Prediction of the Secondary Structure of Proteins Using the Helix–Coil Transition Theory: Reply to Criticisms by Tanaka and Scheraga

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ABSTRACT: The author refutes criticisms of his work (M. Froimowitz and G. D. Fasman, *Macromolecules*, **7**, 583 (1974)) by S. Tanaka and H. A. Scheraga, *Macromolecules*, **9**, 168 (1976).

Tanaka and Scheraga have recently published some work in which they attempt to predict the secondary structure of proteins from their amino acid sequences using a statistical mechanical model.[1–3] Since we have made a similar effort in the past,[4] they have presented a short discussion of our work. In doing so, however, they have seriously misrepresented and distorted our work to the extent that it was felt necessary to reply to their criticisms.

Tanaka and Scheraga criticize our work by claiming that it is based on three "misconceptions".[3] The first of these is that we have mistakenly identified the parameters P_α and P_β of Chou and Fasman[5] with statistical weights and subsequently used them as such in helix–coil transition theory. In actual fact, the parameters P_α and P_β are not mentioned in the paper except in the most peripheral way. Indeed, the statistical weights that were used in our study were obtained in a manner that is conceptually *identical* with that of Tanaka and Scheraga in their work. That is, the assumption was made that the conformation of a residue in a protein is primarily determined by the short-range interactions such that its conformation will be relatively independent of the surrounding amino acids. Under these conditions, the statistical weights can be obtained from the relative occurrence of the various amino acids in their possible states in the crystal structure of proteins (see the Appendix for proof). Unfortunately, Tanaka and Scheraga have not seen fit to acknowledge the similarity of our approaches and instead seem to imply that we have incorrectly obtained the statistical weights.

The second misconception that we have been accused of is that since we used a two-state model (helix and coil states), the amino acids that are experimentally observed to be in the β regions of proteins should have been included in our definition of the coil state. This omission, however, was a conscious and reasonable decision on our part. It was felt that including β-region amino acids in the coil region would result in the underestimation of the helix-forming strength of amino acids that occur frequently in the β region. In any case, Tanaka and Scheraga in their three-state model compute the statistical weight of the helix relative to the coil in an *identical* fashion, though their definitions of a helical and coil state are a little different from ours. It is, therefore, felt that it is incorrect and unnecessarily rigid to label this a misconception.

The third criticism of Tanaka and Scheraga is somewhat justified though it tends to unnecessarily cast doubts on the results that we obtained. In our original formulation, we had erroneously defined the statistical weight of an isolated helical residue (that is, one that has its conformation space restricted to the helix but without any compensating hydrogen bonds) as

$$\sigma = v_n \times v_c \qquad (1)$$

rather than

$$\sigma = (v_n \times v_c)^{1/2} \qquad (2)$$

where v_n and v_c are respectively the statistical weights assigned to the amino and carboxyl ends of a helical section. After this error had been caught by one of the referees, we reasoned that an isolated helical residue would not be expected to contribute significantly to the probability of an amino acid being found in a helical region since it has a relatively unfavorable entropy without the stabilization of hydrogen bonds. We, therefore, did not recompute the helical probabilities. As a result of the criticism by Tanaka and Scheraga, in which they imply that the results would have been different due to the above error, we recomputed the helical probabilities of the amino acids in three representative proteins (cytochrome b_5, lysozyme, and myogen) which appeared in our original study. It appears that using eq 2, which is theoretically correct, does change the computed helical probabilities more than had been anticipated. Nevertheless, the relative probabilities have not changed all that much. This can be seen in Figure 1, in which the helical probabilities for the amino acids in lysozyme have been plotted using both eq 1 and 2. While some individual residues would have been predicted differently with eq 2, the results and conclusions of our work are essentially the same as before. In the three representative proteins, of the 160 residues that had previously been predicted to be helical using eq 1, all but five continue to be predicted as helical using eq 2.

A final and significant misrepresentation by Tanaka and Scheraga occurs when they state that the main improvement of our work was the introduction of asymmetric nucleation statistical weights to the amino and carboxyl ends of a helical segment. While this was an improvement, the major improvement in accurately predicting helical amino acids (see Table IV in ref 4) occurred with the introduction of statistical weights for the helical state that were obtained from the experimentally observed frequency of the various amino acids in the helical and coil states of crystallized proteins. Tanaka and Scheraga apparently also feel that this was a major improvement since they now obtain their statistical weights in a similar fashion, though, as discussed above, they have not referenced our ideas in their recent work.

Appendix

It might be useful at this point to prove that, under the assumption of energetically independent amino acid residues, the statistical weight, s, for a helical residue is indeed equal to the experimentally observed ratio of helical to coil residues. It has been shown[6] that, for the above assumption, the fraction of helical residues in a polypeptide, θ, is equal to

$$\theta \equiv n_h/(n_h + n_c) = s/(s + 1) \qquad (3)$$

where n_h and n_c are respectively the equilibrium number of residues in the helical and coil states, and s and 1 are the sta-

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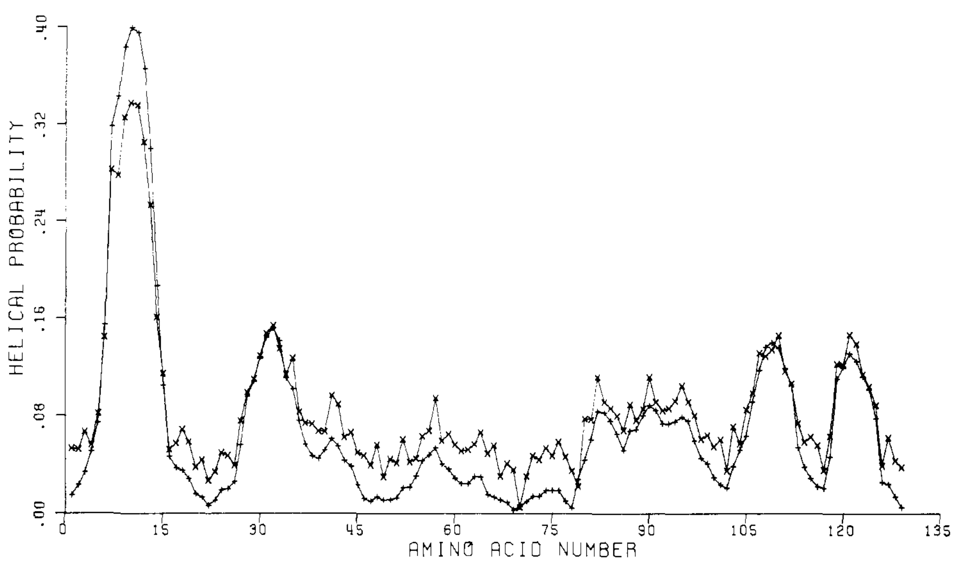

Figure 1. The helical probabilities for the amino acids in lysozyme computed using eq 1 (+) and eq 2 (X).

tistical weights assigned to those states. Dividing the numerator and denominator of the middle term of eq 3 by n_c, it is easy to see that

$$s = n_h/n_c \tag{4}$$

This is the same result obtained by Tanaka and Scheraga (see eq 16 of ref 1) whose formulation is in terms of the residue partition function. It should be noted that n_h/n_c is actually the equilibrium constant for the coil to helix transition and that under the above conditions the statistical weight, s, is equal to the equilibrium constant.

References and Notes

(1) S. Tanaka and H. A. Scheraga, *Macromolecules*, **9**, 142 (1976).
(2) S. Tanaka and H. A. Scheraga, *Macromolecules*, **9**, 159 (1976).
(3) S. Tanaka and H. A. Scheraga, *Macromolecules*, **9**, 168 (1976).
(4) M. Froimowitz and G. D. Fasman, *Macromolecules*, **7**, 583 (1974).
(5) P. Y. Chou and G. D. Fasman, *Biochemistry*, **13**, 211, 222 (1974).
(6) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).

Phenomenology of Short-Range Order in *n*-Alkane Liquids

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ABSTRACT: A fluctuation theory, analogous to de Gennes' treatment for the isotropic liquid crystal phase, is used to compute the effects of enhanced short-range orientational order in *n*-alkane liquids. Anisotropy of shape causes parallel alignment of neighboring chain segments. The segments may belong to the same or to different molecules. The free energy of the extra short-range order is reduced to explicit form by the introduction of two adjustable parameters, T^* and N^*. T^* is a pseudo-critical temperature, below the freezing point for the normal paraffins, at which the free energy density of orientationally ordered fluid would equal that of the disordered fluid if the volume of the liquid remained constant. It appears to be near the orthorhombic–hexagonal solid phase transition observed for *n*-alkanes which have an odd number of carbon atoms. N^* is proportional to the volume integral of the (asymptotic) orientational part of the pair segment correlation function. A compensation of energy and entropy effects is predicted. Both are negative and should behave as $-(T - T^*)^{-\gamma}$ where T is the absolute temperature and γ is expected to be close to one. Corroboration of the theory is found for the excess energy of mixtures of *n*-C_{14} and *n*-C_{16} with inert (noncorrelating) second components. For both alkanes, T^* is about 270 °K and N^* depends on the solute. The excess heat capacity of these mixtures is also computed, and agreement with experiment is reasonable. Small "anomalous" increments in the expansion coefficient and compressibility of the pure liquid *n*-alkanes are anticipated. Effects of the correlation of segment orientations (CSO) on optical properties are briefly discussed. Both the magnetic birefringence and the intensity of depolarized Rayleigh scattering should display a contribution from the CSO which increases strongly with the approach to the freezing point as $(T - T^*)^{-\gamma}$.

The detailed local structure of simple fluids is determined primarily by the repulsive forces acting between the molecules.[1] Attractive forces act over greater distances and do not produce sharp, short-range effects. Repulsive forces should also govern local structure in complex fluids. This is partially verified for liquid crystals where Onsager has shown that the excluded volume between rigid cylinders is able to induce a transition to a fluid phase with long-range orientational order.[2] The important role of the attractive potential in liquid crystals is believed to be a stabilization of the regions of molecular alignment.[3] The excluded volume force between the molecules is reduced by bringing them into a more parallel arrangement, and this permits a closer approach and a resultant lowering of the potential energy.

Barring an unexpected cancellation of repulsions and long-range attractions, chain-molecule systems should exhibit