Design of Helices That Are Stable in Vacuo

Robert R. Hudgins, Mark A. Ratner, and Martin F. Jarrold*

Department of Chemistry, Northwestern University
2145 Sheridan Road, Evanston, Illinois 60208

Received August 24, 1998

The α-helix is the most common short-range structural motif in proteins,[1] and understanding its properties is central to understanding protein folding[2] and enzyme function.[3] Different amino acids have different helix propensities.[2,4,5] Alanine has one of the highest helix propensities, and alanine-rich peptides have been widely studied in solution. However, the factors responsible for the different helix propensities certainly include the effects of the solvent.[6−8] In principle, intrinsic helix propensities can be obtained from gas-phase measurements where solvent interactions are absent. Here we report studies of alanine-based peptides in a solvent-free environment. Protonated polyalanines with up to 20 residues do not form extended helices in a vacuum. However, the addition of a single lysine at the C terminus (to give Ac-Ala_n-$LysH^+$) results in the formation of very stable, monomeric, polyalanine helices. The design of the Ac-Ala_n-$LysH^+$ peptide optimizes hydrogen bonding of the charged lysine with the C-terminal backbone carbonyl groups, and the interaction of the charge with the helix dipole. Helices are observed for Ac-Ala_n-$LysH^+$ peptides with as few as eight residues, which is significantly shorter than the shortest helical peptides found in solution.

A variety of experimental techniques have recently been used to examine the gas-phase conformations of proteins and peptides.[9−17] In the work reported here we have used high-resolution ion mobility measurements.[18,19] The mobility is a measure of how rapidly an ion moves through an inert buffer gas under the influence of a weak electric field. The mobility depends on the ion's collision cross section with the buffer gas. Structural information is deduced by comparing measured cross sections to orientationally averaged cross sections calculated for conformations derived from molecular dynamics (MD) simulations.[12] Our experimental apparatus consists of an electrospray source, a 63-cm long drift tube containing helium buffer gas, and a quadrupole mass spectrometer. Details of the experimental technique are described in a recent publication.[20] MD simulations were performed with the CHARMM force field[21] using the 21.3 parameter set, the united atom approximation, and SHAKE[22] constraints on all explicit bonds. Multiple simulations of 0.25−1.0 ns were performed at 300 K for each peptide. Cross sections were calculated by a trajectory method[23] and averaged over 50 structures taken from an MD simulation at regular time intervals.

Figure 1a shows a mass spectrum obtained by electrospraying a solution of unpurified Ac-Ala_{19}-Lys in formic acid. A progression of peaks at mass to charge (m/z) ratios corresponding to Ac-Ala_n-$LysH^+$, $n = 14-19$, is apparent. The distribution of peptide sizes results from inefficient coupling during solid-phase Fmoc synthesis (Anaspec Inc., San Jose, CA). Figure 1b shows a drift time distribution measured with the mass spectrometer set to transmit Ac-Ala_{19}-$LysH^+$. There are two main peaks; the peak at a drift time of ∼170 ms is assigned to the Ac-Ala_{19}-$LysH^+$ monomer while the one at ∼155 ms is assigned to the (Ac-Ala_{19}-$LysH)_2^{2+}$ dimer, which occurs at the same m/z.[24]

The measured drift times are converted into average collision cross sections.[25] In this work we will use a relative cross section scale given by $\Omega_{av} - 14.50n$ where Ω_{av} (the measured cross section) is in Å^2 and 14.50 Å^2 is the calculated average cross section per residue for an ideal polyalanine α-helix with the torsion angles fixed at $\phi = -57°$ and $\psi = -47°$. With this scale, helical conformations have relative cross sections that are independent of the number of alanine residues, whereas other conformations will have relative cross sections that change with the number of residues. Relative collision cross sections for Ac-Ala_n-$LysH^+$, $n = 5-19$, and Ala_nH^+, $n = 3-20$, are plotted against n in Figure 2. The relative cross sections for the Ala_nH^+ peptides (open points in the figure) clearly decrease with increasing size. This indicates that these peptides have conformations that are more compact than helices. When helices are used as the starting point for MD simulations for the Ala_nH^+ peptides, they rapidly collapse to globular conformations such as that shown for $Ala_{19}H^+$ in Figure 3. The lower dashed line in Figure 2 shows average cross sections determined for the lowest energy globular conformation found in several different MD simulations for each Ala_nH^+ peptide. The relatively large fluctuations in the calculated cross sections result from incomplete conformational averaging. However, the good overall agreement between the calculated cross sections and the measured values indicates that the Ala_nH^+ peptides have globular conformations. A number of the low-energy globular conformations found for the Ala_nH^+ peptides have short helical regions (for example, Figure 3). However, these partially helical structures have cross sections (and energies) which are similar to globular structures without helical regions, and thus they cannot be distinguished in our experiments.

Inspection of the globular conformation for $Ala_{19}H^+$ shown in Figure 3 shows that the charge (which is located at the N terminus) is stabilized by hydrogen-bonding interactions with backbone carbonyl groups. The rest of the peptide has wrapped around the charge to maximize these interactions. Thus, the charge appears to destabilize the helical conformation, and this leads to a lack of definitive secondary structure. Figure 3 (bottom) shows a conformation from an MD simulation for an Ac-Ala_{19}-$LysH^+$

(1) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 222.
(2) Chakrabartty, A.; Baldwin, R. L. *Adv. Protein Chem.* **1995**, *46*, 141.
(3) Hol, W. G. J. *Prog. Biophys. Mol. Biol.* **1985**, *45*, 149.
(4) Marqusee, S.; Robbins, V. H.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5286.
(5) O'Neil, K. T.; DeGrado, W. F. *Science* **1990**, *250*, 646.
(6) Blaber, M.; Zhang, X.-J.; Matthews, B. W. *Science* **1993**, *260*, 1637.
(7) Yang, A.-S.; Honig, B. *J. Mol. Biol.* **1995**, *252*, 351.
(8) DiCapua, F. M.; Swaminathan, S.; Beveridge, D. L. *J. Am. Chem. Soc.* **1991**, *113*, 6145.
(9) Suckau, D.; Shi, Y.; Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 790.
(10) Collings, B. A.; Douglas, D. J. *J. Am. Chem. Soc.* **1996**, *118*, 4488.
(11) Sullivan, P. A.; Axelsson, J.; Altmann, S.; Quist, A. P.; Sundqvist, B. U. R.; Reimann, C. T. *J. Am. Soc. Mass. Spectrom.* **1996**, *7*, 329.
(12) Wyttenbach, T.; vonHelden, G.; Bowers, M. T. *J. Am. Chem. Soc.* **1996**, *118*, 8355.
(13) Clemmer, D. E.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1995**, *117*, 10141.
(14) Valentine, S. J.; Anderson, J. G.; Ellington, A. D.; Clemmer, D. E. *J. Phys. Chem. B* **1997**, *101*, 3891.
(15) Gross, D. S.; Schnier, P. D.; Rodriguez-Cruz, S. E.; Fagerquist, C. K.; Williams, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3143.
(16) Klassen, J. S.; Blades, A. T.; Kebarle, P. *J. Phys. Chem.* **1995**, *99*, 15509.
(17) Kaltashov, I. A.; Fenselau, C. *Proteins: Struct., Funct., Genet.* **1997**, *27*, 165.
(18) Dugourd, P.; Hudgins, R. R.; Clemmer, D. E.; Jarrold, M. F. *Rev. Sci. Instrum.* **1997**, *68*, 1122.
(19) Hudgins, R. R.; Woenckhaus, J.; Jarrold, M. F. *Int. J. Mass. Spectrom. Ion Processes* **1997**, *165/166*, 497.
(20) Hudgins, R. R.; Mao, Y.; Ratner, M. A.; Jarrold, M. F. *Biophys. J.*, submitted.
(21) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
(22) VanGunsteren, W. F.; Berendsen, H. J. C. *Mol. Phys.* **1977**, *34*, 1311.
(23) Mesleh, M. F.; Hunter, J. M.; Shvartsburg, A. A.; Schatz, G. C.; Jarrold, M. F. *J. Phys. Chem.* **1996**, *100*, 16082.
(24) There is also a small dimer peak at a drift time a few percent lower than that of the dominant dimer peak.
(25) Mason, E. A.; McDaniel, E. W. *Transport properties of ions in gases*; Wiley: New York, 1988.

10.1021/ja983021q CCC: $15.00
Published on Web 12/01/1998

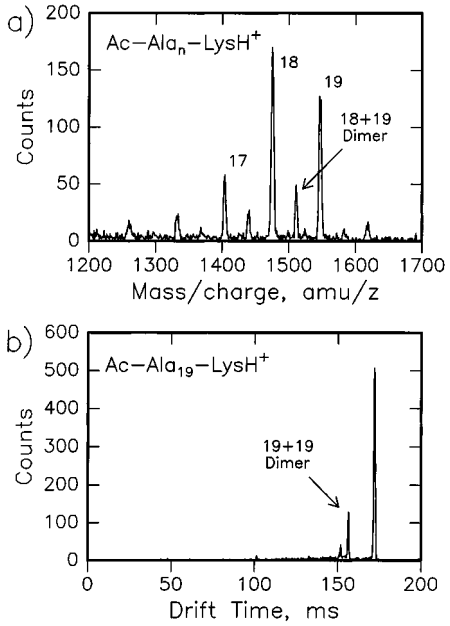

Figure 1. 1. Electrospray mass spectrum of Ac-Ala$_{19}$-Lys in formic acid (a) and drift time distribution measured for Ac-Ala$_{19}$-LysH$^+$ (b).

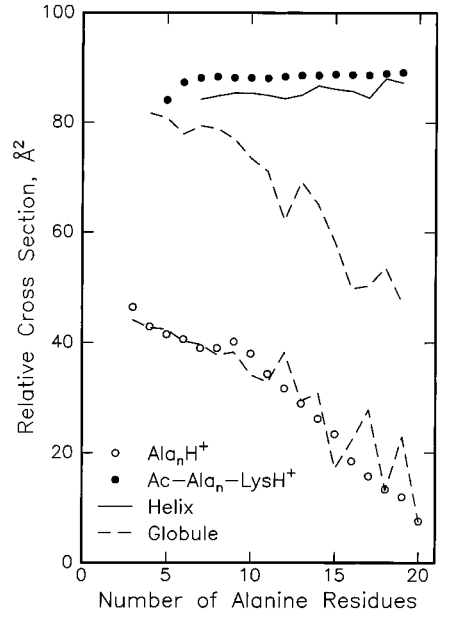

Figure 2. 2. Plot of the relative collision cross section against the number of alanine residues for Ala$_n$H$^+$ (●) and Ac-Ala$_n$-LysH$^+$ monomers (○). The dashed lines show relative collision cross sections calculated for globular structures from MD simulations. The solid line shows cross sections calculated for helical conformations from MD simulations.

peptide that was designed to generate a stable, extended helix in the gas phase. The lysine butylamine side chain is flexible enough that the protonated amine group can be located at the C terminus of the helix where it is coordinated to four backbone carbonyls. In a hypothetical Ala$_n$ helix, the last four carbonyls of the helix do not have hydrogen bond partners. Here the lysine side chain caps the helix. Hydrogen bonding partners are believed to stabilize helices within proteins,[26] and there are indications of similar effects for small peptides in solution.[27] For the structure shown in Figure 3 the charge is also stabilized because it is at the negative end of the helix dipole. Theory[28,29] and solution studies[30,31] show

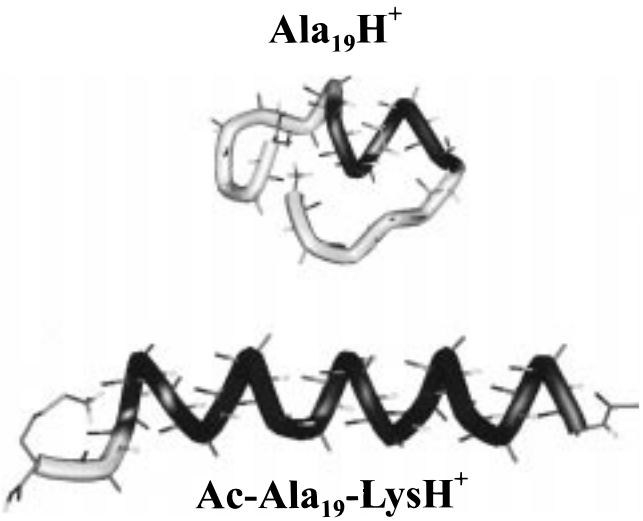

Figure 3. 3. Conformations of an Ala$_{19}$H$^+$ globule and an Ac-Ala$_{19}$-LysH$^+$ helix, taken from MD simulations. Pictures were generated using WebLab Viewer (Molecular Simulations, Inc.).

that amino acids with charged side chains are preferentially located near the helix pole of opposite polarity. In MD simulations the helical conformation of Ac-Ala$_{19}$-LysH$^+$ is stable. Globular conformations generated by starting the MD with an extended ($\phi = \psi = 180°$) structure are much less stable than the helix (by $\sim$150 kJ mol^{-1} for Ac-Ala$_{19}$-LysH$^+$). Antiparallel β-sheets are also less stable in the simulations (by $\sim$200 kJ mol^{-1} for Ac-Ala$_{19}$-LysH$^+$). In a simulation at 600 K the β-sheet converted into the α-helical form within 0.5 ns. In agreement with previous simulations,[32] 3−10 helices were found to rapidly convert (in the first 10 ps) into α-helices.

Experimental evidence that Ac-Ala$_n$-LysH$^+$ peptides form extended helices is provided in Figure 2. The relative cross sections for these peptides are independent of n as expected for an extended helix. The solid line in the figure shows average cross sections calculated for the helical conformations from MD simulations. They are in good agreement with the measured cross sections. Average cross sections calculated for the less stable globular conformations, shown as the upper dashed line in Figure 2, are not in good agreement with the experimental data. The measured relative cross sections remain relatively constant for n $\geq$ 7, suggesting that $n = 7$ (8 residues) is the smallest Ac-Ala$_n$-LysH$^+$ peptide that is an extended helix. However, a helical peptide of eight residues is already much smaller than any helical peptide found in solution. Even small helical peptides such as the 13-residue C-peptide (analogue III) of RNAse A[30] only exhibit $\leq$ 25% helicity in solution at room temperature. The helices observed here are substantially less fluxional and have a much higher helix content. This supports the idea that secondary structure is more stable in vacuo than in solution. In the gas phase, there is no competition from hydrogen bonds between the backbone and the solvent, which usually destabilizes helices in solution.[33]

Acknowledgment. We thank Jiri Kolafa for the use of his Prosis Molecular Modeling Software and Yi Mao for helpful advice. We gratefully acknowledge the National Science Foundation, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the Army Research Office for partial support of this work.

JA983021Q

(26) Presta, L. G.; Rose, G. D. *Science* **1988**, *240*, 1632.
(27) Forood, B.; Feliciano, E. J.; Nambiar, K. P. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 838.
(28) Blagdon, D. E.; Goodman, M. *Biopolymers* **1975**, *14*, 241.
(29) Daggett, V. D.; Kollman, P. A.; Kuntz, I. D. *Chem. Scr.* **1989**, *29A*, 205.
(30) Shoemaker, K. R.; Kim, P. S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Nature* **1987**, *326*, 563.
(31) Richardson, J. S.; Richardson, D. C. *Science* **1988**, *240*, 1648.
(32) Tirado-Rives, J.; Maxwell, D. S.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1993**, *115*, 11590.
(33) Sundaralingam, M.; Sekharudu, Y. C. *Science* **1989**, *244*, 1333.