Intrinsic Size Parameters for Val, Ile, Leu, Gln, Thr, Phe, and Trp Residues from Ion Mobility Measurements of Polyamino Acid Ions

Sheila C. Henderson, Jianwei Li, Anne E. Counterman, and David E. Clemmer*

Department of Chemistry, Indiana University, Bloomington, Indiana 47405

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The conformations of singly charged ions of eight polyamino acids of varying length [polyalanine (3−23 residues), polyglutamine (2−8 residues), polyisoleucine (2−6 residues), polyleucine (2−9 residues), polyphenylalanine (2−7 residues), polythreonine (8−14 residues), polytryptophan (2−9 residues), and polyvaline (2−7 residues)] have been studied by ion mobility methods and molecular modeling simulations. The average amino acid contributions to cross section for 4−9 residue homopolymers agree with intrinsic size parameters for these residues derived from data for tryptic digest peptides [*J. Phys. Chem. B* **1999**, *103*, 1203]. Some variations in residue sizes with changes in oligomer length are apparent for small oligomers and those with polar and aromatic ring side chains. Molecular modeling simulations reveal that all of these homopolymers have roughly spherical (globular) structures. Conformations appear to be influenced by three primary types of interactions: (1) self-solvation of the charge site by backbone carbonyls, a characteristic of all oligomer types; (2) steric hindrance of side chains (leading to efficient ring stacking), a prominent factor for the polyphenylalanine and polytryptophan systems; and (3) hydrogen bonding involving side chains, backbone carbonyls, and the charged residue, apparent in the polyglutamine and polythreonine systems.

Introduction

Defining the intrinsic properties of amino acid residues that are important in the formation of local structural elements such as helices, sheets, and coils is central to understanding factors that govern the conformation and folding of polypeptides. Probabilistic methods for structural prediction have been developed from the propensities of amino acid residues to form α-helices, β-sheets, and turns in proteins.[1] The stabilities of α-helices in various solution environments have been investigated in detail,[2] and β-sheet formation is currently receiving considerable attention.[3] Many questions about roles of solvent and intramolecular interactions in establishing conformation remain. The recent development of new ionization sources[4] for mass spectrometry (MS) makes it possible to examine the conformations of anhydrous proteins and peptides in the gas phase. In vacuo studies of conformation provide information about structural elements that are intrinsic to the polypeptide sequence and complement efforts to understand the formation of structure in the condensed phase.[5] Several MS-based strategies are being developed for these studies, including isotopic hydrogen−deuterium exchange;[6] proton-transfer reactivity,[7] and molecular adduction;[8] kinetic energy release measurements;[9] microscopy of surfaces bombarded with high energy ions;[10] and determination of collision cross sections (or collision integrals) by triple quadrupole[11] and ion mobility methods.[12−14] Some of these studies provide evidence that solution-like conformation can be preserved during ionization;[6b,13d,15] there is also evidence that proteins undergo folding and unfolding transitions that are analogous to thermal and pH driven transitions found in solution.[11,13,14b]

Ion mobility/time-of-flight MS techniques,[16] which allow cross sections for mixtures of ions to be measured in a single experimental sequence, have facilitated the accumulation of a substantial database of sequence/cross section information for peptides.[17] We have recently used these data to examine the average contributions to cross sections (intrinsic sizes)[17] and volumes[18] of individual amino acids in a series of related sequences of peptide ions (tryptic fragments) in the gas phase. The average intrinsic sizes and volumes of individual amino acids in related peptides are reasonably well-conserved, indicating that the overall peptide structures are similar. Molecular modeling indicates that most sequences have compact globular conformations, where the charge site (assigned to the Lys residue) is solvated by interactions with electronegative groups (e.g., the carbonyl backbone and some polar side chains). This is consistent with compact conformations proposed previously for several peptides including singly protonated bradykinin[12b,19] and a range of polyglycine[20,21] and polyalanine sizes.[20,22] Values for many residues can be rationalized by considering the physical and chemical characteristics of the individual residues. Intrinsic sizes of nonpolar residues, such as Val, Ile, and Leu, were substantially larger than those of polar groups (e.g., Gln and Thr). The relatively strong long-range charge−dipole interactions associated with the latter residues lead to more tightly packed conformations. Aromatic residues with bulky side chains, such as Phe, Tyr, and Trp, contributed less than expected; it was suggested that these residues might pack efficiently.[18]

Although the database of tryptic fragments is relevant to sequences found in common proteins, the large sequence heterogeneity makes it difficult to assess variations that arise from differences in peptide length, a factor that should influence packing.[18] In this paper we report ion mobility measurements for varying sizes of eight polyamino acid peptide ions, $[Ala_n + H]^+$ ($n = 3-23$), $[Gln_n + H]^+$ ($n = 2-8$), $[Ile_n + H]^+$ ($n = 2-6$), $[Leu_n + H]^+$ ($n = 2-9$), $[Phe_n + H]^+$ ($n = 2-7$), $[Thr_n + H]^+$ ($n = 8-14$), $[Trp_n + H]^+$ ($n = 2-9$) and $[Val_n + H]^+$ ($n = 2-7$). The average contributions of individual amino acids to cross section as a function of peptide length are determined. For polyamino acids containing four to nine residues, these

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values agree with residue sizes derived from the naturally occurring tryptic digest peptide sequences. For several oligomers, contributions to cross section are notably smaller for peptides having fewer than four residues. In other systems, such as polyglutamine and polytryptophan, residue sizes decrease slightly with increasing peptide length; for these systems, cooperative effects involving the side chains and polar backbone groups lead to remarkably compact structures. Overall, three primary types of interactions appear to influence conformation: (1) charge solvation by backbone carbonyl groups, which is an important factor for all residue types; (2) steric hindrance of bulky side chain groups (leading to efficient ring stacking); (3) hydrogen bonding interactions associated with side chain groups.

Experimental Section

Cross Section and *m*/*z* Measurements. Ion trap[23] and ion mobility/MS[24] methods have been discussed previously. The current experimental approach (described in detail elsewhere)[16,25] is as follows. A continuous beam of ions, formed by electrospray ionization,[4b] is accumulated in an ion trap (R. M. Jordan, model C-1251) for ~100 ms. Concentrated packets of ions (0.6 μs in duration) are injected into a 40.8 cm long drift tube containing ~2−3 Torr of buffer gas. The injection energies used for these studies ranged from 50 to 150 eV. There was no evidence for structural variation with injection energy. Ions drift through the gas and across the tube under the influence of a weak electric field (10.0 V cm^{-1}) and are separated by differences in their mobilities. Compact conformers have higher mobilities than more open ones. As ions exit the drift tube, they enter the source region of a time-of-flight mass spectrometer and are subjected to high-voltage, high-frequency pulses (synchronous with the initial injection pulse) that are used to initiate mass-to-charge (*m*/*z*) measurements. Because flight times in the evacuated flight tube are much shorter than drift times through the buffer gas, it is possible to record hundreds of flight time distributions in the mass spectrometer with respect to each packet of ions that is injected into the drift tube. We refer to this as a *nested drift (flight) time* measurement, as described previously.[16] Flight times at specified drift times are combined to create a three-dimensional data set that contains *m*/*z*-resolved ion mobility distributions for all of the electrosprayed ions.

The experimental collision integral (or collision cross section)[26] is determined from[27]

$$\Omega = \frac{(18\pi)^{1/2}}{16}\frac{ze}{(k_b T)^{1/2}}\left[\frac{1}{m_I}+\frac{1}{m_B}\right]^{1/2}\frac{t_D E}{L}\frac{760}{P}\frac{T}{273.2}\frac{1}{N}$$

where the measured parameters t_D, E, L, P, and T correspond to the average drift time, the electric field strength, the drift tube length, buffer gas pressure (in Torr), and temperature, respectively.[28] The other terms are ze, the ion's charge; N, the neutral number density; k_b, Boltzmann's constant; and m_I and m_B, the masses of the ion and buffer gas, respectively. The reproducibility of measured cross sections is excellent; the relative uncertainty of any two measurements is usually less than 1.5%.

Formation of Polyamino Acid Ions. The following polyamino acids were obtained from Sigma and used without further purification: polyalanine (mol wt = 1000−5000), polyleucine (3000−15 000), polyisoleucine (5000−15 000), polyglutamine (2000−15 000), polyphenylalanine (2000−5000), polythreonine (5000−15 000), polytryptophan (5000−15 000), and polyvaline (5000−10 000). Positively charged (protonated) ions were formed by electrospraying solutions containing 5×10^{-7} to 4×10^{-5} M peptide in 49:49:2 (% volume) water:acetonitrile:acetic acid. The electrospray source and conditions were identical to those described previously.[25]

Molecular Modeling and Cross Section Calculations. Molecular modeling studies were carried out using the Insight II molecular modeling package with the AMBER force field.[29] In these studies, 50 final conformations of each peptide are generated by a two-stage simulated annealing procedure. We start with an extended form of a polypeptide with a single proton assigned to the amino terminus. A process by which this structure is heated from 300 to 1000 K (over 2 ps), equilibrated at 1000 K (for 2 ps), and then cooled to 300 K (over 1 ps) is repeated 100 times. The 100 structures that are generated are energy minimized and the five lowest energy conformers are selected for a second annealing cycle. Each of the five selected conformers was reheated to 500 K (over 2 ps), equilibrated (for 2 ps), and cooled to 300 K (over 1 ps) in a repetitive procedure that generates 100 structures for each selected conformer. The 10 lowest energy structures from each set (50 total) were selected for further study. We have arrived at this procedure by comparing cross sections for the model conformers to experimental values and find that the agreement is generally acceptable for a wide range of small (three to ten residue) sequences. It is unlikely that this approach would find the lowest energy conformer; however, the approach appears to be adequate for generating a distribution of conformers with cross sections near those measured experimentally.

Cross sections for distributions of model conformers have been calculated by the projection approximation method[30] and the exact hard-spheres scattering (EHSS) method.[31] The projection method ignores all scattering and potential interactions between the ion and the buffer gas; however, for relatively small ions (mol wt ≤ 1500) this method should be accurate to within a few percent of the true collision integral.[12,30] The EHSS method ignores potential interactions but includes a scattering term that is important in calculating accurate cross sections for large ions as well as capturing concave shapes.[31] The results have been calibrated to values obtained from the trajectory method[32] by Jarrold and co-workers.[33] Average calculated cross sections for model oligomers from the projection method were within 4% of experimental values for all but one of the peptides (Trp); calculated values from the EHSS method were larger than the projection values by an average of 4%. However, deviations in the values from EHSS and projection methods are similar (although not identical) for all oligomers; thus, we believe that the differences between residues are due primarily to contributions of residues to the shapes of the oligomer rather than differences associated with residue-helium scattering dynamics for different side chains. Below, we use an average of results from the two methods for comparison with experimental values.

Results and Discussion

General Features of Experimental Data. Nested drift (flight) time data were recorded for all eight oligomer systems on at least five different days over a 3 month period. Cross sections for the parent $[M + H]^+$ polyamino acid ions were determined from the drift times recorded at the appropriate flight times (*m*/*z* ratios). In some cases, peaks corresponding to $[M - H_2O + H]^+$, $[M + 2H]^{2+}$, and $[M - H_2O + 2H]^{2+}$ ions were observed. These peaks are easily identified on the basis of the mobilities and *m*/*z* measurements, but the structures of these ions are not considered further here. Figure 1 shows a plot of experimental cross sections for the different sizes of

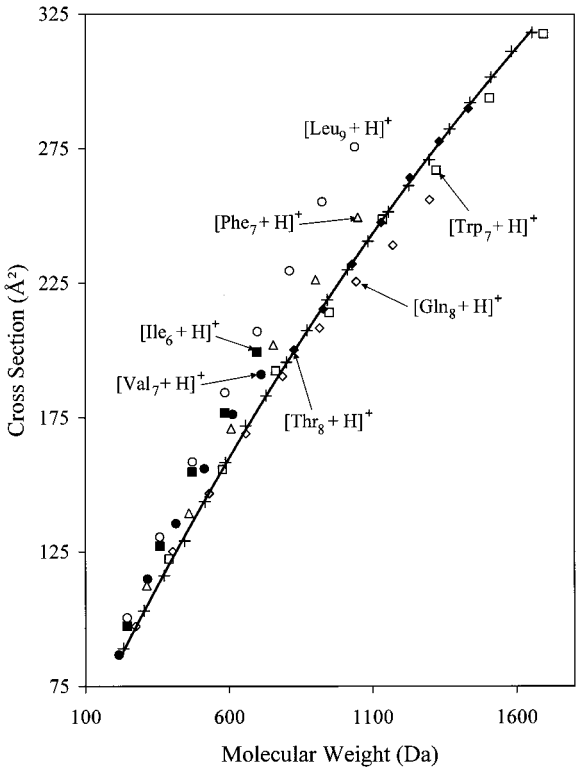

Figure 1. Plot of experimental cross section as a function of molecular weight for singly charged ions of polyalanine (+), polyglutamine (open diamonds), polyisoleucine (filled squares), polyleucine (open circles), polyphenylalanine (open triangles), polythreonine (filled diamonds), polytryptophan (open squares), and polyvaline (filled circles). The solid line corresponds to a polynomial fit to the polyalanine cross sections.

polyamino acid systems studied. At a given molecular weight, nonpolar oligomers (Ile, Leu, and Val) have relatively large cross sections, consistent with our expectations based on the intrinsic size parameters derived from tryptic fragments.[17] Oligomers with polar side chains (Gln, Thr, and Trp) have cross sections that are similar to those for polyalanine. Cross sections for polyphenylalanine fall between those for polyalanine and the other nonpolar polyamino acids. Differences among cross sections for different systems increase with molecular weight.

Previous work has shown that polyalanine ions have roughly spherical (globular) conformations where the protonated N-terminal amino group is self-solvated by a large portion of the peptide chain (primarily through contacts with electronegative backbone carbonyl groups).[22,34] To compare oligomers of different sizes (molecular weights), we have defined a reduced cross section: each measured value divided by the value for polyalanine at an identical molecular weight (as determined from a polynomial fit to the polyalanine data).[17] Figure 2 shows a plot of the reduced cross sections for the oligomers studied here. A value of 1.0 indicates an intrinsic contribution to cross section that is identical to that of an alanine residue in polyalanine. The Val, Ile, and Leu oligomers (with nonpolar aliphatic side chains) have larger reduced cross sections than do the polar aliphatic residues (Thr and Gln). Although it is somewhat surprising that the intrinsic size of the tryptophan residue (having a bulky indole side chain) is smaller than that for alanine, this result is consistent with size parameters that we derived previously from tryptic fragment ions;[17] the small size requires that side chains pack efficiently.

Comparison of reduced cross sections derived from these homopolymers with values that were extracted from the heterogeneous tryptic fragment sequences shows remarkably

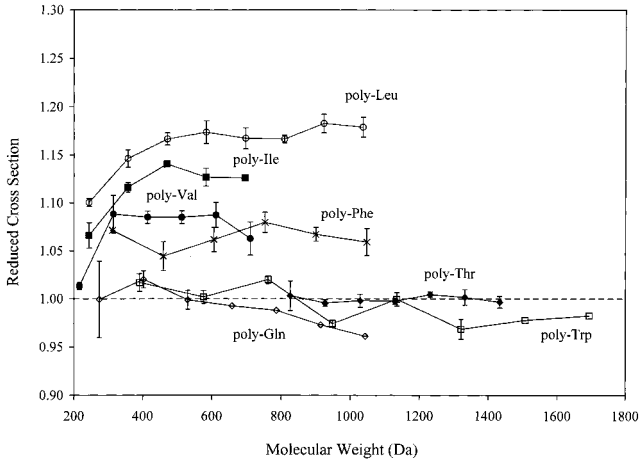

Figure 2. Plot of the experimental reduced cross sections obtained by dividing the experimental cross section of each ion by evaluating a fit to polyalanine cross sections at the same molecular weight. Uncertainties correspond to one standard deviation about the mean.

good agreement. Most of the reduced cross sections for the homopolymers containing four to nine residues are in quantitative agreement with size parameters determined previously from similarly sized tryptic fragments: Trp (0.96 ± 0.03) < Gln (0.98 ± 0.03) < Thr (1.00 ± 0.02) < Phe (1.05 ± 0.02) < Val (1.08 ± 0.02) < Ile (1.12 ± 0.02) < Leu (1.19 ± 0.02).[17] In the homopolymers, variations of intrinsic contributions to size with changes in length are readily observed. For example, the Val, Ile, and Leu size parameters decrease substantially (by ~5−7%) for oligomers with fewer than four residues. However, for oligomers with more than four residues, the cross section contributions for these nonpolar groups are relatively insensitive to oligomer length. This indicates that growth of these peptides is similar to the polyalanine system. That is, they favor globular conformations. Intrinsic size parameters for polar side chains, such as the $CH_2CH_2CONH_2$ groups of polyglutamine and the indole side chain of polytryptophan, appear to decrease slightly with increasing peptide length (by 3−4% over the range of lengths studied). As the length of these oligomers increases, they favor conformations that are more tightly packed than those of polyalanine.

Overall, the agreement of size parameters from homopolymers with values determined from heterogeneous sequences suggests that small (four to ten residue) peptide structures are dominated by solvation of the charge. Charge solvation by backbone carbonyl groups has the effect of exposing side chains at the peptide surface. The net result is that the exact composition or sequence has little bearing on the intrinsic contributions to cross sections of individual residues. Although this appears to hold for four to ten residue singly protonated peptides with globular conformations that are dominated by charge solvation effects, it should not be the case for smaller or larger systems where other structures are favored. Our early attempts to extract size parameters as a function of tryptic fragment length show that parameters vary with peptide length.[17b]

Molecular Modeling Studies. More detail regarding the factors that influence the structures of polyamino acid ions can be obtained from molecular modeling studies. We have carried out simulated annealing studies for oligomers ranging from five to nine residues in length for all residues studied experimentally. Figure 3 shows a comparison of experimental reduced cross sections with reduced cross sections determined from model conformers of polyleucine, polyglutamine, and polytryptophan produced by the simulated annealing procedure

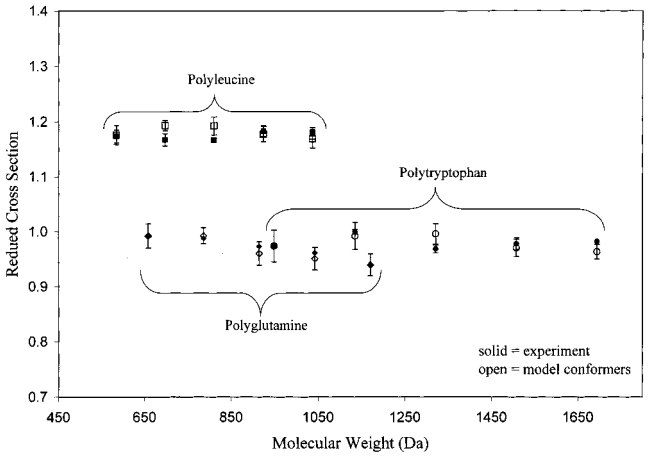

Figure 3. Plot of average experimental reduced cross sections (filled symbols) and average calculated reduced cross sections for structures generated by simulated annealing studies (open symbols) for $[Gln_n + H]^+$, $[Leu_n + H]^+$, and $[Trp_n + H]^+$ peptides where $n = 5-9$. Calculated cross sections are an average of the results obtained from projection approximation and EHSS methods. Uncertainties correspond to one standard deviation about the mean. In cases where the uncertainties are not apparent, the error bars are small relative to the symbol size and are obscured. See text for discussion.

described above. The average cross sections for structures found by simulated annealing are consistent with the experimental results. We have compared the data in this normalized fashion because small variations that occur with changes in length are easily discernible. As described above, absolute comparisons of calculated and experimental cross sections depend on the method used to calculate the collision integral. The normalized comparison is sensitive to the relative changes between amino acid types, as can be seen from the observation that the relative ordering of sizes (Gln < Trp < Leu) agrees with the experimental results. The calculated values also capture the ~4% decrease in the size contribution associated with the increasing length of polyglutamine that is observed experimentally.

Figure 4 shows typical model structures for nonamers of each of the polyamino acids studied here. All of the structures have compact roughly spherical conformations, and calculated cross sections are consistent with the experimental values. Jarrold and co-workers have previously noted that polyalanine with more than nine residues begins to show evidence of small helical regions of structure.[21] Presumably, this is because when the charge is fully solvated by smaller portions of the peptide chain, weaker interactions between residues are not disrupted. In some cases, we observe hydrogen bonding interactions such as $i \rightarrow i + 4$ interactions found in helical turns, side chain−backbone interactions, as well as bonding between side chain pairs. However, these interactions appear to occur randomly, involving only a few sequential residues at most.

The contribution to cross section of nonpolar aliphatic residues in small peptides (four to nine residues) is relatively invariant with oligomer length. This comes about because the polar carbonyl backbone solvates the charged amino terminus; in the resulting conformation the nonpolar side chains extend radially outward from the solvated protonated core (Figure 4, parts a−d). Nonpolar residue side chains are largely spectators in defining the structures of these oligomers; however, their accessibility at the surface of the peptide has a pronounced effect on the average collision cross section. We note that the alanine contribution (by definition, 1.0) is smaller than the 1.07 ± 0.01 value determined for tryptic fragments,[17] suggesting that there are some differences in charge solvation at the amino terminus

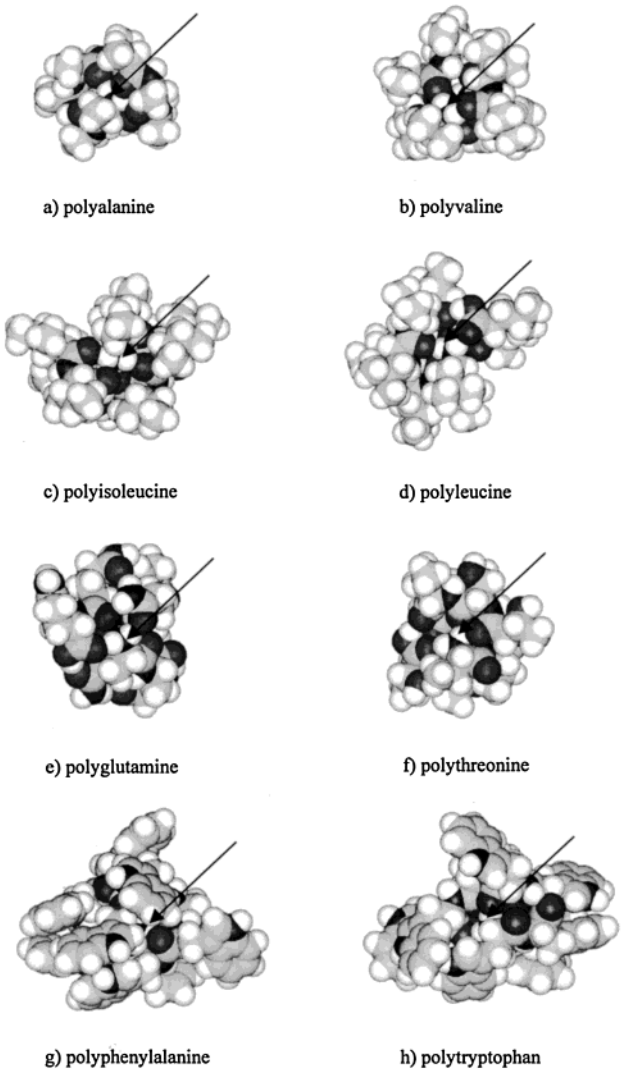

Figure 4. Atomic coordinates for trial structures generated for (a) $[Ala_9 + H]^+$, (b) $[Val_9 + H]^+$, (c) $[Ile_9 + H]^+$, (d) $[Leu_9 + H]^+$, (e) $[Gln_9 + H]^+$, (f) $[Thr_9 + H]^+$, (g) $[Phe_9 + H]^+$, and (h) $[Trp_9 + H]^+$. Different atom types are depicted as follows: H (white), N and C (light gray), and O (black). The arrows indicate the position of the protonated N-terminus. Nonpolar polyamino acid side chains extend radially outward, as can be seen from the knobby protrusions on the polyvaline, polyisoleucine, and polyleucine structures. Side chains of polar residues pack more tightly around the charged site.

and the Lys residue at the C-terminal end. Thus, the fact that the Ala values are different probably has little meaning. Side chain contributions to cross sections increase with aliphatic chain length: CH_3 (Ala) < C_3H_7 (Val) < C_4H_9 (Ile and Leu). This ordering is the same as that observed previously from analysis of tryptic fragments.[17]

Oligomers of polar aliphatic residues (Gln and Thr; Figure 4, parts e and f, respectively) pack more tightly than polyamino acids with nonpolar side chains. Examination of numerous conformers shows close range interactions of carbonyl backbone groups and polar side chains with the charge site as well as side chain−side chain and side chain−backbone hydrogen bonding interactions. Reduced cross section plots (Figures 2 and 3) define oligomer growth rates relative to the polyalanine reference system. As systems with polar side chains increase in length, the magnitude of structural differences with polyalanine will increase, consistent with the decrease in intrinsic cross section observed with increasing polyglutamine length.

Aromatic ring residues are substantially smaller than expected on the basis of consideration of the side chain size. The structures shown in Figure 4 (parts g and h) show that carbonyl groups solvate the protonated amino terminus of the oligomer. Additionally, the close range interactions of multiple carbonyl groups with the charge site force the bulky ring systems to align. For sizes as large as the nonamer, sterically allowed structures for the closely packed system of side chains almost always involve some side chains stacking. Ring stacking interactions should further stabilize structures. We note that in Figure 2, there is a reproducible alternation in reduced cross section for polytryptophan that occurs with even or odd numbers of residues. We suspect that this results from differences in stacking between oligomers with even and odd numbers of residues; however, we have been unable to identify the origin of this effect in our model structures.

Summary and Conclusions

The conformations of singly charged ions of eight polyamino acids of varying length [polyalanine (3−23 residues), polyvaline (2−7 residues), polyisoleucine (2−6 residues), polyleucine (2−9 residues), polyglutamine (2−8 residues), polythreonine (8−14 residues), polyphenylalanine (2−7 residues), and polytryptophan (2−9 residues)] have been examined by ion mobility and molecular modeling techniques. Intrinsic size parameters derived for oligomers containing four to nine residues were found to agree with values extracted from a series of cross sections for peptides generated by tryptic digestion of common proteins.[17]

The structures of model conformers generated by molecular modeling provide insight into the nature of the conformations for the different oligomers and the intrinsic contributions to cross section of different side chain groups. Oligomer structures are dominated by self-solvation of the charged amino terminus by electronegative groups along the backbone; polar side chain groups may also contribute to charge solvation. For oligomers with nonpolar side chains (Ala, Val, Ile, and Leu), self-solvation of the protonation site is accomplished through interactions with electronegative carbonyl groups along the peptide backbone. For small oligomers (four to nine residues) this favors structures in which side chains extend radially away from the solvated protonation core of the peptide.

Oligomers with polar side chains (polyglutamine and polythreonine) favor compact structures because of the large number of charge−dipole and dipole−dipole interactions associated with the polar backbone moieties and side chains. The intrinsic contribution to cross sections of polar residues decreases with increasing oligomer length, a result that emphasizes the importance of intramolecular hydrogen bonding. Results of molecular modeling indicate that hydrogen bonding among side chains and backbone groups is not cooperative. That is, there is no strong long-range ordering of side chains. Oligomers with aromatic ring residues contribute surprisingly little to cross section; bulky ring side chains appear to be tighly packed, and rings are often stacked.

There is currently significant interest in understanding the relationship of polypeptide structure in the gas phase with solution conformation. It has been suggested[5a] that the vacuum environment can be thought of as an apolar solvent, in which case the polypeptide may favor structures that are effectively inside-out (relative to their expected solution state). The results presented here for small singly charged peptides are consistent with this picture.

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