

## Commentary

### Helix is a helix is a helix?

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There is much activity directed at quantifying the contribution of specific interactions to the stabilization of protein structure. The paper by Myers *et al.* (1) in this issue of the *Proceedings* addresses a central and much studied issue: The experimental measure of the intrinsic  $\alpha$ -helix forming propensities of the amino acids. What is an intrinsic  $\alpha$ -helix forming propensity? The idea that the different amino acids might have different abilities to adopt an  $\alpha$ -helical conformation is derived from the observation that in statistical surveys of proteins of known structure there are distinct, nonrandom distributions of the amino acids in  $\alpha$ -helices versus  $\beta$ -sheets (2). This suggests that certain amino acids may be energetically more favored in  $\alpha$ -helices, whereas others are more favored in  $\beta$ -sheets. A quantitative understanding of such rankings would greatly enhance our ability to rationally modify the stability and properties of proteins and could be of tremendous practical significance for therapeutically important proteins.

The experimental approach has been to perform “host–guest” experiments in which the effect of individually substituting each “guest” amino acid on the stability of a “host” protein or peptide is measured. As a result of such studies, a quantitative ranking of the propensity of each amino acid to adopt a  $\alpha$ -helical or  $\beta$ -sheet conformation is obtained (3, 4). Of key importance is the selection of the host protein or peptide and the local environment of the guest site. If such measurements are to be meaningful the aim, in so far as it is experimentally feasible, is to isolate the guest site from interactions with neighboring residues. The goal is to measure *only* the free energy associated with the unfolded-to-helix transition. To date, several different hosts have been employed, initially block copolymers (5) and more recently short, designed peptides (6–9) and small natural proteins (10, 11). The different host systems have generated similar, but certainly not identical, thermodynamic scales for the  $\alpha$ -helix forming tendencies of the amino acids. In addition, the range of free energies between the best and worst  $\alpha$ -helix forming residues differs quite significantly between the different studies. What is the source of the differences that have been observed? Do they derive from the methods used to calculate free energies? Do they reflect the influence of local interactions between the guest position and neighboring host residues that are superimposed upon intrinsic propensities? Are they related to the position of the guest site in the helix? Are there fundamental differences between helical peptides and helices in proteins?

The paper by Myers *et al.* (1) addresses the last possibility directly with an elegantly simple study. Their focus is a solvent-exposed  $\alpha$ -helix in the protein ribonuclease T<sub>1</sub> (RNase T<sub>1</sub>). The novel feature of their work is that they aim to measure the  $\alpha$ -helix forming propensities of the nonpolar amino acids at a guest site within this helix in the protein and at the same site in a synthetic peptide corresponding to the helix alone. The structure of RNase T<sub>1</sub> is shown in Fig. 1 on page 2833, with the chosen helix toward the front. The sequence of the helix is:

SSDVSTAQAAGYKHLLED (Ser-13 through Asp-29).

There is a site in this helix that has several features that are attractive for propensity measurements. Ala-21 (underlined above) is on the solvent-exposed face of the helix, in the exact center, and no residues from other regions of the protein come close to it. It is important to note, however, the identity of the residues at the i-3 (Thr), i-4 (Ser) and i+3 (Tyr), i+4 (Lys) positions relative to the guest site (i) within the helix itself. In the folded helix these residues will be nearest neighbors to the guest site and could potentially interact with the guest residue. In a direct comparison of the same guest site in a peptide versus a protein, such local effects should effectively cancel. The identity of such neighbors becomes important, however, when comparing the results of this study with those of other host–guest systems.

The admirable idea of directly comparing peptides and proteins was almost thwarted at the outset, because a synthetic peptide with a sequence corresponding to that of the wild-type helix was essentially completely lacking in helicity. Fortunately, the authors were able to overcome this obstacle by changing Gly-23 to alanine. Introduction of this additional helix-enhancing residue was sufficient to increase the helicity of the peptide to about 30%, making it well suited for the propensity studies. All further work in both the peptide and the protein was performed in this wt\* background.

The amino acids that comprise this study are alanine, leucine, methionine, isoleucine, phenylalanine, valine, and glycine. These amino acids were substituted individually into position 21 of the protein and the stability of each variant was determined from urea-induced denaturation curves, assuming a two-state transition (12, 13). Calculation of the stability of the corresponding peptides was more complex, because the transition is not two-state. The authors used circular dichroism to determine the fractional helicity of each peptide and from that value used Lifson–Roig helix–coil theory to calculate free energy (14).

The free energy propensity ranking from the peptide study compared very well with that of the protein study, both in rank ordering and in the magnitudes of the free energy change associated with a particular substitution. It is, for example, approximately 0.6 kcal/mol less favorable for phenylalanine to adopt a helical conformation than it is for alanine, regardless of whether the helix is in the protein or the isolated peptide. Interestingly, even in this closely matched system, although the correlation is strong it is not perfect. Methionine and leucine have essentially equal propensities to adopt a helical conformation in the protein, whereas in the peptide leucine is measurably poorer. With this exception, the rank orders are identical. In addition, the free energy range between the best and worst helix-forming residues is the same, the implication being that peptides are good models for proteins and that there is no inherent difference in being in a helical conformation within a peptide compared with within a protein.

There are more issues to discuss when the results of this study are compared with those obtained in other protein and peptide host systems. Should we expect a perfect one-to-one correlation between different scales? Factors that may contribute to imperfect correlations include the influence of local host-specific interactions, positional effects within the helix,

the appropriateness of the method used to calculate free energies, and intrinsic differences between helices in peptides and helices in proteins. The data presented in this study suggest that this last point is likely not a significant source of difference.

The authors mainly compare their results with earlier propensity measurements, which used the proteins barnase (10) and T<sub>4</sub> lysozyme (11) as the hosts, and with two earlier peptide studies that used either alanine-based (6) or salt-bridged monomeric helices as the hosts (8) (Fig. 3 on page 2836). What should we be looking for when we compare the different scales? The following are all important components when considering the similarity between the results in two different host systems: The correlation coefficient of a linear fit of the energies in the two scales, which is influenced by both the rank order of the amino acids in each scale, the size of the energy gaps between different pairs of amino acids, and finally the overall range between the best and worst helix formers (does the linear fit pass through the origin with a slope of one?).

How do the comparisons between the RNase T<sub>1</sub> data and the other studies fare in these areas? The correlation coefficients range from 0.88 for barnase to 0.97 for the alanine and salt-rich peptides. None of the pair-wise comparisons have identical rank orders, although in all the studies alanine is clearly the best  $\alpha$ -helix former and glycine the worst. Interestingly, all the studies reverse the ranking of methionine and leucine relative to the RNase T<sub>1</sub> peptides. Isoleucine and phenylalanine are reversed relative to RNase T<sub>1</sub> in the barnase study and phenylalanine and valine in the T<sub>4</sub> lysozyme study. The energy spacing between pairs of amino acids is similar, with all studies reporting a larger gap between phenylalanine and valine and the other amino acids. The range of free energies between the best and worst  $\alpha$ -helix formers is approximately 1 kcal/mol for the RNase T<sub>1</sub> peptide and for the barnase and T<sub>4</sub> lysozyme, whereas as it is greater, 1.97, for the alanine-based peptides and less, 0.74, for the salt-bridge peptides.

What should be the most important consideration when comparing two scales? The authors propose that a similar overall free energy range is most important and conclude that the results of the RNase T<sub>1</sub> peptide study are most similar to those of the barnase and T<sub>4</sub> lysozyme protein studies. It is interesting to note that the overall range is heavily weighted by

the free energy associated with glycine, an extremely poor  $\alpha$ -helix forming residue. The T<sub>4</sub> lysozyme and salt-bridge peptides, for example, have much similar ranges if glycine is excluded from the comparison.

The authors speculate as to what might be the cause of the difference in ranges observed in the alanine-rich and salt-bridge peptides versus the RNase T<sub>1</sub> and barnase and lysozyme studies. They consider the local helix environment (the i-3, i-4 and i+3, i+4 positions relative to the guest site) and note that the range of propensities seems to scale with the size of these neighboring residues. Could this be the cause of the differences? Further experimental and theoretical studies are required to address this and other differences between propensity scales in greater detail.

The results presented by Myers *et al.* (1) are of great interest and will doubtless stimulate much discussion. Let us end by not losing site of one clear conclusion of the paper: Helix propensities can make equivalent energetic contributions in both peptides and proteins.

1. Myers, J. K., Pace, C. N. & Scholtz, J. M. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 2833–2837.
2. Chou, P. Y. & Fasman, G. (1974) *Biochemistry* **13**, 211–222.
3. Chakrabarty, A. & Baldwin, R. L. (1995) *Adv. Protein Chem.* **46**, 141–176.
4. Smith, C. K. & Regan, L. (1997) *Acc. Chem. Res.*, in press.
5. Wojcik, J., Altman, K. H. & Scheraga, H. A. (1990) *Biopolymers* **30**, 121–134.
6. Rohl, C. R., Chakrabarty, A. & Baldwin, R. L. (1996) *Protein Sci.* **5**, 2623–2637.
7. Kallenbach, N. R., Lyu, P. & Zhou, H. (1996) in *Circular Dichroism and the Conformational Analysis of Biopolymers*, ed. Fasman, G. D. (Plenum, New York), pp. 201–259.
8. Park, S.-H., Shalongo, W. & Stellwagen, E. (1993) *Biochemistry* **32**, 7048–7053.
9. O'Neil, K. T. & DeGrado, W. F. (1990) *Science* **250**, 646–651.
10. Horovitz, A., Matthews, J. M. & Fersht, A. R. (1992) *J. Mol. Biol.* **227**, 560–568.
11. Blaber, M., Zhang, X.-J. & Matthews, B. W. (1993) *Science* **260**, 1637–1640.
12. Santoro, M. M. & Bolen, D. W. (1988) *Biochemistry* **27**, 8063–8068.
13. Scholtz, J. M. (1995) *Protein Sci.* **4**, 35–43.
14. Lifson, S. & Roig, A. (1961) *J. Chem. Phys.* **34**, 1963–1974.