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Thermodynamic fluctuations in protein molecules

(stochastic and relaxation processes/"breathing")

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ABSTRACT **Apparently conflicting views of the physical nature of globular proteins, and other macromolecules, may be reconciled by consideration of the inevitable thermodynamic fluctuations inherent in microscopic systems. Discrete protein molecules, considered singly, undergo sizeable fluctuations in thermodynamic properties which are manifest in their stochastic properties. This is not incompatible with time-averaged studies of ensembles of proteins from which a more compact, rigid, and static view of these molecules may be obtained.**

There are still major conceptual problems involved in the visualization of the nature of globular proteins, and other macromolecules, in solution, and different types of experiment can lead to quite different views of the same molecule. Experimental techniques such as fluorescence quenching (1, 2) and relaxation (3), phosphorescence (4), nuclear magnetic resonance (5–7) point to a rather fluid, dynamic structure for globular proteins involving rapid conformational fluctuations which allow relatively easy, if somewhat transient, accessibility of interior groups to solvent and molecular probes (1). Some aspects of the dynamics of protein molecules have been recently reviewed (8). There are, in addition, indications from hydrogen exchange experiments (9) and studies of molecular fragments (10) of somewhat slower structural relaxations of importance, i.e., "breathing".

On the other hand, analyses of data from x-ray crystallography (11–13) indicate that the packing densities of groups within globular proteins are as high as those found for solid, crystalline amino acids (12, 13) and small organic compounds (11), suggesting a rather compact, rigid, and static view of these molecules. The gross thermodynamic properties of proteins seem to confirm this. Thermal denaturation transitions of many globular proteins are highly cooperative (14) and reminiscent of the melting of pure, microcrystalline solids. In addition, the heat capacities (C_p) of a range of proteins in aqueous solutions lie in the range 0.30–0.35 cal $g^{-1}deg^{-1}$ (1.26–1.47 kJ·g^{-1} K^{-1}) (14, 15), which is somewhat higher than found for simple organic liquids but compares well to the heat capacities of solid, crystalline amino acids (0.316 ± 0.026 cal $g^{-1}deg^{-1}$ at 25°) (16–19).

Thus, experiment presents us with two, apparently conflicting views: one, a compact structure in which the polypeptide chain is precisely folded to give a tightly interlocking, rigid molecule; the other, a "kicking and screaming stochastic molecule" (20) in which fluctuations are frequent and dramatic. These fluctuations produce a seemingly fluid and flexible system. The intention of this note is to point out that no real paradox is involved and that, though it is difficult to conceive macroscopic systems having both fluid and solid-like behavior at one and the same time, these properties are perfectly compatible with the microscopic nature of individual protein molecules.

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The distribution functions for thermodynamic parameters in macroscopic systems are usually extremely sharp and, except in special cases near critical points, deviation of these parameters from the mean are extremely small (see ref. 21, for example, or any advanced thermodynamic text). Individual protein molecules, however, are very small systems consisting of relatively few discrete particles (atoms) in comparison to familiar macroscopic objects, and in such cases statistical fluctuations in thermodynamic properties assume much greater importance. General procedures are available from statistical thermodynamics to estimate the magnitude of these fluctuations in any given system (21). Of particular interest here, since they are readily calculated from known properties of proteins in solution, are fluctuations about the mean of the internal energy, U, and total volume, V. General expressions for the mean square fluctuations (second moments of the distribution function) of U and V are (21):

$$\overline{\delta U^2} = kT^2mC_V \quad [1]$$

$$\overline{\delta V^2} = kTV\beta_T \quad [2]$$

where m and V are the mass and volume of the system, respectively, C_v is the heat capacity at constant volume, β_T the isothermal bulk compressibility, T the absolute temperature, and k is Boltzmann's constant.

The third moment of the energy distribution is also of interest, and is given by

$$\overline{\delta U^3} = 2k^2T^3mC_V + k^2T^4m\frac{\partial C_V}{\partial T}. \quad [3]$$

A representative globular protein of molecular weight 25,000 has a mass of 4.2×10^{-20} g and a volume of about 3.2×10^{-20} cm^3. Taking a mean heat capacity of 0.32 cal $g^{-1}deg^{-1}$ (C_p and C_v are not significantly different) gives, for the root mean square energy fluctuations in an individual molecule at 25°, $\delta U_{RMS} = 6.4 \times 10^{-20}$ calories per molecule. This would be equivalent to energy fluctuations of about 38 kcal mol^{-1}, if all molecules were to fluctuate in synchrony. These are surprisingly large fluctuations, and are comparable to the mean enthalpy changes on thermal denaturation of proteins (14). It must be remembered, however, that these are the fluctuations within a single molecule and are uncorrelated with similar fluctuations in other molecules so that, in a population of many protein molecules, fluctuations will cancel to give a sharp, essentially nonfluctuating, measurement of the thermodynamic parameters.

The compressibility (β_T) of proteins in solution is not known, but is probably less than 5×10^{-6} atm^{-1} (0.5 Pa) (22) (i.e., much less than β_T for organic liquids, and approaching that of solids). The change in compressibility on denaturation (22) is in the region of 2×10^{-6} atm^{-1}, and we might take this as an order of magnitude estimate for β_T of native proteins. Using this in

Eq 2 gives root mean square volume fluctuations for one protein, $\delta\bar{V}_{RMS}$, of about 5×10^{-23} cm^3 per molecule, equivalent to about 30 cm^3 mol^{-1}. Once again this is sizeable and comparable to the volume changes observed on denaturation (22–24). Volume fluctuations of this order correspond to very small changes in overall dimensions of a globular protein, but if concentrated in one area would produce cavities or channels in the proteins sufficient to allow entry of solvent or probe molecules.

The third moment of the energy distribution is of interest since it gives a measure of the asymmetry of the distribution, and should be zero for a symmetric distribution. Evaluation of Eq. 3, ignoring the second term, which is usually small and positive (14), gives a value for the cube root, $(\delta U^3)^{1/3}$, equivalent to about +12 kcal mol^{-1}. This implies that the energy distribution function is not symmetric and is weighted to higher energies. More important, it implies that the *most probable* value for the internal energy of an individual protein molecule is not the same as its *mean* energy. In other words, the mean state we normally observe in solutions of large numbers of protein molecules is not the state that would be most likely seen if we could observe the individual molecules and, in the case of energy at least, the difference is quite marked.

In summary, we see that even for a system having mean properties equivalent to those of a macroscopic solid, small size implies that large, transient fluctuations are thermodynamically inevitable, even at thermodynamic equilibrium. It should be emphasized that this is not a unique property of proteins. Indeed, any system, or part of a system, having a similar size and similar gross thermal properties would exhibit similar fluctuations.

Thermodynamics can tell us little about the precise molecular form of the fluctuations, or of their kinetics. Presumably, the majority of fluctuations involve small, rapid changes in bond lengths and angles of individual groups in the polypeptide chain, but these may readily combine to give gross changes in configuration. It is apparent from studies involving relaxation processes (1–7, 9, 10) that sizeable conformational fluctuations are possible and cover a time range from nanoseconds, or less, up to minutes or hours.

It is clear that complete understanding of the nature and function of protein molecules will require knowledge not only of their mean properties, but also of their dynamic characteristics, and that static descriptions of molecular structure are incomplete and may be misleading when applied to observations of stochastic processes.

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