Commentary

Are denatured proteins ever random coils?

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To polymer chemists, the term random coil means a chain molecule whose backbone coils randomly in three-dimensional space, with a conformation described by the Gaussian probability function (1). To protein chemists, the term random coil carries an added special meaning: the backbone conformation of every amino acid residue, described by its phi, psi pair of backbone angles, is independent of the conformations of neighboring residues. This is Flory's isolated-pair hypothesis (2). If it is valid for denatured proteins in specified solvent conditions, then the random coil protein in these conditions has no structure characteristic of the native protein, which could be used to guide the first steps of the refolding process. Evidently, study of the folding problem would be greatly simplified if conditions could be found in which denatured proteins assume the random coil conformation. In proposing the isolated-pair hypothesis, Flory was motivated by his own experimental analysis of the chain dimensions of some peptides: see especially his later work on poly-L-alanines (3).

Pappu *et al.* in this issue of PNAS (4) now find that the isolated-pair hypothesis is valid for alanine peptides only within a restricted region of the Ramachandran map, near the β-strand or extended region. For example, steric exclusion by contacts between residues separated by 3–6 peptide bonds is pronounced near the right-hand α-region of the map. The result is to stiffen the peptide chain and reduce the number of possible conformations. Pappu *et al.* obtain their results by exhaustive enumeration of all possible conformations, using a simple potential function in which the only variable parameter is the strength of an interaction treated as a peptide hydrogen bond, which might include contributions from related interactions.

Tanford (5) studied whether denatured proteins are in fact random coils in 6 M guanidinium chloride, a denaturant that unfolds nearly all water-soluble proteins. He used hydrodynamic properties such as intrinsic viscosity to characterize the overall dimensions of the polypeptide chain, after reducing any disulfide bonds present, and he used optical rotatory dispersion, the forerunner of circular dichroism used today, to detect secondary structure. Tanford found that denatured proteins in 6 M guanidinium chloride can be described as random coils in the sense of being devoid of all secondary and tertiary structure. He pointed out, however, that thermally denatured proteins in water have optical rotatory dispersion spectra indicative of some secondary structure, possibly residual native structure.

Modern NMR studies of denatured proteins are able to use ^{13}C- and ^{15}N-isotopic labeling, combined with three-dimensional spectral resolution approaches, to assign resonances and measure coupling constants (6) in individual amino acid residues. Using this approach, Hennig *et al.* (7) find no evidence for nonrandom structure in denatured hen lysozyme (8 M urea, pH 2.0) except for hydrophobic clusters, and they suggest that their results can be described to a good approximation by a statistical random coil model. The distribution of residue conformations found in this way agrees satisfactorily with a random coil model for denatured proteins (COIL) derived from the Protein Data Bank, by making a library of residue torsion angles from all residues except those included in regular secondary structures (8).

The concept of denatured proteins as random coils in specified solvent conditions allows great freedom in hypothesizing different models for the initial events in folding. However, the recognition that denatured proteins are not in fact random coils focuses interest on possible structures in the denatured protein. Pappu *et al.* (4) find that steric clashes among residues separated by 3–6 units eliminate large numbers of backbone conformations in certain regions of the Ramachandran map. Their result is particularly striking because it is obtained with alanine peptides, because alanine has only a methyl group for a side chain. Steric clash should be enhanced with larger side chains, especially β-branched side chains. The calculations of Pappu *et al.* almost certainly will stimulate experimental efforts to find preferred conformations in short peptides. A main result from their work is that

the denatured protein is predisposed to adopt specific backbone conformations at the start of refolding, when stronger hydrogen bonds come into play (compare ref. 9).

Their view of the denatured protein connects with the hierarchic model (reviews, refs. 10–12) in which folding begins in the backbone by forming specific local structures, and these then interact during subsequent stages to make tertiary interactions that establish the native topology. The hierarchic model implies that the denatured protein is poised, ready to adopt specific backbone structures once the denaturant is diluted out. The results of Pappu *et al.* show that favored backbone conformations already preexist in the denatured protein. The hierarchic model is being used with some success currently to predict folding rates and transition state structures (reviews, refs. 13 and 14). A main reason for some skepticism about the hierarchic model has been that local backbone structure cannot be detected in peptides from the small protein CI2 while mutational evidence ("phi values") points to nucleation of folding at the level of tertiary interactions (15). However, a recent mutational study (16) of the structure responsible for the formation of the 14–38 disulfide bond at the start of refolding, when pancreatic trypsin inhibitor refolds oxidatively, reveals that a backbone β-hairpin, present at a level too low to be detected by ordinary methods, is responsible. Formation of the 14–38 disulfide bond stabilizes the hairpin structure and the overall process acts as a funnel to direct folding.

Pappu *et al.* (4) point out an additional reason for testing the validity of the isolated-pair hypothesis. When a denatured protein folds to its unique native conformation, there is a large decrease in backbone conformational entropy, which is difficult to measure or estimate accurately. Its value is needed to quantitate the thermodynamics of folding. Most estimates of the entropy change on folding are based on the assumption that the denatured protein is a random coil. First

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thoughts on this entropy problem took the synthetic polymer chain as a guide. Consider, for example, a chain of N carbon atoms. There are three rotational conformations at each carbon atom: a "trans" state and two "gauche" states. In the simplest carbon polymer, polymethylene, all three have approximately the same energy within about 1 kcal/mol. Then a random coil of length N has approximately 3^N independent states. If N is about 100, representative of a very small poly-

mer, then the number of states in the random coil is astronomical (3^{100} is about 5×10^{47}), and it is inconceivable that the chain could ever find its way to fold into a unique "native" structure without the aid of steering interactions.

Clearly some caution should be used by those inclined to make rough calculations of the backbone entropy change on folding by using the exponential function. The study by Pappu *et al.* shows that the number of states accessible to an

unfolded polypeptide with 100 peptide units is much, much less than 3^{200} (there are two backbone bonds per peptide unit about which free rotation might take place). Further, there are important steering effects arising from hydrogen bonds and related interactions between residues. Understanding these and related mysteries will require more studies like that of Pappu *et al.* and doubtless will require an intimidating amount of computer power.

1. Flory, P. J. (1953) *Principles of Polymer Chemistry* (Cornell Univ. Press, Ithaca, NY).
2. Flory, P. J. (1969) *Statistical Mechanics of Chain Molecules* (Wiley, New York).
3. Conrad, J. C. & Flory, P. J. (1976) *Macromolecules* **9,** 41–47.
4. Pappu, R. V., Srinivasan, R. & Rose, G. D. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 12565–12570.
5. Tanford, C. (1968) *Adv. Protein Chem.* **23,** 121–282.
6. Hu, J.-S. & Bax, A. (1996) *J. Am. Chem. Soc.* **118,** 8170–8171.

7. Hennig, M., Bermel, W., Spencer, A., Dobson, C. M., Smith, L. J. & Schwalbe, H. (1999) *J. Mol. Biol.* **288,** 705–723.
8. Smith, L. J., Bolin, K. A., Schwalbe, H., MacArthur, M. W., Thornton, J. M. & Dobson, C. M. (1996) *J. Mol. Biol.* **255,** 494–506.
9. Srinivasan, R. & Rose, G. D. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 14258–14263.
10. Baldwin, R. L. & Rose, G. D. (1999) *Trends Biochem. Sci.* **24,** 26–33.

11. Baldwin, R. L. & Rose, G. D. (1999) *Trends Biochem. Sci.* **24,** 77–83.
12. Honig, B. (1999) *J. Mol. Biol.* **293,** 283–293.
13. Baker, D. (2000) *Nature (London)* **405,** 39–42.
14. Eaton, W. A., Muñoz, V., Hagen, S. J., Jas, G. S., Henry, E. R. & Hofrichter, J. (2000) *Annu. Rev. Biophys. Biomol. Struct.* **29,** 327–359.
15. Itzhaki, L. S., Otzen, D. E. & Fersht, A. (1995) *J. Mol. Biol.* **254,** 260–288.
16. Zdanowski, K. & Dadlez, M. (1999) *J. Mol. Biol.* **287,** 433–445.