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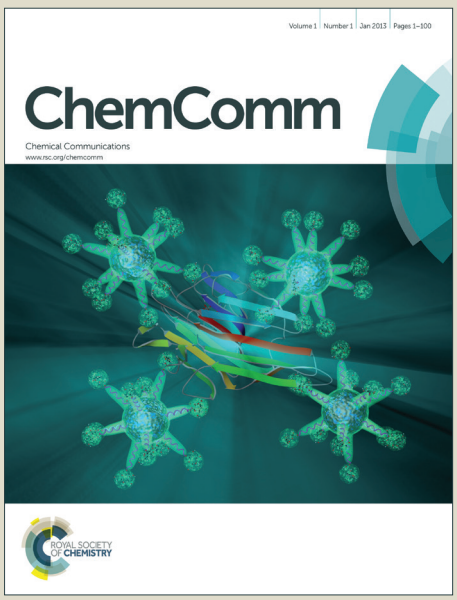

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Negatively-Charged Helices in the Gas Phase

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A polyalanine-based peptide which forms a stable, negatively-charged α-helix in the gas phase is reported. Addition of an N-terminal acidic residue forms a stabilizing hydrogen bond network and an electrostatic interaction with the helical dipole. Formation of this secondary structure was demonstrated using ion mobility-mass spectrometry and molecular modelling techniques.

A quantitative description of protein folding must rely on a fundamental understanding of the contribution of amino acids to secondary structure.[1-3] Due to its prevalence in nature,[4] the α-helix has garnered significant attention. In an early effort to relate sequence to structure, Chou and Fasman created an empirical scale of helix propensity of individual amino acids based on the probability of their occurrence in the helices of 15 crystal structures.[4, 5] These studies were followed by solution-phase denaturation measurements, which allowed the determination of relative (de)stabilization energies of individual amino acids in otherwise helix-forming peptides.[6, 7] However, the interpretation of these measurements is complicated by interactions of the peptide with aqueous solvent.[8] Additionally, it has been suggested that water, with a dielectric constant[9] of 80, is a poor mimetic of the biological setting of α-helices, mainly found in protein interiors or cell membranes, with dielectric constants of 2-20.[10-12] In contrast, gas-phase techniques, such as electrospray (ESI) ion mobility (IM)-mass spectrometry (MS), provide a low dielectric medium, devoid of solvent interactions to more directly measure the relative stability of helices. In IM spectrometry, ions travel through a buffer gas in a weak electric field. The measured drift time (t_D) is directly related to an ion's collisional cross section (CCS) with the buffer gas. The measurements can then be compared to theoretical cross sections for model structures generated by molecular dynamics (MD) simulations.[13, 14] Jarrold and coworkers[15, 16] measured polyalanine in the gas phase by ESI(+)-IM-MS and observed that singularly charged polyalanine, $[Ala_n+H]^+$, with $n \leq 20$ residues, adopted a globular shape. To create a stable helix in the gas-phase, a lysine residue was appended to the C-terminus. The protonated form of the side chain was favored by acetylation of the N-terminus, maximizing the interaction of this charge with the partially-negative helix dipole at the C-terminus. This charged side chain also has adequate flexibility to interact with the backbone carbonyl oxygens, otherwise lacking hydrogen-bonding partners at the C-terminus of the helix as evident in Figure 1(D). In this engineered peptide, Ac-Ala_n-Lys, helices were observed in sequences as short as 9 residues in length.[17]

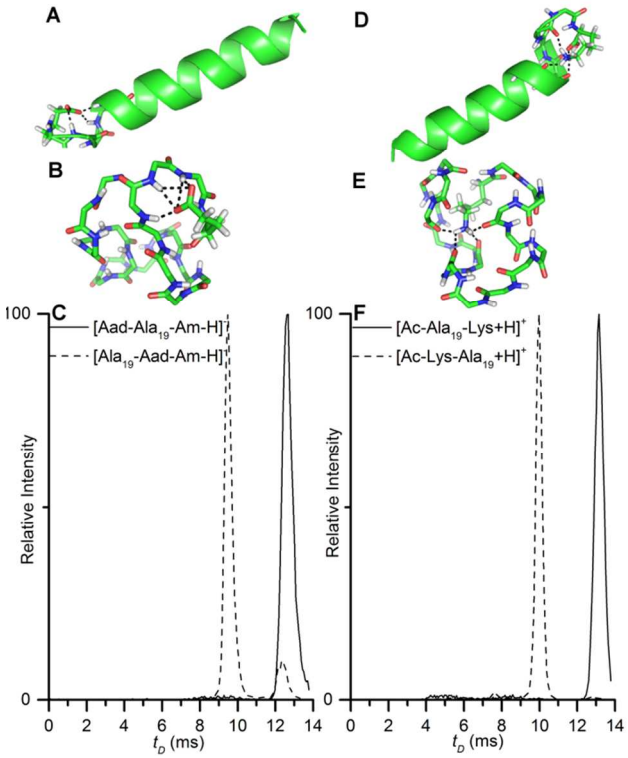

Figure 1. Representative structures of MD simulations using InsightII. (A) [L-α-Aminoadipic acid (Aad)-Ala_{19}-Am-H]$^-$ and (B) [Ala_{19}-Aad-Am-H]$^-$ adopt a helical and globular structure, respectively, during simulated annealing. Dashed lines represent putative hydrogen bonds with the charged side chain. Images were created in PyMol. (C) Experimental drift time distributions of the two negatively-charged peptides. (D) [Ac-Ala_{19}-Lys+H]$^+$ and (E) [Ac-Lys-Ala_{19}+H]$^+$ adopt a helical

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and globular conformation, respectively, during simulated annealing experiments. (F) Experimental drift time distributions of the positively charged peptides.

Building upon this initial study, IM-MS has been used to relate changes in peptide structure to the number and position of charges,[18] cation,[19] metal coordination,[20, 21] and charged side chain groups,[22] as well as the systematic study of side-chain helical propensities of individual amino acids.[23, 24] To our knowledge, all IM-MS studies of helices have been performed on positively-charged peptides, yielding incomplete exploration of the variables contributing to protein structure in gas-phase studies. While ESI(+) favors the neutral form of acidic side chains,[1] energetics of these functionalities may bear little resemblance to their physiological, deprotonated form. In larger structures, peptides can often form zwitterions[25-27] and intramolecular salt bridges.[28, 29] While more similar to physiological conditions, it is well-demonstrated that multiple charges can have profound effects on peptide structure in the absence of solvating water.[18, 22] To provide a complimentary approach to the study of peptide helicity, we report the design of a 20 residue peptide bearing a single negative charge that forms a stable helix in the gas phase,[30] demonstrated by ion mobility measurements performed on a Waters Synapt G2 IM-MS instrument.

Initial design of the helix included three key features: (1) an amidated C-terminus to influence the position of the charge on the ion; (2) an N-terminal acidic residue capable of a stabilizing electrostatic interaction with the helix dipole; and (3), favorable interactions of the side chain with the backbone amides lacking hydrogen bonding partners. A number of commercially-available amino acid analogues were assessed for their ability to form a helix in simulated annealing experiments. The best candidate was Ala_{19}-Am with an N-terminal L-α-aminoadipic acid residue. L-α-aminoadipic acid (Aad) is an unnatural, carboxylic acid-functionalized residue with four side-chain carbons, roughly analogous to the length of lysine. We envisioned that the Aad side chain could mirror the behavior of lysine in the positively-charged helix of Jarrold and coworkers,[15, 16] with the side chain being flexible enough to enable the charged group to be solvated by the backbone, as is illustrated in Figure 1A.

In order to demonstrate the formation of a negatively-charged helix, we performed comparative ESI(-)-IM-MS measurements of the helical peptide, $[Aad\text{-}Ala_{19}\text{-}Am\text{-}H]^-$ to the reverse peptide, $[Ala_{19}\text{-}Aad\text{-}Am\text{-}H]^-$. If our model was correct, the peptide with the charge on the N-terminus would be unable to form stabilizing interactions with the helical dipole, and there would be no hydrogen bond donors present from the backbone to solvate the charge in a helical conformation. Thus, we anticipated this sequence would promote a globular structure (Figure 1B). Drift time distributions, under identical instrument conditions, of the two negative ions are shown in Figure 1C. For the helical peptide, $[Aad\text{-}Ala_{19}\text{-}Am\text{-}H]^-$ t_D = 12.65 ± 0.05 ms: significantly longer than that of $[Ala_{19}\text{-}Aad\text{-}Am\text{-}H]^-$, t_D = 9.56 ± 0.11 ms. This is consistent with $[Ala_{19}\text{-}Aad\text{-}Am\text{-}H]^-$ adopting the predicted globular structure, and $[Aad\text{-}Ala_{19}\text{-}Am\text{-}H]^-$ a more elongated, helical structure. We attribute the presence of a minor population of an elongated structure evident in the drift time distribution of $[Ala_{19}\text{-}Aad\text{-}Am\text{-}H]^-$ to a secondary conformer possible in peptides of sufficient length to overcome the destabilizing forces alluded to above. Further characterization of this minor structure is provided in the Supporting Information (Figure S1). The acidic peptides were similarly measured in ESI(+)-IM-MS. The change in polarity leaves $[Aad\text{-}Ala_{19}\text{-}Am\text{+}H]^+$ devoid of the stabilizing forces possessed as a negative ion and would thus be expected to adopt a globular shape. Indeed, the t_D of $[Aad\text{-}Ala_{19}\text{-}Am\text{+}H]^+$ = 9.48 ms, similar to that of the intended globular structure of $[Ala_{19}\text{-}Aad\text{-}Am\text{-}H]^-$ or $[Ala_{19}\text{-}Aad\text{-}Am\text{+}H]^+$ (t_D = 9.39; Supporting Information Figure S2). The drift time distributions of the acidic peptides measured in ESI(+) demonstrate a minor population of an elongated structure, likely for the same reasons outlined above for $[Ala_{19}\text{-}Aad\text{-}Am\text{-}H]^-$.

To more definitively demonstrate the secondary structure of our designed helix, we also synthesized and measured the drift time distributions of a model, gas-phase, positively-charged helix, $[Ac\text{-}Ala_{19}\text{-}Lys\text{+}H]^+$ and the corresponding reverse peptide, $[Ac\text{-}Lys\text{-}Ala_{19}\text{+}H]^+$. Calculated structures for these species are shown in Figure 1D and 1E, respectively. In order to directly compare the ESI(-)-generated peptide structures, we measured the drift time distributions of the peptides described by the Jarrold group, previously reported in a linear-field instrument,[15, 16] using conditions that were identical to our analysis of the negative peptides, save the polarity (Figure 1F). The difference in t_D between the positively charged model helix (t_D = 12.99 ± 0.06 ms) and the reverse-peptide globule (t_D = 9.79 ± 0.14 ms) are of similar magnitude to our engineered peptide, indicating that $[Aad\text{-}Ala_{19}\text{-}Am\text{-}H]^-$ adopts a helical structure in the gas phase.

The peptides in this study were generated using solid-phase synthetic techniques. Direct analysis of the crude reaction mixtures of the desired 20-mers allowed the measurement of a series of deletion sequences missing one or several alanine residues. The measured t_D values, placed on a *m/z* scale, demonstrate the striking similarity in CCS of the negatively- and positively-charged helices, as well as the corresponding globules (Figure 2A). These data clearly show that the additional CCS of the positively charged structures is accounted for by a difference in mass, and that both the positively- and negatively-charged structures follow very similar trends.

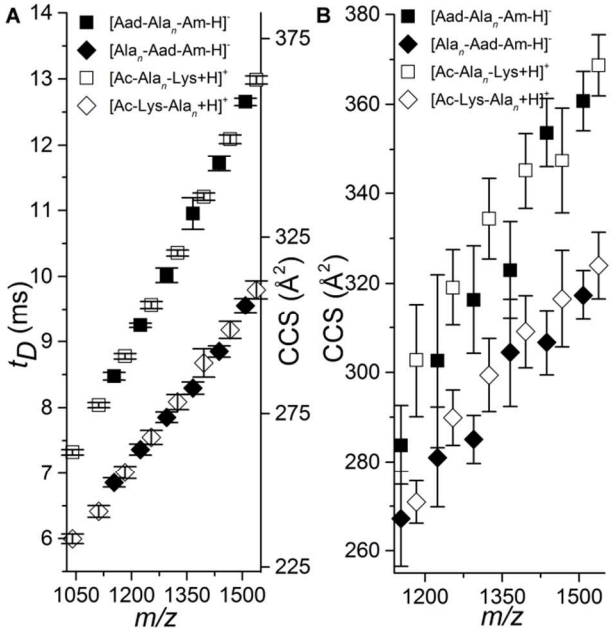

Figure 2. (A) t_D of negatively-(filled shapes) and positively-(open shapes) charged peptides. Helical structures (squares) have t_D values that are significantly longer than the more compact, globular structures (diamonds). Error bars represent the standard deviation of triplicate measurements. The right axis is approximated based on the published CCS values for $[Ac\text{-}Ala_n\text{-}Lys\text{+}H]^+$.[16] (B) Estimated CCS values for peptides obtained by MD simulations.[14] Error bars represent the standard deviation among the five lowest energy structures of 100 simulations of ~50 ps.

We corroborated the similarity in CCS for the negatively- and positively-charged structures by MD simulations performed on

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the four target peptides, and corresponding deletion sequences (15-19 alanine residues; Figure 2B). The CCSs were calculated for the five lowest energy structures of 100 MD simulations for each peptide using the trajectory.[14] At low *m/z*, there is less differentiation between the globular and helical structures of both polarities in the model. However, as the length of the peptides increase, a clear divergence between the two secondary structures occurs, similar to the experimental data, with the negatively- and positively-charged peptides following the same trends.

Conclusions

In summary, we have demonstrated the design and implementation of an α-helix stabilized in the gas phase by the addition of an acidic residue at the N-terminus. This group forms a favorable electrostatic interaction with the helical dipole and satisfies hydrogen bonding partners. This stabilized secondary structure was verified using IM-MS, where the similarity between positively-charged helices, as well as MD simulations, is consistent with the formation of a stable α-helix. This system should draw interest to the complimentary study of peptide structure under negative ionization for those systems that warrant it. In particular, structures containing acidic residues in which negative ionization may more closely approximate the protonation state of biologically-relevant residues.

Notes and references

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† Electronic Supplementary Information (ESI) available: Synthetic scheme, details of IM-MS and molecular dynamics simulations, partial characterization of elongated Ala_n-Aad-Am. See DOI: 10.1039/c000000x/

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