Communications to the Editor

Helix Formation in Unsolvated Peptides: Side Chain Entropy Is Not the Determining Factor

Brian S. Kinnear and Martin F. Jarrold*

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

Received April 6, 2001

Understanding the factors that stabilize α-helices is critical to understanding protein folding.[1] Structure and sequence information for naturally occurring proteins has revealed a preference for certain amino acids in α-helices.[2] Thus, many studies have focused on the individual effects of the amino acids on α-helix stability,[3,4] and algorithms have been developed that can predict helix content.[5] Monte Carlo simulations have indicated that side chain entropy opposes helix formation and can account for most of the differences in the helix-stabilizing/destabilizing effects of the natural amino acids.[6,7] Specifically, side chain entropy predicts a helix propensity scale with Ala > Leu > Val. The main complication in solution studies of α-helix stability is that the solvent environment can change the helix-stabilizing/destabilizing tendencies of the amino acids.[8,9] Factors such as the solvation of the helix backbone may also play a role in the ability of a particular amino acid to stabilize an α-helix in solution.[10] By studying unsolvated peptides, the intrinsic factors leading to α-helix stability can be elucidated.[11] Here we report on the conformations of unsolvated leucine-based peptides and compare them to previous studies of the conformations of unsolvated glycine-, alanine-, and valine-based peptides.[12−14] The results show that leucine forms helices more readily than alanine, but less readily than valine (Val > Leu > Ala). This indicates that side chain entropy is not the determining factor in helix formation in unsolvated peptides. The results of molecular dynamics simulations suggest that the controlling factor is the stability of the globular state (a compact three-dimensional geometry). Residues that make poor (high energy) globules are good at making helices.

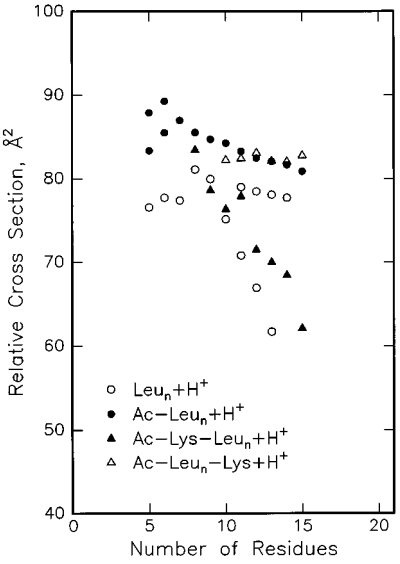

Figure 1. Relative cross-sections plotted against the total number of residues (excluding the acetyl group). The relative cross-section scale (in Å^2) is given by $\Omega_{rel} = \Omega_{meas} - 21.03N_L - 22.08N_K$, where N_L is the number of leucine residues and N_K is the number of lysine residues.

Information about the conformations of unsolvated peptides was obtained from high-resolution ion mobility measurements.[15] The mobility of an ion in the gas phase depends on its average collision cross section with a buffer gas, which in turn depends on its structure.[16−18] The experimental apparatus consists of an electrospray source followed by a 5 cm long ion gate and a 63 cm long drift tube containing helium buffer gas. The pressure in the drift tube is slightly above atmospheric pressure so that a steady flow of helium (∼2000 sccm) through the ion gate prevents neutral molecules from entering the drift tube. A drift voltage of 10 000 V is divided across 46 guard rings, providing a uniform electric field along the drift tube axis. After traveling through the drift tube, some of the ions exit through a small aperture (0.125 mm in diameter), where they are then focused into a quadrupole mass spectrometer and detected by an off-axis collision dynode and dual microchannel plates. The measured drift times are subsequently converted into collision cross sections for further analysis. All the peptides studied here were synthesized on an Applied Biosystems Model 433A peptide synthesizer using *FastMoc* (a variant of Fmoc) chemistry.

Relative cross sections for Leu$_n$+H$^+$, Ac-Leu$_n$+H$^+$, Ac-Lys-Leu$_n$+H$^+$, and Ac-Leu$_n$-Lys+H$^+$ are shown in Figure 1. The relative cross section scale used is $\Omega_{rel} = \Omega_{meas} - 21.03N_L - 22.08N_K$, where N_L and N_K are the number of leucine and lysine residues, respectively, 21.03 Å^2 is the cross section per residue determined for an ideal polyleucine α-helix, and 22.08 Å^2 is the average increment in the cross section for a C-terminus lysine.

(1) Horovitz, A. H.; Matthews, J. M.; Fersht, A. R. *J. Mol. Biol.* **1992**, *227*, 560−568.
(2) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 211−222.
(3) Pace, C. N.; Scholtz, J. M. *Biophys. J.* **1998**, *75*, 422−427.
(4) Chakrabartty, A.; Baldwin, R. L. *Adv. Protein Chem.* **1995**, *46*, 141−176.
(5) Muñoz, V.; Serrano, L. *Biopolymers* **1997**, *41*, 495−509.
(6) Creamer, T. P.; Rose, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5937−5941.
(7) Piela, L.; Nemethy, G.; Sheraga, H. A. *Biopolymers* **1987**, *26*, 1273−1286.
(8) Wang, C.; Liu, L.-P.; Deber, C. M. *Phys. Chem. Chem. Phys.* **1999**, *1*, 1539−1542.
(9) Krittanai, C.; Johnson, W. C., Jr. *Proteins: Struct., Funct., Genet.* **2000**, *39*, 132−141.
(10) Luo, P.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4930−4935.
(11) For some examples of studies of unsolvated peptides and proteins, see: Suckau, D.; Shi, Y.; Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. *Proc. Natl. Acad. Sci.* **1993**, *90*, 790−793. Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1995**, *117*, 12840−12854. Schnier, P. D.; Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1996**, *118*, 7178−7189. Kaltashov, I. A.; Fenselau, C. *Proteins: Struct. Func. Genet.* **1997**, *27*, 165−170. Valentine, S. J.; Clemmer, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 3558−3566. Wyttenbach, T.; Bushnell, J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098−5103. Arteca, G. A.; Velázquez, I.; Reimann, C. T.; Tapia, O. *Phys. Rev. E* **1999**, *59*, 5981−5986. Schaaff, T. G.; Stephenson, J. L.; McLuckey, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 8907−8919.
(12) Hudgins, R. R.; Jarrold, M. F. *J. Phys. Chem. B* **2000**, *104*, 2154−2158.
(13) Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1999**, *121*, 3494−3501.
(14) Kinnear, B. S.; Kaleta, D. T.; Kohtani, M.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **2000**, *122*, 9243−9256.
(15) Dugourd, P.; Hudgins, R. R.; Clemmer, D. E.; Jarrold, M. F. *Rev. Sci Instrum.* **1997**, *68*, 1122−1129.
(16) Hagen, D. F. *Anal. Chem.* **1979**, *51*, 870−874.
(17) Von Helden, G.; Hsu, M.-T.; Kemper, P. R.; Bowers, M. T. *J. Chem. Phys.* **1991**, *95*, 3835−3837.
(18) Clemmer, D. E.; Jarrold, M. F. *J. Mass Spectrom.* **1997**, *32*, 577−592.

10.1021/ja010891q CCC: $20.00
Published on Web 07/18/2001

Table 1. Average Potential Energies for Leucine-, Valine-, Alanine-, and Glycine-Based Peptides in Helical and Globular Conformations

peptide	average potential energy,[a] kJ mol^{-1}		
	α-helix[b]	globule[c]	helix − globule[d]
Ac-Leu_{19}-$LysH^+$	−2890	−2739	−151
Ac−Val_{19}-$LysH^+$	−2827	−2665	−162
Ac−Ala_{19}-$LysH^+$	−2819	−2690	−129
Ac-Gly_{19}-$LysH^+$	−2846	−2800	−46

[a] Average potential energy from the last 35 ps of the simulations. [b] Lowest average potential energy from up to ten 300 K simulations started from an ideal α-helix. [c] Lowest average potential energy from up to 60 simulated annealing runs started from an extended string. [d] These differences in the average potential energies may change slightly if more simulations are run.

When plotted in this way, α-helices have relative cross sections that are nearly independent of the peptide size, while globules have relative cross sections that decrease sharply with increasing size.

Of the leucine-based peptides we have examined, Ac-Leu_n-$Lys+H^+$ is the most likely to form a helix as the protonated lysine side chain can cap the C-terminus and interact favorably with the helix macrodipole.[19] The relative cross sections for Ac-Leu_n-$Lys+H^+$ (see Figure 1) are almost independent of peptide size, which is characteristic of an α-helical conformation. For $n > 8$, Ac-Leu_n+H^+ is also helical. In this peptide, the proton must be on the backbone near the C-terminus so that it can interact favorably with the helix macrodipole. The alanine and valine analogues to these peptides, Ac-Ala_n-$Lys+H^+$, Ac-Ala_n+H^+, Ac-Val_n-$Lys+H^+$, and Ac-Val_n+H^+, also form helices.[12,17,20] The glycine analogues do not.[10]

The results for Ac-Lys-Leu_n+H^+ are characteristic of a globule. When the lysine is located at the N-terminus and it is protonated, the helix is destabilized by an unfavorable interaction between the positive charge and the helix macrodipole and by the lack of end-capping at the C-terminus. The alanine analogue to this peptide, Ac-Lys-Ala_n+H^+, also forms globules.[17] However, the valine analogue, Ac-Lys-Val_n+H^+, forms both a random globule and helix for $n = 12$ and 13, and only a helix for $n > 13$.[12] As the proton must be near the C-terminus for the helix to form, the free energy gained from forming a polyvaline helix must be large enough to compensate for moving the proton from the N-terminus to the C-terminus. As Ac-Lys-Leu_n+H^+ does not form a helix, the free energy gained by forming a helix must not be as large as that gained by Ac-Lys-Val_n+H^+. Thus it can be concluded that leucine does not form a helix as readily as valine in the absence of a solvent.

For Leu_n+H^+ with $n > 10$, both a helical state and a globular state are formed, with the globule becoming negligible for $n > 13$. The alanine analogue to this peptide, Ala_n+H^+, forms only random globules,[21] while the valine analogue, Val_n+H^+, also formed both random globules and helices for $n > 11$.[12] The most basic site in these peptides is the N-terminus, but in the helical conformation the proton must be located on the backbone near the C-terminus so that the charge can interact favorable with the helix macrodipole. The proton affinity of the N-terminus of a peptide is slightly lower than that for the lysine side chain, so it requires slightly less free energy to relocate the proton in these peptides than it does to relocate the proton in the peptides with lysine at the N-terminus. As leucine is able to relocate the proton and make a helix while alanine does not, unsolvated leucine forms helices more readily than alanine does.

On the basis of the results described above, we obtain the following ranking of helix-forming tendencies in unsolvated peptides: Val > Leu > Ala ≫ Gly. This is quite different from the consensus rank ordering found in solution studies: Ala > Leu ≫ Val > Gly, which clearly demonstrates that the solvent plays an important role in modifying the helix-forming tendencies of the different amino acids. The rank ordering predicted from consideration of side chain entropy is identical with that found in solution. Clearly, side chain entropy alone cannot account for the helix-forming tendencies of the different amino acids in the gas phase.

In an effort to understand the experimental results, a series of molecular dynamics (MD) simulations were performed. The simulations were done using the MACSIMUS suite of programs[22] with the CHARMM force field (21.3 parameter set).[23] Two types of simulations were performed: 960 ps simulations at 300 K starting from an ideal α-helix and simulated annealing with a schedule of 240 ps at 600, 500,and 400 K and 480 ps at 300 K starting from an extended string. The latter usually yielded globular conformations. The temperature was maintained by re-scaling the kinetic energies every 0.1 ps. Average potential energies were obtained from the last 35 ps of each simulation.

A compilation of the lowest average potential energies found in the simulations for leucine-, valine-, alanine-, and glycine-based peptides in helical and globular conformations is shown in Table 1. Comparisons cannot be made between the energies of the helices and globules for the different peptides; however, the energy differences between the helix and globule can be compared (note that these are not free energy differences).[24] The differences are in the order Val > Leu > Ala ≫ Gly, which is identical to the ranking of helix-forming tendencies in unsolvated peptides described above. This ordering roughly follows the physical size of the side chain: residues with bigger side chains generate less compact and higher energy globular states, which makes the helix appear more favorable (the energy difference between the helix and globule is larger). Leucine has a slightly larger side chain than valine, but the leucine side chain (which is not β-branched) is more flexible and it can move more easily than the valine side chain to optimize hydrogen bonding within the globule. These results suggest that the stability of the globular state is more important than the stability of the helical state in controlling helix formation in unsolvated peptides.

Acknowledgment. We thank Jiri Kolafa for use of his MACSIMUS molecular modeling programs. We gratefully acknowledge the support of the National Institutes of Health.

JA010891Q

(19) Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1998**, *120*, 12974−12975.
(20) Kaleta, D. T.; Jarrold, M. F. *J. Phys. Chem. B* **2001**, *105*, 4436-4440.
(21) Hudgins, R. R.; Mao, Y.; Ratner, M. A.; Jarrold, M. F. *Biophys. J.* **1999**, *76*, 1591−1597.
(22) Kolafa, J. http://www.icpf.cas.cz/jiri/macsimus/default.htm.
(23) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187−217.
(24) In the MD simulations for the glycine peptide the helix is 46 kJ mol^{-1} lower in energy than the globule, while the globule is observed in the experiments. The helical state is expected to be disfavored entropically for the glycine peptide, and so when the entropic contribution is taken into account, the globule is expected to have the lowest free energy.