(12) before conformations with stability comparable to kT can be attained, leaving only a minor residue of geometrically self-consistent structures to be explored.

That proteins can be hierarchically subdivided into domains (Fig. 4) shows that parts of the polypeptide chain that are near in sequence will be close together in space as the hierarchy is traversed from bottom to top. In other words, all folding interactions are local ones at some step in the cooperative self-assembly of the protein; but local interactions at the ith step are not yet local at the $(i-1)$st step, because at each step the intermediates will include an increasing measure of the polypeptide chain.

Proteins in cells assume the same conformations as proteins in test tubes (13), in many, if not in all, instances. The local nature of folding events seems plausible in the case of an in vivo nascent chain in which the N-terminus may begin to fold before the C-terminus is completed. But even a denatured protein in vitro would experience this effect, because the likelihood that two sites along the chain will interact decreases dramatically as a function of the distance between them due to diffusion (14). Thus, the model can serve to explain the existence of similar folded end-products in dissimilar folding conditions.

This work was supported by National Institutes of Health grant GM27370.

Received for publication 24 January 1980.

REFERENCES

1. Wetlaufer, D. B. 1973. *Proc. Natl. Acad. Sci. U.S.A.* **70**:697–701.
2. Liljas, A., and M. G. Rossman. 1974. *Annu. Rev. Biochem.* **43**:475–507.
3. Ptitsyn, O. B. 1978. *Fed. Eur. Biochem. Soc. Lett.* **93**:1–4.
4. Gilbert, W. 1978. *Nature (Lond.).* **271**:501.
5. Blake, C. C. F. 1979. *Nature (Lond.).* **277**:598.
6. Rose, G. 1979. *J. Mol. Biol.* **134**:447–470.
7. Levitt, M., and C. Chothia. 1976. *Nature (Lond.).* **261**:552–558.
8. Crippen, G. M. 1978. *J. Mol. Biol.* **126**:315–332.
9. Levinthal, C. 1968. *J. Chim. Phys.* **65**:44–45.
10. Rose, G. 1978. *Nature (Lond.).* **272**:586–590.
11. Kauzmann, W. 1959. *Adv. Protein Chem.* **14**:1–63.
12. Cohen, F. E., T. J. Richmond, and F. M. Richards. 1979. *J. Mol. Biol.* **132**:275–288.
13. Anfinsen, C. B. 1973. *Science (Wash. D. C.).* **181**:223–230.
14. Karplus, M., and D. L. Weaver. 1979. *Biopolymers* **18**:1421–1437.

LOCAL CONFORMATIONAL RELAXATIONS AND PROTEIN FOLDING-UNFOLDING TRANSITION

Tian Yow Tsong and Minoru I. Kanehisa, *Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 U.S.A.*

A protein molecule is a small system; as such its conformation constantly fluctuates (1). These events cover a broad time range: picoseconds for bond vibrations, oscillations and rotations, nanoseconds to microseconds for chain motions at a local level, and milliseconds to seconds for folding-unfolding transition. In a recent study we examined how events associated with rapid intramolecular motions or local conformational relaxations might affect the dynamics of the chain folding. We have proposed a cluster model (2–5) which postulates that

a peptide chain folds, under a favorable solvent condition, by first forming structural nuclei at locals, and second, merging or coalescing these nuclei into larger and more stable structural domains. The merging of local clusters is driven by solvent exclusion, i.e., by a hydrophobic effect. Fabrication of the detailed three dimensional arrangements within the newly forged hydrophobic core(s) is a consequence of this merging process. Calculations show that the cluster model is a two state model. However, each macrostate is a composite of microscopic states. Kinetics of interconversion of the two macroscopic states, as in the case of the folding-unfolding transition, are complex. These kinetics are determined by analyzing the initial distributions of the population and their movements toward the final distribution after a change in solvent conditions. Two pieces of information will be added to further clarify some basic features of the model.

The first one concerns the molecular nature of the local clusters. We have examined 47 proteins of known x-ray structures to see whether or not α-helix and β-sheet propensities of amino acid residues depend on the hydrophobicity of their immediate environments. Specifically we ask, does the probability of α-helix or β-sheet occurrences of each amino acid increase as the hydrophobicity of its microenvironment increases? The hydrophobicity of the microenvironment is defined as the sum of the Nozaki-Tanford free energy of transfer (6) for the 4 neighboring residues. The result indicates that for the 20 amino acids, taken together, there is a threefold increase in both α-helix and β-sheet residue frequencies when the residue's environment changes from least hydrophobic (zero transfer energy) to very hydrophobic (transfer energy $>$ 5 kcal). We have also observed that helical segments generally are amphipathic, i.e., they possess a hydrophobic surface and a hydrophilic surface at the opposite side. Thus, appearance of hydrophobic core(s) or hydrophobic contacts tends to stabilize the secondary structures.

The second experiment involves temperature-jump measurements of protein conformational relaxations in the native and the unfolded states. We use a colorimetric indicator, phenol red, that monitors pK change of ionizable groups (7). Three relaxations ranging from

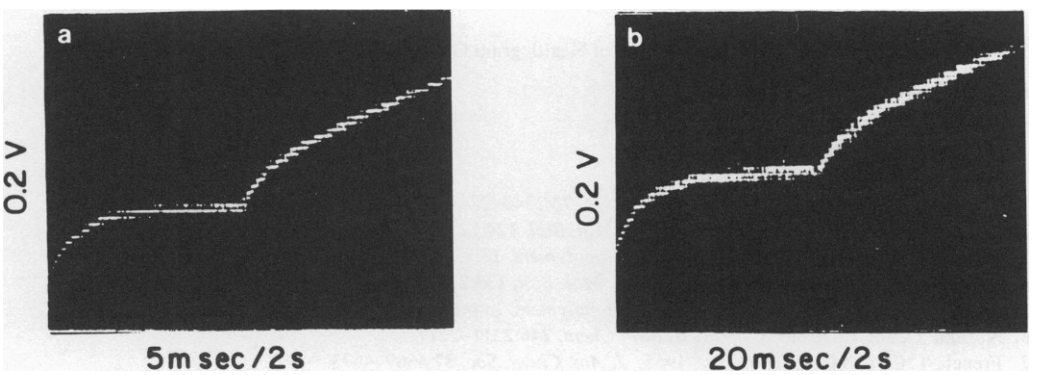

Figure 1 A proton absorption process of ribonuclease A after a rapid temperature jump at pH 7.3. (*a*) A t-jump, from 21.0 to 25.0°C, in 0.1 N NaCl, containing no buffer, but 0.04 mM of phenol red as a colorimetric indicator. The signal follows a color change at 560 nm (1 V = 0.15 ΔAbs). The first half records kinetics in the first 5 ms, and the second half records the event of the next 2 s. Enzyme concentration was 0.29 mM, and the intial pH was 7.3. The same reactions can be detected at 75°C. (*b*) Similar to *a* except that the solution contains 30% D-glucose, and the first half records event occurring in 20 ms. The viscosity of the glucose solution relative to that of the aqueous solution is 4.6. Although the fast event in *a* ($\tau_f \sim$ 0.9 ms) is significantly slowed down (to 3.0 ms), the slow reactions are little affected. The slow reactions resemble the two folding reactions of ribonuclease A under the same condition. See text for details.

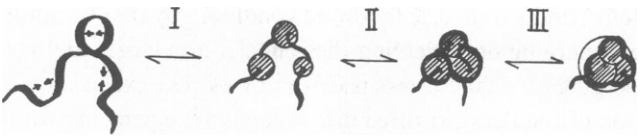

Figure 2 A cluster model of protein chain folding. Nucleations or initiations of local clusters (step I) involve segmental motions that are rate-limited by external frictions. Thus, their rate is dependent on the solvent viscosity. Growth and merging of local clusters into larger structure domains (step II) are rate-limited by internal frictions, and their rate are independent of the solvent viscosity. Interlocking and fine-tuning of a variety of intramolecular interactions occur at the third step. This final step probably includes topoisomeric reactions, such as proline isomerizations, β-bendings, etc. Most of the protein activity should appear at this step. Because of the increased steric hindrance these fine-tuning reactions are slow. The free energy gain or the stability of the native structure is largely achieved at the second step. The cluster model is a quasi-two-state, multi-nucleation-sites model (see References for details).

submilliseconds to seconds have been detected for ribonuclease A in the pH range 6–8, for both the low and the high temperature (75°C or higher) states. They are shown to be due to local conformational changes of the protein. Of these, the rapid reaction (τ_f = 0.9 ms, pK 6) depends strongly on the microscopic viscosity of the solvent. The other two slower reactions (55 ms and 10 s) do not (Fig. 1). Interestingly, neither the fast nor the slow folding reactions of ribonuclease A, also in the same time ranges for the two slower reactions reported here, depends on the solvent viscosity (8).

The above results support the following picture of the protein chain folding (Fig. 2). Local conformational relaxations and nucleations of local clusters in the peptide chain are rate-limited by the external friction; thus, their rate depends on the solvent viscosity. On the other hand, folding-unfolding transition, beginning at the cluster mergings, most likely is rate-limited by internal friction. Thus, its rate is independent of the solvent viscosity. The cluster model also favors formation of a compact globular form to precede formation of a relatively rigid framework of the protein secondary structures in the chain folding process. This latter concept can be put to experimental test.

This work was supported by National Institutes of Health grant GM 24077.

Received for publication 14 December 1979.

REFERENCES

1. Cooper, A. 1976. *Proc. Natl. Acad. Sci. U.S.A.* **73**:2740–2741.
2. Kanehisa, M. I., and T. Y. Tsong. 1978. *J. Mol. Biol.* **124**:177–194.
3. Kanehisa, M. I., and T. Y. Tsong. 1979a. *Biopolymers.* **18**:1375–1388.
4. Kanehisa, M. I., and T. Y. Tsong. 1979b. *J. Mol. Biol.* **133**:279–283.
5. Kanehisa, M. I., and T. Y. Tsong. 1979c. *Biopolymers.* In press.
6. Nozaki, Y., and C. Tanford. 1971. *J. Biol. Chem.* **246**:2211–2217.
7. French, T. C., and G. G. Hammes. 1965. *J. Am. Chem. Soc.* **87**:4669–4673.
8. Tsong, T. Y., and R. L. Baldwin. 1978. *Biopolymers* **17**:1669–1678.