability of a β-hairpin or β-sheet conformation. In a helix, the C terminus is the negative end of the helix macro-dipole, and a positive charge near the C terminus stabilizes the helix by favorable interaction with the macro-dipole.[38−40] Thus a basic residue (such as K or R) at the C terminus is expected to stabilize the helical state, and a basic residue at the N terminus is expected to destabilize the helix through unfavorable interactions with the helix macro-dipole.

Two main conformations have emerged from the MD simulations performed on the peptides studied here: helices and globules. A variety of different helical conformations were found, and we comment on this further below. At this point, the important result is that the helices are expected to have larger cross sections than the globules and that the calculated cross sections for the helices are close to the measured values for the second main feature in the drift-time distributions. β-Hairpins, a conformation that seems plausible for peptides in the size range examined here, are not a favorable structure for charged peptides in vacuo. We have not yet been able to design a stable β-hairpin or a stable S-shaped antiparallel β-sheet, even when up to two disulfide bridges are incorporated.[41] For protonated peptides, self-solvation of the charge drives the hairpin to collapse into a globule. On the other hand, recent electric deflection measurements suggest that β-structures are the preferred room-temperature conformations for neutral (i.e. uncharged) alanine-based peptides in the gas phase.[42] Only the β-structures have low enough dipole moments to match the measured values.

In summary, all the available evidence is consistent with the assignment of the two main features observed in the drift-time distributions of the peptides studied here to a globular conformation and a helix. The nature of the helix (α, π, or some combination thereof) still needs to be resolved.

Table 1shows the helix content measured for the peptides **1**−**10** at a drift-tube temperature of 213 K, where the conversion between the globular and helical structures is slow compared to the experimental time scale. The globule is present for all peptides while the helix is only present for some of them. At higher temperatures the peak due to the helix melts and a single peak is observed in the drift-time distribution for most peptides. Only peptide **3** (the substituted peptide with the largest percent helix at 213 K) shows a helical peak at room temperature. At 393 K, the drift time distributions for all the peptides consist of a single narrow peak, indicating that there is only one conformation present or that rapid conformational averaging occurs. If the time scale for isomerization is much shorter than the drift time, the measured drift time is a time average of the conformations sampled during transit through the drift tube. The measured cross section for peptide **3** at 373 K is 246 Å^2, while for the other sequence isomers it is 237−238 Å^2, suggesting a significant helix content for peptide **3** even at high temperatures.

The results in Table 1 clearly show that the introduction of E and K residues reduces the helical content of the substituted AG peptides compared to that of the unsubstituted peptide **1**. Even a single isolated glutamic acid placed in the middle of peptide **8** significantly decreases the amount of helix present. In solution, the helix propensities for E and K are higher than for G.[43,44] This is evidently not the case here. E and K are clearly more helix-disrupting than G. E and K both have polar side chains and can disrupt helix formation by competing for backbone hydrogen bonding sites and by helping to stabilize the globular conformation. In the case of peptide **8**, the E residue and the K at the C terminus are *i*,*i*+9 which is not favorable for helix formation, so this interaction could also help disrupt helix formation. Capping the side chain of the glutamic acid in peptide **8** with a benzyl group increases the helix content at

(37) Damsbo, M.; Kinnear, B. S.; Hartings, M. R.; Ruhoff, P. T.; Jarrold, M. F.; Ratner, M. A. An Application of Evolutionary Algorithm Methods to Polypeptide Folding: Comparison with Experimental Results for Unsolvated Ac-(Ala-Gly-Gly)$_5$-Lys+H$^+$. *Proc. Natl. Acad. Sci. U.S.A.*, submitted for publication.
(38) Blagdon, D. E.; Goodman, M. *Biopolymers* **1975**, *14*, 241−245.
(39) Ihara, S.; Ooi, T.; Takahashi, S. *Biopolymers* **1982**, *21*, 131−145.
(40) Shoemaker, K. R.; Kim, P. S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Nature* **1987**, *326*, 563−567.
(41) Sudha, R.; Schneider, J.; Jarrold, M. F. Unpublished results.
(42) Dugourd, Ph.; Antoine, R.; Breaux, G.; Broyer, M.; Jarrold, M. F. Entropic Stabilization of Isolated β-Sheets. *Phys. Rev. Lett.*, submitted for publication.
(43) Chakrabartty, A.; Kortemme, T.; Baldwin, R. L. *Protein Sci.* **1994**, *3*, 843−852.
(44) O'Neil, K. T.; DeGrado, W. F. *Science* **1990**, *250*, 646−651.

213 K to 19%. This helix content is still significantly less than for unsubstituted peptide **1**, presumably because of the presence of a bulky benzyl group. However, the observed increase in helix content on blocking the E residue is consistent with the explanation given above that the polar side chains disrupt helix formation in vacuo.

The helix content of peptide **10**, which has an R residue at the C terminus, is similar to that of peptide **4** at 213 K. Peptides **4** and **10** are identical except the C-terminal K in **4** is replaced with R in peptide **10**. Arginine is significantly more basic than lysine. Hence, in peptide **10**, the protonation site should definitely be the R residue at the C terminus and not the lysine residue at the 11th position. In the helical form of peptide **4**, the protonation site is still expected to be the C-terminus lysine, because protonation at this site allows favorable interactions with the helix macro-dipole that stabilize the helical state. The experimental observation that the helix content of peptides **4** and **10** are similar supports this idea. As we discuss below, the EK pair in peptides **4** and **10** may form an ion pair (at least in the helical conformations); thus, it is possible that both K residues in peptide **4** are protonated.

Formation of a zwitterion in the gas phase is known to be marginal with arginine,[45−47] so formation of an unsolvated EK ion pair seems unlikely (because lysine is considerably less basic than arginine). However, in the present case the ion pair may be stabilized by interactions with the helix macro-dipole. When the positions of the E and K are reversed in peptide **7** (so that interactions with the helix macro-dipole destabilize the ion pair), no helix is observed. This observation is consistent with ion-pair formation in the E7K12 peptide, but not proof. In the simulations, the EK pair did not couple in their neutral forms, and it was necessary to assume that the EK pair formed an ion pair in order to get them to interact significantly (as indicated by the experimental results). However, the fact that the neutral EK pair do not interact strongly enough may be due to a deficiency in the force field rather than an indication of ion-pair formation.

The helix content of AG peptides in Table 1 varies from 0 to 24%, depending on the relative position of the E and K residues. The highest amount of helix is observed when the EK pair is in an $i,i+5$ arrangement. For $i,i+2$ and $i,i+6$ no helix is observed. If the K residue of the EK pair is removed, the helix content decreases significantly. Finally, no helix is observed for peptide **7**, where the EK pair is $i,i+4$, but their positions are reversed. All these observations are consistent with the notion that some arrangements of the EK pair are helix-stabilizing and some are helix-destabilizing. The fact that the largest percent helix is observed where the EK pair are registered $i,i+5$, rather than $i,i+4$, suggests that the preferred helical conformation is a π-helix rather than an α-helix (at least in the center of the peptide where the EK pair are located). As described above, for an ideal α-helix the $i,i+5$ arrangement has the E and K side chains on opposite faces of the helix and pointing away from each other, while for an ideal π-helix the $i,i+5$ arrangement allows favorable interactions between the EK pair. The $i,i+4$ arrangement also allows favorable interactions between the EK pair in a π-helix.

Because of the size of the systems studied here, the MD simulations must be performed with a classical force field. Force fields are known to be deficient in a number of ways, so the results must be treated with caution. In the present case we are concerned with identifying the main conformations (which have been identified as helices and globules) and in interpreting the cross section to obtain more information about the nature of the helices that are present: α-helices and π-helices are expected to have significantly different collision cross sections. Note, however, that there is already a strong indication that there is a π-helix preference for these peptides from the studies of the percent helix as a function of the relative positions of the EK pair. The cross sections for the helical peaks of peptides **3** and **4** lie between those calculated for ideal α- and π-helices. For peptide **3** (the peptide with the largest percent helix) the conformation in the lowest energy simulation is π-helical in the middle and α-helical at the ends. The calculated average cross section for this conformation is in good agreement with the measured cross section. The presence of the π-helical section in the middle of this conformation is consistent with the percent helix measurements that indicate a π-helix preference for these peptides. For peptide **4**, the measured cross section is slightly smaller than for peptide **3**; the simulations suggest that this may be due to a distortion of the N terminus. The average cross section from the lowest energy simulation for peptide **4** matches the cross section for the helix peak of this peptide (extrapolated to room temperature). There is also a substantial π-helical component in the simulations for peptide **4**. Thus, it appears that even when the EK pair are $i,i+4$ there is still a π-helix preference. Small low-intensity peaks emerge at large drift times for peptides **4** and **10** at low temperatures. Both these peptides have the EK pair in an $i,i+4$ arrangement that can stabilize both α- and π-helices. The extrapolated cross sections for these peaks are close to the value expected for an ideal α-helix. Thus, it seems likely that these peaks are due to conformations that are predominantly α-helical and that these conformations become frozen in at low temperatures. The abundance of the α-helical component is significantly smaller than the abundance of the π-helical component that occurs at shorter drift time. This is consistent with the idea that the π-helix is the preferred helical conformation for the alanine/glycine peptides.

Summary and Conclusions

Ion-mobility measurements, substitution studies, and molecular dynamics simulations have been used to probe helix formation in a series of alanine/glycine peptides. Incorporation of residues with polar side chains into the $Ac\text{-}A_3G_{12}K+H^+$ peptide reduces the amount of helix present, presumably because hydrogen bonding between the backbone and the polar side chains stabilizes the nonhelical globular conformations. Glutamic acid and lysine may form an ion pair that is stabilized by interactions with the helix macro-dipole.

Percent helix measurements performed as a function of the relative positions of an EK pair indicate a π-helix preference for these peptides. The largest percent helix is observed for the E7K12 peptide which has an $i,i+5$ EK pair. This peptide is the only one studied here where the helix survives to above room temperature. Cross sections measured for the helical conforma-

(45) Schnier, P. D.; Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1996**, *118*, 7178−7189.
(46) Strittmatter, E. F.; Williams, E. R. *J. Phys. Chem. A* **2000**, *104*, 6069−6076
(47) Julian R. R.; Hodyss, R.; Beauchamp, J. L. *J. Am. Chem. Soc.* **2001**, *123*, 3577−3583.

tions of peptides with an $i,i+4$ and $i,i+5$ EK pair lie between those calculated for ideal α- and π-helices and are in agreement with average cross sections derived from molecular dynamics simulations. In the molecular dynamics simulations the E7K12 peptide has a central π-helical region with α-helical ends. There is evidence for the formation of a predominantly α-helical conformation at low temperature when the EK pair is $i,i+4$.

Previous work suggests that the α-helix is the preferred helical form for alanine-based peptides.[22,23] So it appears that the preference for a π-helical arrangement is residue-dependent. We are currently examining α-/π-helix preferences for other residues, particularly those that are abundant in transmembrane helices where α/π transitions could have important consequences.

Acknowledgment. We gratefully acknowledge the support of this work by the National Institutes of Health. We thank Jiri Kolafa for the use of his MACSIMUS programs.

JA0381353