Communications to the Editor

Free Energy Profile of a 3_{10}- to α-Helical Transition of an Oligopeptide in Various Solvents

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The α-helix is a well-known secondary structural element in proteins. The 3_{10}-helix is not as prevalent as the α-helix, but it is still relatively common in proteins (10% of helical residues observed in proteins).[1] These 3_{10}-helices are usually short, ~4 residues in length, though 3_{10}-helices of 7–12 residues in length have been reported,[2,3] and are commonly found on the surfaces of proteins, where they may form binding surfaces or active sites.[4] In fact, two α- to 3_{10}-helical transitions have been reported in crystal structures of enzymes[5,6] as a result of substrate binding.

The factors governing the relative stabilities and transitions between α- and 3_{10}-helices are quite subtle[7] and not thoroughly understood. In crystal structures of peptides containing α-methylalanine (MeA, aminoisobutyric acid, Aib), both helical types have been observed in the same crystal.[8-10] Other crystallographic data[8,10-12] illustrate that simply changing the protection group (Ac vs Boc) or the solvent of crystallization (water, 2-propanol, or no solvent) can result in the preferential formation of one helix over the other. Nuclear magnetic resonance data have shown that hexapeptides of similar sequence are 3_{10}-helical in $CDCl_3$ and α-helical in $(CD_3)_2SO$.[13] Due to these observations and the role of peptides containing MeA (peptaibol antibiotics) in causing voltage-dependent conductance changes in membranes, there has been a great deal of interest in trying to further understand the α- to 3_{10}-helical transition.[7,14-16]

As reported here, theoretical methods have been applied to obtain free energy profiles, or potentials of mean force (pmfs), for the α- to 3_{10}-helical transition of an MeA oligomer in various solvents in order to elucidate the effect of solvent on the relative stability of the two helical forms and the transitions between them. MeA was chosen rather than Ala because the conformational space of this constrained residue is restricted to regions centered about the α- and 3_{10}-helices.[17] Computationally, this makes an MeA oligomer much more appealing than, for example, an Ala oligomer of the same length, due to the restricted conformational space that must be sampled for reasonable thermodynamic results.

Pmfs of an MeA decamer (CH_3CO-MeA_{10}-NMe) were calculated using the method of umbrella sampling.[18] A well-defined reaction coordinate for the transition has been previously determined,[15,19] and it correlates well with a sequential change of the end-to-end distance ($r_{C\alpha 1}$–$r_{C\alpha 10}$) from the 3_{10}-helical region (19 Å) to the α-helical region (13 Å). Molecular dynamics calculations were carried out at 298 K using a modified version of the AMBER 4.0[20] software package and the AMBER/OPLS[21,22] force field. Three different environments were simulated including *in vacuo*, water, and CH_3CN. For the *in vacuo* simulations, the dielectric constant was fixed at 1.0, a time step of 2 fs was used, and no nonbonded cutoff was used. Seven overlapping windows were used, and the end-to-end distance was restrained in each with a force constant of 5 kcal/mol/Å^2. Each window of the simulation was run for a total of 900 ps. The solution simulations used conditions similar to those in the *in vacuo* simulation, except periodic boundary conditions, a force constant of 3 kcal/mol/Å^2 in each window, an 8.0-Å cutoff, and a total simulation time of 400 ps per window were used. The conformations of the α- and 3_{10}-helical endpoints were found to possess a high percentage of helical character, as characterized by averaging the conformations of the endpoints and by a hydrogen-bond analysis.

The resultant pmfs are shown in Figure 1. The pmf of the *in vacuo* simulation shows a free energy minimum for the 3_{10}-helix at 18.5 Å (Figure 1 insert). The α-helix is more stable than the 3_{10}-helix by 3.3 kcal/mol, and a small barrier of 0.2 kcal/mol was calculated for the 3_{10}- to α-helix transition. As the dielectric of the system increases, the α-helix becomes more thermodynamically stable in comparison to the 3_{10}-helix (by 8.0 kcal/mol in CH_3CN and water). To some extent, these data can be explained by considering the relative geometries of the α- and 3_{10}-helices. Due to the side-chain arrangements of the 3_{10}-helix, this conformation contains several short, though not sterically forbidden, contacts in comparison to the α-helix. However, the ideal 3_{10}-helix has one more intramolecular hydrogen bond than the α-helix. Upon increasing the solvent dielectric, the effective strength of this extra hydrogen bond will be reduced, and the close contacts of the 3_{10}-helix may disfavor this conformation.[7] Therefore, one would expect that the α-helix would become more stable than the 3_{10}-helix as the solvent dielectric increases, as observed in our pmf calculations. This conclusion is also consistent with experimental data that show MeA-containing peptides to be 3_{10}-helical in $CDCl_3$ and α-helical in $(CD_3)_2SO$.[13]

The potential relevance of these calculations to helices containing the usual amino acids, such as Ala, is based on the following arguments. Clark *et al.*[15] investigated helical transitions

(1) Barlow, D. J.; Thornton, J. M. *J. Mol. Biol.* **1988**, *201*, 601–619.
(2) Robbins, D. J.; Stout, C. D. *Proteins: Struct., Funct. Genet.* **1989**, *5*, 289–312.
(3) Pathak, D.; Ollis, D. *J. Mol. Biol.* **1990**, *214*, 497–525.
(4) Karpen, M. E.; De, H. P. L.; Neet, K. E. *Protein Sci.* **1992**, *1*, 1333–1342.
(5) McPhalen, C. A.; Vincent, M. G.; Picot, D.; Jansonius, J. N.; Lesk, A. M.; Chothia, C. *J. Mol. Biol.* **1992**, *227*, 197–213.
(6) Gerstein, M.; Chothia, C. *J. Mol. Biol.* **1991**, *220*, 133–149.
(7) Marshall, G. R.; Hodgkin, E. E.; Langs, D. A.; Smith, G. D.; Zabrocki, J.; Leplawy, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 487–91.
(8) Karle, I. P.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Proteins: Struct., Funct. Genet.* **1990**, *7*, 62–73.
(9) Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, H.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 765–9.
(10) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–56.
(11) Karle, I. L.; Sukumar, M.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 9284–88.
(12) Karle, I. L.; Flippen-Anderson, J. L.; Sukumar, M.; Balaram, P. *Int. J. Pept. Protein Res.* **1988**, *31*, 567–76.
(13) Vijayakumar, E. K. S.; Balaram, P. *Biopolymers* **1983**, *22*, 2133–40.
(14) Hodgkin, E. E.; Clark, J. D.; Miller, K. R.; Marshall, G. R. *Biopolymers* **1990**, *30*, 533–546.
(15) Clark, J. D.; Hodgkin, E. E.; Marshall, G. R. In *Molecular Conformation and Biological Interactions (Prof. G. N. Ramachandran Festschrift)*; Balaram, P., Ramaseshan, S., Eds.; Indian Academy of Sciences: Bangalore, India, 1991; pp 503–510.
(16) Aleman, C.; Subirana, J. A.; Perez, J. J. *Biopolymers* **1992**, *32*, 621–631.
(17) Marshall, G. R.; Bosshard, H. E. *Circ. Res. Suppl. II* **1972**, *30/31*, 143–150.
(18) Valleau, J. P.; Torrie, G. M. In *Statistical Mechanics, Part A*; Berne, B. J., Ed.; Plenum: New York, 1977; Chapter 5.
(19) Huston, S. E.; Marshall, G. R., *Biopolymers* **1994**, in press.
(20) Pearlman, D. A.; Case, D. A.; Caldwell, J. C.; Seibel, G. L.; Singh, U. C.; Weiner, P.; Kollman, P. *AMBER 4.0*; University of California: San Francisco, CA, 1991.
(21) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657–1666.
(22) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 765–784.

0002-7863/93/1515-11594$04.00/0

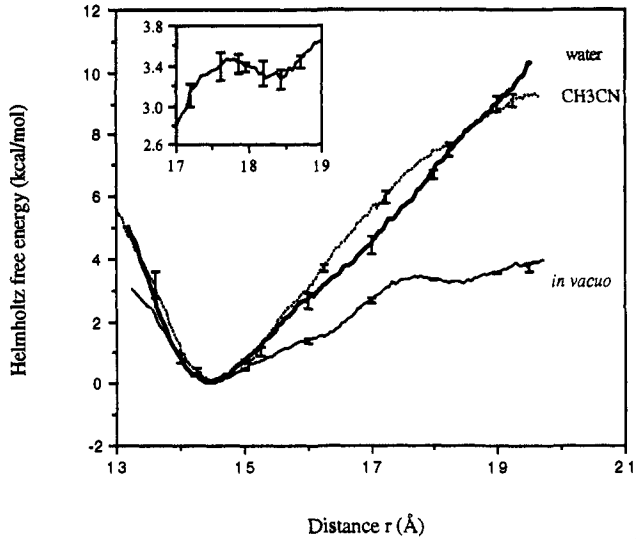

Figure 1. Computed potentials of mean force for the α- to 3_{10}-helical transition in various solvents. The inset is an expansion of the 3_{10}-helical region *in vacuo*. Statistical uncertainties were calculated according to the method of block averages.

of both Ala and MeA decamers using the AMBER united atom force field *in vacuo*. The results indicate that the α-helices of Ala and MeA are more stable than the 3_{10}-helices by 15.8 and 8.3 kcal/mol, respectively. This result for decamethylalanine is significantly different from the 3.3 kcal/mol of differential stability calculated here. Using the protocol described here, we have recently repeated the calculations for decamethylalanine using the AMBER united atom force field and found that the α-helix is more stable than the 3_{10}-helix by 5.7 kcal/mol. This is in closer agreement with the results reported here, and the remaining discrepancies (2.4 kcal/mol) must be due to the different force fields employed. One could conclude on the basis of the Clark *et al.*[15] results that in isolated peptides, 3_{10}-helices of α-monosubstituted amino acids are thermodynamically unstable. However, environmental effects may play a role in stabilizing the 3_{10}-helix of these peptides. In fact, molecular dynamics studies of helices comprising α-monosubstituted amino acids in water have shown that helix unfolding may take place *via* a 3_{10} intermediate.[23-25] As well, crystal structures of peptides containing mixtures of α,α-disubstituted and α-monosubstituted amino acids illustrate that subtle environmental effects can influence helical formation.[8-12] Such data are consistent with the fact that α- and 3_{10}-helices (of α-monosubstituted and α,α-disubstituted amino acids) are located in close-lying, low-energy regions of conformational space. Environmental effects would therefore play an important role in stabilizing either the α- or the 3_{10}-helix. To a certain extent, this has been illustrated by the pmfs calculated here.

It is clearly demonstrated by these calculations that aqueous conditions strongly favor the α-helix over the 3_{10}-helix; this raises questions concerning the reported experimental observation[26] of an isolated 3_{10}-helix in aqueous solution. In addition, our results indicate that the low dielectric environment of proteins, membranes, and crystals could provide appropriate conditions for stabilizing the 3_{10}-helix. Furthermore, it is possible that local side-chain interactions could stabilize one helical form over the other. This is supported by the sequence differences between 3_{10}- and α-helices observed in proteins.[4] Based on the minimal activation energy barrier to helical transitions, one can expect that relatively subtle changes in the protein environment could result in α- to 3_{10}-helical transitions. Overall, the present results emphasize the effects of environment[7] on relative helix stability and indicate a much more flexible helical structure than commonly assumed.[27] Detailed thermodynamic and structural results are currently being interpreted in order to gain more insight into the α- to 3_{10}-helical transition in peptides and its possible role in protein chemistry.

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(23) Tirado-Rives, J.; Jorgensen, W. L. *Biochemistry* **1991**, *30*, 3864–3871.
(24) Tobias, D. J.; Brooks, C. L., III. *Biochemistry* **1991**, *30*, 6059–6070.
(25) Soman, K. V.; Karimi, A.; Case, D. A. *Biopolymers* **1991**, *31*, 1351–1361.
(26) Miick, S. M.; Martinez, G. V.; Fiori, W. R.; Todd, A. P.; Millhauser, G. L. *Nature (London)* **1992**, *359*, 653–5.
(27) After this work was completed we became aware of a similar study using a Monte Carlo approach. Tirado-Rives, J.; Maxwell, D. S.; Jorgensen, W. L. *J. Am. Chem. Soc.*, previous paper in this issue.