Structural fluctuations in proteins

Hans Frauenfelder

Department of Physics, University of Illinois,
1110 West Green Street, Urbana, Il 61801 USA

Proteins are flexible and fluctuating systems. Evidence is accumulating that the internal motion is essential for their biological function (1,2). While a complete understanding of the nature and role of fluctuations is not yet available, some basic features are becoming clearer. Here I will first sketch the fundamental concepts and describe the relevant reaction theory. I will then show how a simple biological process, the binding of small ligands to heme proteins, can be understood.

1. Concepts. The characteristic size of proteins implies large fluctuations in energy, entropy, and volume (3). Moreover, in contrast to solids, binding is highly anisotropic in proteins. Along the peptide backbone, bonds are covalent and cannot be broken by thermal fluctuations. The cross connections, however, are mainly through hydrogen bonds and they can break and reform. The large fluctuations and the anisotropic bonds together lead to conformational substates: A given primary sequence does not produce a single tertiary structure, but a very large number of structurally related, but in detail different substates (4,5). At temperatures below about 200 K, each protein molecule is frozen in a particular substate; at physiological temperatures, a given protein fluctuates rapidly from one substate to another.

Evidence for conformational substates comes from many experiments. Rebinding of small ligands (O_2, CO) to heme proteins after photodissociation is nonexponential in time below about 200 K and implies the existence of many substates (4). The Debye-Waller factor in X-ray diffraction yields the mean-square displacement of all non-hydrogen atoms and shows that some parts of the protein look like a solid, others like a semi-liquid (6). The rate of transitions among substates can be explored by the Mössbauer effect, for instance, and data indicate that the transitions become very slow around 200 K and occur in about ns around 300 K (7,8).

2. Reaction Theory. The fluctuations in proteins can be expected to be strongly influenced by the properties of the protein environment, particularly its viscosity. Standard reaction theory does not consider the solvent viscosity and consequently is not the best framework for the treatment of bimolecular reactions. A theory that takes the influence of the environment into account was proposed by Kramers (9); in a slightly generalized form, Kramers' approach can be used for proteins (10).

3. Ligand Binding to Heme Proteins. If proteins were rigid and nonfluctuating systems, small ligands such as dioxygen could not reach the binding site at the iron in heme proteins. Fluctuations thus are essential for this simplest biological process. Flash photolysis experiments over wide ranges in time, temperature, and solvent viscosity lead to the following picture for the binding process (4,10,11). The ligand (O_2, CO)

undergoes three-dimensional diffusion in the solvent until it encounters and enters the heme protein. Once inside, it migrates, possibly by two-dimensional diffusion, through the protein matrix to the heme pocket. In the final binding step the ligand approaches the heme iron, the iron changes spin state and moves into the heme plane, the heme becomes more planar, and a covalent Fe = CO or Fe = OO bond is established. The association rate is controlled both by the migration through the protein matrix and the final binding step. Without fluctuations, migration through the protein would be greatly hindered or even stopped.

1) Gurd, F.R.N., and Rothgeb, T.M., (1979), Adv. Protein Chem. 33, 73.
2) Karplus, M., and McCammon, J.A., (1981), CRC Critical Reviews in Biochemistry 9, 293.
3) Cooper, A., (1976), Proc. Natl. Acad. Sci. USA, 73, 2740.
4) Austin, R.H., Beeson, K.W., Eisenstein, L., Frauenfelder,H., and Gunsalus, I.C., (1975), Biochemistry 14, 5355.
5) McCammon, J.A., Gelin, B.R., and Karplus, M., (1977), Nature 267, 585.
6) Frauenfelder, H., Petsko, G.A., and Tsernoglou, D., (1979), Nature 280, 585.
7) Keller, H., and Debrunner, P.G., (1980), Phys. Rev. Letters 45, 68.
8) Parak, F., Frolov, E.N., Mössbauer, R.L., and Goldanskii, V.I., (1981), J. Mol. Biol. 145, 825.
9) Kramers, H.A., (1940), Physica 7, 284.
10) Beece, D., Eisenstein, L., Frauenfelder, H., Good, D., Marden, M.C., Reinisch, L., Reynolds, A.H., Sorensen, L.B., and Yue, K.T., (1980), Biochemistry 19, 5147.
11) Case, D.A., and Karplus, M., (1979), J. Mol. Biol. 132, 343.