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DYNAMICS OF PROTEINS: ELEMENTS AND FUNCTION

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INTRODUCTION

The classic view of proteins has been static in character, primarily because of the dominant role of the information provided by high-resolution X-ray crystallography for these very complex systems. The intrinsic beauty and

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remarkable detail of the drawings of protein structures led to an image in which each protein atom is fixed in place; an article on lysozyme by Phillips (1), the books by Dickerson & Geis (2), and by Perutz & Fermi (3), and the review by Richardson (4) give striking examples. Stating clearly the static viewpoint, Tanford (5) suggested that as a result of packing considerations "the structure of native proteins must be quite rigid." Phillips (6) wrote recently ". . . the period 1965–75 may be described as the decade of the rigid macromolecule. Brass models of double helical DNA and a variety of protein molecules dominated the scene and much of the thinking."

Most attempts to explain enzyme function have been based on the examination of the average structure obtained from crystallography; e.g. the high specificity of enzymes for their substrates has been likened to the complementarity of two pieces of a jigsaw puzzle. Cases in which conformational changes were known from X-ray data to be induced by ligand or substrate binding (e.g. the allosteric transition in hemoglobin) were generally treated as abrupt transitions between otherwise static structures.

The static view of protein structure is being replaced by a dynamic picture. The atoms of which the protein is composed are recognized to be in a state of constant motion at ordinary temperatures. From the X-ray structure of a protein, the average atomic positions are obtained, but the atoms exhibit fluidlike motions of sizable amplitudes around these average positions. Crystallographers have acceded to this viewpoint and have come so far as to sometimes emphasize the parts of a protein molecule they do not see in a crystal structure as evidence of motion or disorder (7).

The new understanding of protein dynamics subsumes the static picture in that use of the average positions still allows discussion of many aspects of protein function in the language of structural chemistry. However, the recognition of the importance of fluctuations opens the way for more sophisticated and accurate interpretations of protein function. The dynamic picture incorporates a variety of phenomena known to be involved in the biological activity of proteins, but whose detailed description was not possible under the static view. Transient packing defects due to atomic motions play an essential role in the penetration of oxygen to the heme-binding site in myoglobin and hemoglobin (8, 9). Functional interactions of flexible ligands with their binding sites often require conformational adjustments in both the ligand and the binding protein; the ligands involved include drugs, hormones, and enzyme substrates (10, 11). The structural changes in the binding proteins regulate the activity of many of these molecules through induced fit and allosteric effects (12–14). The chemical transformations of substrates by enzymes typically involve significant atomic displacements in the enzyme-substrate complexes. The mechanisms and rates of such transformations are sensitive to the dynamic properties of these systems; for example, the differences in the vibrational modes of the initial and transition

states affect the free energies of activation and catalytic rates (13–15). Electron transfer processes may depend strongly on vibronic coupling and fluctuations that alter the distance between the donor and acceptor (16–19). The relative motion of distinct structural domains is important in the activities of myosin (20–22), other enzymes (23–25), and antibody molecules (26–28), as well as in the assembly of supramolecular structures such as viruses (29).

Any attempt to understand the function of proteins requires an investigation of the dynamics of the structural fluctuations and their relation to activity and conformational change. The review deals primarily with theoretical approaches to protein dynamics. This rapidly developing field of study is founded on efforts to supplement our understanding of protein structure with concepts and techniques from modern chemical theory, including reaction dynamics and quantum and statistical mechanics. From a knowledge of the potential energy surface, the forces on the component atoms can be calculated and used to determine phase space trajectories for a protein molecule at a given temperature. Such molecular dynamics simulations, which have been successfully applied to gases and liquids containing a large number of atoms, provide information concerning the thermodynamic properties and the time-dependence of processes in the system of interest (30). More generally, statistical mechanical techniques have succeeded very well in characterizing molecular motion and chemical reaction in condensed phases (31–33). The application of these methods to proteins is natural in that proteins contain many atoms, are densely packed, and function typically in liquid environments (34).

In this review we present first a brief overview of the wide range of motions that occur in proteins. We then outline the methods that can be used to study the various motions, and review the results obtained so far. We emphasize the role of the motions in the biological activity and compare with experiments where data exist. We conclude with an outlook for the future of this new and exciting field.

A number of reviews of the theory of protein dynamics has already appeared (35–39a). Specific aspects of protein dynamics, including the rapidly growing body of experimental data, have been reviewed (36, 40–50). The proceedings of a Ciba Foundation meeting (March 2–4, 1982) devoted to *Protein Motion and Its Relation to Function* are to be published (51). Two detailed reviews surveyed protein folding recently from the structural (52) and dynamic viewpoints (53).

OVERVIEW

Globular proteins have a wide variety of internal motions. They can be classified for convenience in terms of their amplitude, energy, and time

scale, or by their structural type. Table 1 lists the ranges involved for these quantities; Careri, Fasella & Gratton (54) give a complementary summary. One expects an increase in one quantity (e.g. the amplitude of the fluctuation) to correspond to an increase in the others (e.g. a larger energy and a longer time scale). This is often true, but not always. Some motions are slow because they are complex, involving the correlated displacements of many atoms. An example might be partial-to-total unfolding transitions, in which the correlation of amplitude, energy, and time scale is expected to hold. However, in much more localized events, often involving small displacements of a few atoms, the motion is slow because of a high activation barrier; an example is the aromatic ring flips in certain proteins (55–60). In this case the macroscopic rate constant can be very slow ($k \sim 1$ sec^{-1} at 300°K), not because an individual event is slow (a ring flip occurs in $\sim 10^{-12}$ sec), but because the probability is very small ($\sim 10^{-12}$) that a ring has sufficient energy to get over an activation barrier on the order of 16 Kcal.

At any given time, a typical protein exhibits a wide variety of motions; they range from irregular elastic deformations of the whole protein driven by collisions with solvent molecules to chaotic librations of interior groups driven by random collisions with neighboring atoms in the protein. Considering only typical motions at physiological temperatures, the smallest effective dynamical units in proteins are those that behave nearly as rigid bodies because of their internal covalent bonding. Examples include the phenyl group in the side chain of phenylalanine, the isopropyl group in the side chains of valine or leucine, and the amide groups of the protein backbone. Except for the methyl rotations in the isopropyl group, these units display only relatively small internal motions owing to the high energy cost associated with deformations of bond lengths, bond angles, or dihedral angles about multiple bonds. The important motions in proteins involve relative displacements of such groups associated with torsional motions about the

Table 1 Classification of internal motions of globular proteins

Scales of motions (300°K)	
Amplitude	0.01 to 100 Å
Energy	0.1 to 100 Kcal
Time	10^{-15} to 10^{3} sec
Types of motions	
Local	Atom fluctuations, side chain oscillations, loop and "arm" displacements
Rigid body	Helices, domains, subunits
Large-scale	Opening fluctuation, folding and unfolding
Collective	Elastic-body modes, coupled atom fluctuations, soliton and other non-linear motional contributions

rotationally permissive single bonds that link the groups together. High frequency vibrations occur within the local group, but these are not of primary importance in the relative displacements.

Most groups in a protein are tightly encaged by atoms of the protein or of the surrounding solvent. At very short times ($\lesssim 10^{-13}$ s), such a group may display a rattling motion in its cage, but such motions are of relatively small amplitude ($\lesssim 0.2$ Å). More substantial displacements of the group occur over longer time intervals; these displacements involve concomitant displacements of the cage atoms. Broadly speaking, such "collective" motions may have a local or rigid-body character. The former involves changes of the cage structure and relative displacements of neighboring groups, while the latter involves relative displacements of different regions of the protein but only small changes on a local scale.

The presence of such motional freedom implies that a native protein at room temperature samples a range of conformations. Most are in the general neighborhood of the average structure, but at any given moment an individual protein molecule is likely to differ significantly from the average structure. This in no way implies that the X-ray structure, which corresponds to the average in the crystal, is not important. Rather, it suggests that fluctuations about that average are likely to play a role in protein function. In a protein, as in any polymeric system in which rigidity is not supplied by covalent cross-links, significant fluctuations cannot be avoided; they must, therefore, have been taken into account in the evolutionary development.

Although the existence of the fluctuations is now well established, our understanding of their biological role in specific areas is incomplete. Both conformational and energy fluctuations with local to global character are expected to be important. In a protein, as in other nonrigid condensed systems, structural changes arise from correlated fluctuations. Perturbations, such as ligand binding, that produce tertiary or quaternary alterations do so by introducing forces that bias the fluctuations in such a way that the protein makes a transition from one structure to another. Alternatively, the fluctuations can be regarded as searching out the path or paths along which the transition takes place.

In considering the internal motions of proteins, one must separate the dynamic from the thermodynamic aspects; in the latter, the presence of flexibility is important (e.g. entropy of binding), while in the former the directionality and time scale play a role. Another way of categorizing the two is that in the second, equilibrium behavior is the sole concern, while in the first, the dynamics is the essential element. In certain cases, some

aspects of the dynamics may be unimportant because they proceed on a time scale that is much faster than the phenomenon of interest. An example might be the fast local relaxation of atoms involved in a much slower hinge bending motion; here only the time scale of the latter would be expected to be involved in determining a rate process, though the nature of the former would be of considerable interest. In other situations, the detailed aspects of the atomic fluctuations may be the essential factor.

DYNAMICS METHODOLOGY

To study theoretically the dynamics of a macromolecular system, one must have a knowledge of the potential energy surface, the energy of the system as a function of the atomic coordinates. The potential energy can be used directly to determine the relative stabilities of the different possible structures of the system (30). The forces acting on the atoms of the systems are obtained from the first derivatives of the potential with respect to the atom positions. These forces can be used to calculate dynamical properties of the system, e.g. by solving Newton's equations of motion to determine how the atomic positions change with time (30, 31, 61). From the second derivatives of the potential surface, the force constants for small displacements can be evaluated and used to find the normal modes (62); this serves as the basis for an alternative approach to the dynamics in the harmonic limit (62, 63).

Although quantum mechanical calculations can provide potential surfaces for small molecules, empirical energy functions of the molecular mechanics type (64–67) are the only possible source of such information for proteins and their solvent surroundings. Since most of the motions that occur at ordinary temperatures leave the bond lengths and bond angles of the polypeptide chains near their equilibrium values, which appear not to vary significantly throughout the protein (e.g. the standard dimensions of the peptide group first proposed by Pauling et al in 1951; 68), the energy-function representation of the bonding can be hoped to have an accuracy on the order of that achieved in the vibrational analysis of small molecules. Where globular proteins differ from small molecules is that the contacts among nonbonded atoms play an essential role in the potential energy of the folded or native structure. From the success of the pioneering conformational studies of Ramachandran et al in 1963 (69), which used hardsphere nonbonded radii, it is likely that relatively simple functions (Lennard-Jones nonbonded potentials supplemented by a special hydrogen-bonding term and electrostatic interactions) can adequately describe the interactions involved.

The energy function used for proteins are generally composed of terms representing bonds, bond angles, torsional angles, van der Waals interac-

tions, electrostatic interactions, and hydrogen bonds. The resulting expression has the form (64–67, 70):

$$E(\mathbf{R}) = \frac{1}{2} \sum_{\text{bonds}} K_b(b - b_0)^2 + \frac{1}{2} \sum_{\substack{\text{bond} \\ \text{angles}}} K_\theta (\theta - \theta_0)^2 \qquad 1.$$

$$+ \frac{1}{2} \sum_{\text{torsional}} K_\phi [1 + \cos(n\phi - \delta)]$$

$$+ \sum_{\substack{nb \text{ pairs} \\ r < 8 \text{ Å}}} \frac{A}{r^{12}} - \frac{C}{r^6} + \frac{q_1 q_2}{Dr} + \sum_{\text{H bonds}} \frac{A'}{r^{12}} - \frac{C'}{r^{10}} .$$

The energy is a function of the Cartesian coordinate set, **R**, specifying the positions of all the atoms involved, but the calculation is carried out by first evaluating the internal coordinates for bonds (b), bond angles (θ), dihedral angles (ϕ), and interparticle distances (r) for any given geometry, **R**, and using them to evaluate the contributions to Equation 1, which depends on the bonding energy parameters K_b, K_θ, K_ϕ, Lennard-Jones parameters A and C, atomic charges q_i, dielectric constant D, hydrogen-bond parameters A' and C', and geometrical reference values b_o, θ_o, n, and δ. For most protein atoms an extended atom representation is used; i.e., one extended atom replaces a nonhydrogen atom and any hydrogens bonded to it. However, although the earliest studies employed the extended atom representation for all hydrogens, present calculations treat hydrogen-bonding hydrogens explicitly and generally use a more accurate function to represent hydrogen bonding interactions (e.g. angular terms are included) than that given in Equation 1 (70).

Given a potential-energy function, one may take any of a variety of approaches to study protein dynamics. The most exact and detailed information is provided by molecular-dynamics simulations, in which one uses a computer to solve the Newtonian equations of motion for the atoms of the protein and any surrounding solvent (70–73). With currently available computers, it is possible to simulate the dynamics of small proteins for up to a few hundred ps. Such periods are long enough to characterize completely the librations of small groups in the protein and to determine the dominant contributions to the atomic fluctuations. To study slower and more complex processes in proteins, it is generally necessary to use methods other than straightforward molecular dynamics simulation. A variety of dynamical approaches, such as stochastic dynamics (74–78), harmonic dynamics (63, 79–81), and activated dynamics (59, 82–86), can be introduced to study particular problems.

Molecular Dynamics

To begin a dynamical simulation, one must have an initial set of atomic coordinates and velocities. These are obtained from the X-ray coordinates of the protein by a preliminary calculation that serves to equilibrate the system (72, 73). The X-ray structure is first refined using an energy-minimization algorithm to relieve local stresses caused by nonbonded atomic overlaps, bond length distortions, etc. The protein atoms are then assigned velocities at random from a Maxwellian distribution corresponding to a low temperature, and a dynamical simulation is performed for a period of a few ps. The equilibration is continued by alternating new velocity assignments, chosen from Maxwellian distributions corresponding to successively increased temperatures, with similar intervals of dynamical relaxation. The temperature, T, for this microcanonical ensemble is measured in terms of the mean kinetic energy for the system composed of N atoms as:

$$\frac{1}{2}\sum_{i=1}^{N} m_i <v_i^2> = \frac{3}{2} N k_B T. \qquad 2.$$

In this equation, m_i and $<v_i^2>$ are the mass and average velocity squared of the i^{th} atom, and k_B is the Boltzmann constant. Any residual overall translational and rotational motion can be removed to simplify analysis of the subsequent conformational fluctuations. The equilibration period is considered finished when no systematic changes in the temperature are evident over a time of about 10 ps (slow fluctuations could be confused with continued relaxation over shorter intervals). It is necessary also to check that the atomic momenta obey a Maxwellian distribution and that different regions of the protein have the same average temperature. The actual dynamical simulation results (coordinates and velocities for all the atoms as a function of time) for determining the equilibrium properties of the protein are then obtained by continuing to integrate the equations of motion for the desired length of time.

Several different algorithms for integrating the equations of motion in Cartesian coordinates are used in protein molecular dynamics calculations. Most common are the Gear predictor-corrector algorithm, familiar from small molecule trajectory calculations (72) and the Verlet algorithm, widely used in statistical mechanical simulations (87).

Stochastic Dynamics

In certain cases it is advantageous to simplify the dynamical treatment by separating the system under study into two parts. One part is that whose dynamics are to be examined and the other serves as a heat bath for the first;

this could be a protein in a solvent or one portion of a protein with the surrounding protein serving as the heat bath. In such an analysis (e.g. of a tyrosine sidechain in a protein) the displacement of the part whose dynamics is to be studied relative to its neighbors is presumed to be analogous to molecular diffusion in a liquid or solid. The allowed range of motion can be characterized by an effective potential-energy function termed the "potential of mean force" (30, 72); this potential corresponds to the free energy of displacement of the elements being studied in the average field due to surrounding bath atoms. The motion of the group under study is determined largely by the time variation of its nonbonded interactions with the neighboring atoms. These interactions produce randomly varying forces that act to speed or slow the motion of the group in a given direction. In favorable cases, these dynamical effects can be represented by a set of Langevin equations of motion (30, 72, 74). For a particle in one dimension, we can write:

$$m\frac{\mathrm{d}^2x}{\mathrm{d}t^2} = F(x) - f\frac{\mathrm{d}x}{\mathrm{d}t} + R(t) \qquad 3.$$

Here, m and x are the mass and position of the particle, respectively, and t is the time; thus, the term on the left is simply the acceleration of the particle. The term $F(x)$ represents the systematic force on the particle derived from the potential of mean force. The terms $-f\mathrm{d}x/\mathrm{d}t$ and $R(t)$ represent the effects of the varying forces caused by the bath acting on the particle; the first term is the average frictional force caused by the motion of the particle relative to its surroundings (f is the friction coefficient), and $R(t)$ represents the remaining randomly fluctuating force. The Langevin equation and its generalized forms are phenomenological in character but they are consistent with more detailed models for the atomic dynamics.

The Langevin equation also provides a useful focal point in the discussion of large-scale motions (88, 89). For displacements of whole sections of polypeptide chain away from protein surface (local denaturation), the terms corresponding to the one on the left of Equation 3 are typically negligible in comparison to the others (78). The motion then has no inertial character and the chain displacements have the particularly erratic character of Brownian motion. For elastic deformations of the overall protein shape, such as those involved in interdomain or hinge-bending motions, the potential of mean force may have a simple Hooke's law or springlike character (88). Finally, the larger-scale structural changes involved in protein folding (e.g. the coming together of two helices connected by a coil region to form part of the native structure) are also likely to have Brownian character (90, 91).

Harmonic Dynamics

Harmonic dynamics provides an alternative approach to the dynamics of a protein or one of its constituent elements (e.g. an α-helix). Early attempts to examine dynamical properties of proteins or their fragments used the harmonic approximation. They were motivated by vibrational spectroscopic studies (92), in which the calculation of normal mode frequencies from empirical potential functions has long been a standard step in the assignment of infrared spectra (62). One assumes that the vibrational displacements of the atoms from their equilibrium positions are small enough that the potential energy can be approximated as a sum of terms that are quadratic in the displacements. The coefficients of these quadratic terms form a matrix of force constants which, together with the atomic masses, can be used to set up a matrix equation for the vibrational modes of the molecule (62). For a molecule composed of N atoms, $3N$-6 eigenvalues provide the internal vibrational frequencies of the molecule; the associated eigenvectors give the directions and relative amplitudes of the atomic displacements in each normal mode.

Although the harmonic model may be incomplete because of the contribution of anharmonic terms to the potential energy (Equation 1), it is nevertheless of considerable importance because it serves as a first approximation for which the theory is highly developed. Further, the harmonic model is essential for some quantum mechanical treatments of vibrational contributions to the heat capacity and free energy (81, 93) and for certain approaches to unimolecular reactions (94).

Activated Dynamics

Enzyme catalyzed reactions generally involve some processes in which the rate is limited by an energy barrier. In many cases the phenomenological time scale of such activated events is a microsecond or longer. Such processes that are intrinsically fast but occur rarely (i.e. with an average frequency much less than 10^{11} sec^{-1}) are not observed often enough for adequate characterization in an ordinary molecular dynamics simulation. To study such processes, alternative dynamical methods can be employed.

It is often possible to identify the particular character of the structural change involved (e.g. the reaction path) and then to approximate the associated energy changes. In the adiabatic-mapping approach, one calculates the minimized energy of the protein consistent with a given structural change (57, 95, 96). Minimization allows the remainder of the protein to relax in response to the assumed structural change, so that the resulting energy provides a rough approximation to the potential of mean force. Accurate potentials of mean force can be calculated by means of specialized

molecular-dynamics calculations (59), but the computational requirements are greater. To analyze the time-dependence of the process, the potential of mean force is incorporated into a model for the dynamics such as the familiar transition state theory (57, 83). A more detailed understanding of the process can be obtained by analyzing trajectories chosen to sample the barrier region (59, 85, 86). The trajectory analysis displays the space and time correlations of the atomic motions involved and provides experimentally accessible quantities such as rate constants and activation energies.

Simplified Model Dynamics

To simulate processes that are intrinsically complicated (i.e. that involve the sampling of many configurations), it is sometimes possible to use simplified models for the structure and energetics of the protein. In one model of this kind, each residue in the protein is represented by a single interaction center and these centers are linked by virtual bonds (97, 98). The energy function for this model is obtained by averaging interresidue interactions over all the local atomic configurations within each residue (78, 98–100). Thus, the model incorporates the assumption of separated time scales for local and overall chain motions. The reduced number of degrees of freedom allows rapid calculation of the energy and forces, so that significantly longer dynamical simulations are possible than with a more detailed model. Such an approach may be particularly useful for studying local unfolding or folding of proteins and their secondary structural elements (78).

ATOMIC FLUCTUATIONS

Figure 1 gives a qualitative picture of the fluctuations observed in the molecular dynamics simulation of the basic pancreatic trypsin inhibitor (PTI), a small protein with 58 amino acids and 454 heavy atoms; only the α-carbon atoms plus the three disulfide bonds are shown. The left-hand drawing represents the X-ray structure and the right-hand drawing an instantaneous picture of the equilibrated structure after 3 ps (71). The two structures are very similar, but there are small differences throughout. The largest displacements appear in the C-terminal end, which interacts with a neighboring molecule in the crystal, and in the loop in the lower left, which has rather weak interactions with the rest of the molecule. Corresponding behavior and deviations from the X-ray structure would be observed in "snap shots" taken at any other time during the simulation.

Mean-Square Fluctuations and Temperature Factors

A more quantitative measure of the motions is obtained from the mean-square fluctuations of the atoms from their average positions. These can be

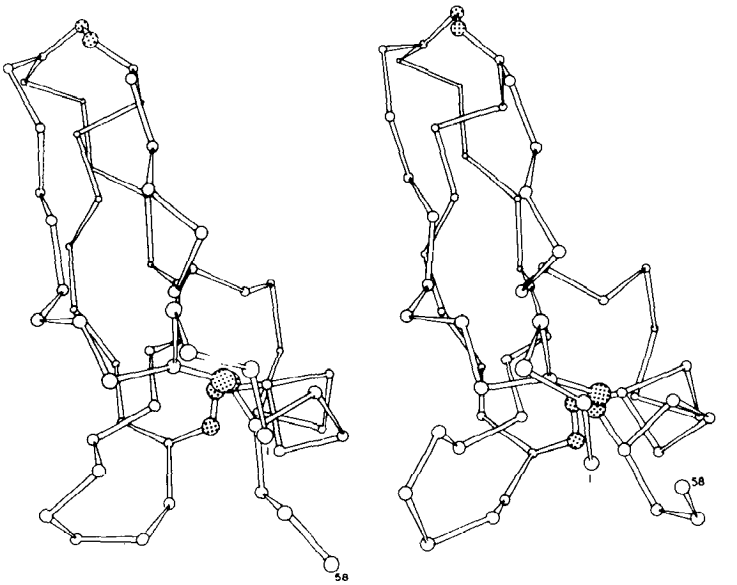

Figure 1 Drawing of α-carbon skeleton plus S–S bonds of PTI; left-hand drawing is the X-ray structure and right-hand drawing is a typical "snapshot" during the simulation.

related to the atomic temperature or Debye-Waller factors, B, determined in an X-ray diffraction study of a protein crystal (101–105). The mean-square positional fluctuation, $<\Delta r^2>_{dyn}$, with the assumption of isotropic and harmonic motion can be written:

$$<\Delta r^2>_{dyn} = \frac{3B}{8\pi^2} - <\Delta r^2>_{dis}. \qquad 4.$$

$<\Delta r^2>_{dis}$ is the contribution to B from lattice disorder and other effects that are difficult to evaluate experimentally. For a number of proteins at ambient temperatures (101–105), the measured value of $(3B/8\pi^2)$ averaged over all of the nonsurface atoms of the protein is in the range 0.48–0.58 Å^2. Comparison of this result with the mean value of $<\Delta r^2>_{dyn}$ from protein simulations (0.28–0.36 Å^2; 101, 106, 107) suggests that the nonmotional contribution to the B-factor $<\Delta r^2>_{dis}$, is in the range 0.20–0.25 Å^2. The only experimental estimate of $<\Delta r^2>_{dis}$ is from Mössbauer data for the heme iron in myoglobin (102); for that one atom a somewhat smaller value (0.14 Å^2) was obtained. Thus, in the cases examined, approximately half of the experimental B-factor is associated with thermal fluctuations in

the atomic positions and half with other sources. However, some protein crystals, particularly those with a high percentage of water, appear to have a larger disorder contribution (109).

There is generally an increase in the magnitude of the experimental and theoretical fluctuations with distance from the center of the molecule. The magnitudes of the rms fluctuations range from $\sim$0.4 Å for backbone atoms to $\sim$1.5 Å for the ends of long sidechains. The hydrogen-bonded secondary structural elements (α-helices, β-sheets) tend to have smaller fluctuations than the random coil parts of the protein (106, 108). The magnitude of the fluctuations vary widely throughout the protein interior, suggesting that the system is inhomogeneous and that some regions are considerably more flexible than others.

To examine the importance of bond length and bond angle fluctuations, simulations were performed on PTI in which the bond lengths or both the bond lengths and the bond angles were fixed at their average values (73). It was found that use of fixed bond lengths (normal fluctuations $\pm$0.03 Å) does not significantly alter the dynamical properties on a time scale longer than 0.05 ps, but that constraint of the bond angles (normal fluctuations, $\pm$5°) reduces the mean amplitude of the atomic motions by a factor of two. This result demonstrates that in a closely packed system, such as a protein in its native configuration, the excluded volume effects of repulsive van der Waals interactions introduce a strong coupling between the dihedral angle and bond-angle degrees of freedom.

Figure 2 shows a comparison of the calculated and experimental rms fluctuations on a residue-by-residue basis for reduced cytochrome c (101). The experimental values were corrected for an estimated disorder contribution by subtracting from all of them $<\Delta r^2>_{dis} = 0.25$ Å^2, obtained from the average calculated results for the protein interior. There is generally good agreement between the experimental and theoretical values. This correlation confirms the reliablity of both the simulation results and the temperature factors as detailed measures of the internal mobility of proteins.

Since most of the molecular dynamics simulations have been done for a protein in vacuum, it is expected that, particularly for the exterior residues, the results will be in error owing to the absence of solvent and, with regard to X-ray temperature factors, the absence of the crystal environment. For cytochrome c, the most prominent differences between theoretical and experimental mean displacements (Figure 2) involve the residues calculated to have very large fluctuations; these are all charged sidechains (particularly lysines) that protrude from the protein and so are not correctly treated in the vacuum simulation. This result is confirmed by molecular-dynamics

simulations of PTI in a Lennard-Jones solvent and in a crystal environment. The simulations show that the motion of the outside residues is significantly perturbed by the surrounding medium (107, 110); in particular, the interaction between charged sidechains of a given protein and its crystal neighbors can produce a reduction in the rms values (66, 110). Such results for the external residues contrast with those for the protein interior, where the environmental effects on the amplitude of fluctuations are found to be small. The dominant medium effect on the equilibrium properties of the PTI molecule is that the average structure in the solvent or crystal field is significantly closer to the X-ray structure than is the vacuum result (e.g. for the C^{α}-atoms, the vacuum simulation has an rms deviation from the X-ray structure equal to 2.2 Å, while those for the solvent and crystal simulations are 1.35 Å and 1.52 Å, respectively).

Recently, a crystal simulation of PTI, including the water molecules, was completed (111). Although the simulation is too short (12 ps) for definitive conclusions, the magnitude of the fluctuations corresponded to those found in the earlier simulations of PTI, while the dynamic average structure was somewhat closer to the X-ray result (C^{α}-atom rms deviation is 1.06 Å). That the integrity of the dynamic average structure is necessary to obtain the

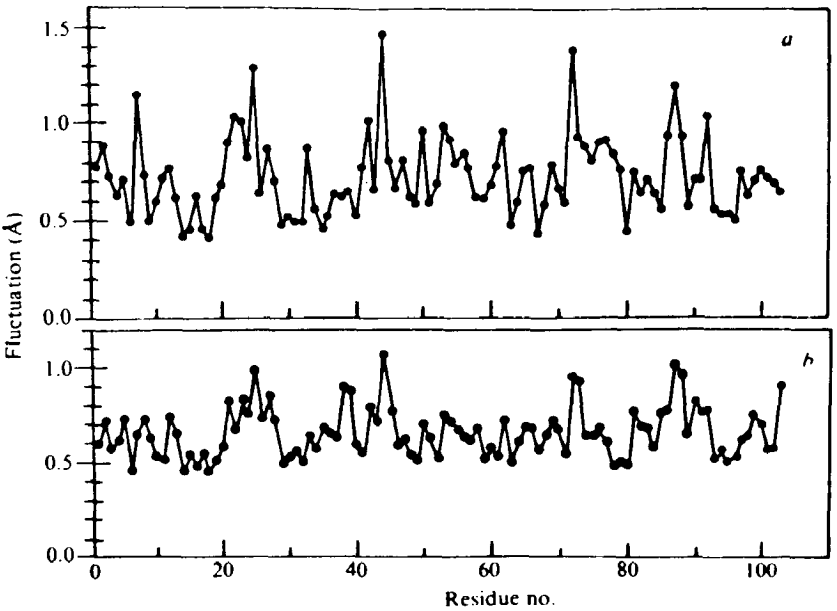

Figure 2 Calculated and experimental rms fluctuations of ferrocytochrome c; residue averages are shown as a function of residue number: (*a*) molecular-dynamics simulation; (*b*) X-ray temperature factor estimation corrected for mean disorder contribution.

correct rms fluctuations was noted in a simulation of rubredoxin (112);the agreement between calculated and experimental (113) temperature factors was poor, apparently because of a significant perturbation in the structure during the simulation.

Of interest also are the results from the dynamic simulation concerning deviations of the atomic motions from the isotropic, harmonic behavior assumed in most X-ray analyses of proteins. The motions of many of the atoms were found in the simulations to be highly anisotropic and somewhat anharmonic. The rms fluctuation of an atom in its direction of largest displacement is typically twice that in its direction of smallest displacement; larger ratios are not uncommon (106, 110, 114, 115). It is sometimes possible to rationalize these directional preferences in terms of local bonding, e.g. torsional oscillation of a small group around a single bond (115). In most cases, however, the directional preferences appear to be determined by larger-scale collective motions involving the atom and its neighbors (115–118). The atom fluctuations are generally also anharmonic; that is, the potentials of mean force for the atom displacements deviate from the simple parabolic forms that would obtain at sufficiently low temperature (106, 110, 119). The most markedly anharmonic atoms are those having multiple minima in their potentials of mean force. The shape of the PTI potential surface in the region of the native structure indicates that the anharmonicity is primarily associated with the softest collective modes of displacement in the protein (120).

Time-Dependence: Local and Collective Effects

Analyses of the time development of the atomic fluctuations were made for PTI (117, 118) and cytochrome c (116). The atomic fluctuations that contribute to the temperature factor (thermal ellipsoid) can be separated into local oscillations superposed on motions with a more collective character. The former have a subpicosecond time scale; the latter, which can involve only a few neighboring atoms, a residue, or groups of many atoms in a given region of the protein, have time scales ranging from 1–10 ps or longer ($\nu \cong 3$ to 30 cm^{-1}). By following the time development of the atomic fluctuations of PTI from 0.2–25 ps, it was shown that the high-frequency oscillations, which contribute about 40% of the average rms fluctuations of mainchain atoms, tend to be uniform over the structure. It is the longer-time-scale, more collective motions that introduce the variations in the fluctuation magnitudes that characterize different parts of the protein structure (117, 118). The correlations of anisotropy with local bonding are often destroyed by these large amplitude collective motions (115, 116).

The time dependence of atom and group motions in proteins can be characterized more fully by calculating appropriate time correlation func-

tion (72, 117, 118). The time correlation function of a fluctuating quantity describes the average manner in which a typical fluctuation decays (30, 31). For the positional fluctuations of individual atoms in the protein interior, the time correlation function has a partial loss of amplitude within the first 0.2 ps, followed by much slower decay on a time scale of several ps; the slow component has significant oscillations in many cases (72, 116–118). The decay times of the correlation functions are increased by including external solvent in the dynamic simulation; this effect is most pronounced for atoms at the protein surface (110, 117, 118). An analysis of the relaxation times for the atoms in PTI plus solvent yields a wide range (0.45–10 ps); in vacuum, the times shift to somewhat shorter values (0.2–6 ps).

Although there is no direct experimental measure of the time scale of the atomic fluctuations, it has been shown that NMR-relaxation parameters (T_1, T_2, and NOE values) are sensitive to picosecond motions. Of particular relevance are ^{13}C-NMR data since for protonated carbons, the C–H bond reorientation provides the dominant relaxation mechanism. Preliminary comparisons of ^{13}C-relaxation data for PTI suggest that the α-carbon mobility has a small effect on the relaxation parameters and that increasing effects are expected as the observed carbon is further out along a sidechain (121, 122). The effect of internal motions on other NMR parameters, such as chemical shifts (123) and vicinal-coupling constants, was also examined by molecular-dynamics simulations.

Biological Function

Although many of the individual atom fluctuations observed in the simulations or obtained from temperature factors may in themselves not be important for protein function, they contain information that is of considerable significance. The calculated fluctuations are such that the conformational space available to a protein at room temperature includes the range of local structural changes observed on substrate or inhibitor binding for many enzymes. There may be a correlated directional character to the active-site fluctuations that play a role in catalysis. Further, the small amplitude fluctuations are essential to all other motions in proteins; they serve as the "lubricant" which makes possible larger-scale displacements, such as domain motions (see Table I), on a physiological time scale. It may be possible to extrapolate from the short time fluctuations to larger-scale protein motions. This is suggested by the approximate correspondence between the rms fluctuations of hydrogen bond lengths in a dynamical simulation of PTI (124) and the relative exchange rate of the hydrogens as measured by NMR (42). Changes in the fluctuations induced by perturbations, e.g. ligand binding, are likely to be important as well, e.g. the entropy differences, for the study of which molecular-dynamics techniques were developed (125), may make a significant contribution to the binding free energy (15).

The collective modes are likely to be of particular significance in the biological function; they may be involved in the displacements of sidechains, loops or other structural units required for the transition from an inactive to the active configuration of a globular protein and in the correlated fluctuations that play a direct role in enzyme catalysis. Further, the extended nature of these motions makes them more sensible to the environment, e.g., differences in the simulation results between vacuum and solution results for PTI (117, 118). Because they involve sizable portions of the protein surface, the collective motions may be involved in transmitting external solvent effects to the protein interior (45). They might also be expected to be quenched at low temperature by freezing of the solvent. Their contribution to the mean-square fluctuations could explain the transition observed near 200°K in the temperature dependence of the fluctuations in proteins like in myoglobin (126).

SIDECHAIN MOTIONS

The motions of aromatic sidechains serve as a convenient probe of protein dynamics. The sidechain motions span a time range from picoseconds, during which local oscillations occur, to milliseconds or longer required for 180° rotations. To cover this range of motions requires use of a variety of approaches that complement each other in the analysis of protein dynamics. Further, the results obtained are typical of a class of motional phenomena that play a significant role.

Tyrosines in PTI

The torsional librations of buried tyrosines in PTI were studied in some detail (72). We focus on a particular aromatic sidechain, Tyr 21, whose ring is surrounded by and has a significant nonbonded interaction with atoms of its own backbone and of surrounding residues that are more distant along the polypeptide chain. Figure 3 shows a potential energy contour map for the sidechain dihedral angles χ_1 and χ_2 of Tyr 21 in the free dipeptide (*top*) and in the protein (*bottom*) (57). The minimum energy conformations are very similar in the two cases; this appears to be true for most interior residues of proteins. Where the plots differ is that the sidechain is much more rigidly fixed in position by its nonbonded neighbors in the protein than it is by interactions with the backbone of the chain in the dipeptide.

Figure 4 (*top*) shows the torsional fluctuations of Tyr 21 observed during a PTI simulation (72); the quantity plotted is $\Delta\phi = \phi - <\phi>$ where $<\phi>$ is the time average of the ring torsional angle. Figure 4 (*bottom*) shows corresponding torsional fluctuation history for the ring in an isolated tyrosine fragment simulation.

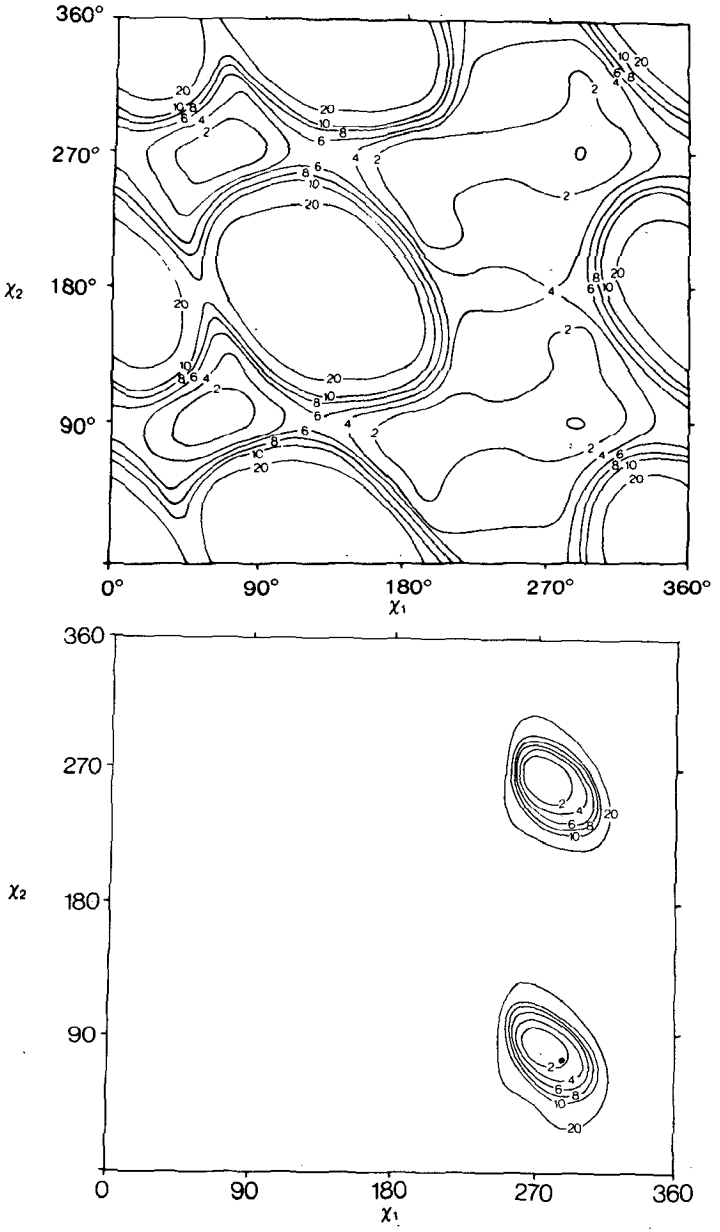

Figure 3 (κ_1,κ_2)) maps for Tyr-21 in PTI: (*top*) free peptide; (*bottom*) peptide in protein; the black dot corresponds to the X-ray value for (κ_1,κ_2) in the protein; energy contours in kcal/-mol.

The torsional motion of the ring is less regular when it is surrounded by the protein matrix than in the separated fragment. In PTI, the rms fluctuation of the Tyr 21 torsion angle is 12°, while that for the tyrosine fragment is 15°. This relatively small difference in amplitudes as compared with the forms of the rigid rotation potentials (Figure 3) indicates that protein relaxation involving correlated fluctuations must play an important role in

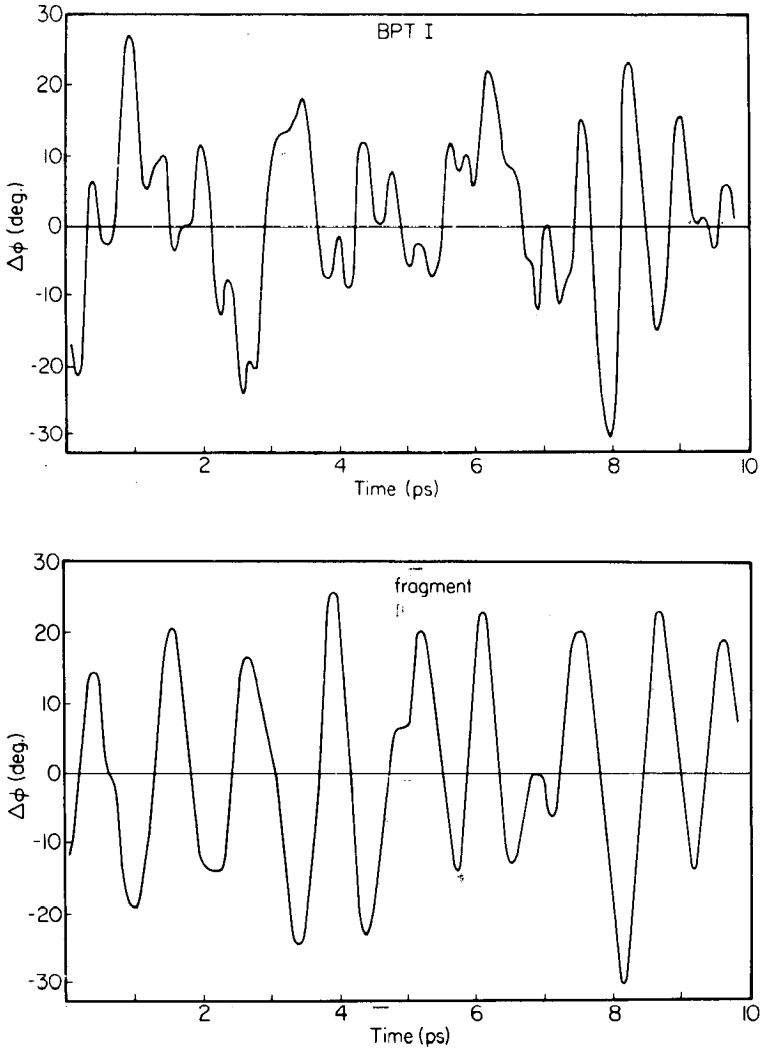

Figure 4 Evolution of the Tyr-21 ring torsional angle during 9.8 ps of dynamical simulation: (*top*) in the protein; (*bottom*) in the isolated tyrosine fragment.

the ring oscillations. The short time, local motion in the protein is consistent with a torsional Langevin equation that contains a harmonic restoring force (see Equation 3). The frictional random force terms are similar to those expected for ring rotation in an organic solvent; this is consistent with the hydrophobic environments of the rings in the protein. The time correlation functions for the torsional fluctuations decay to small values in a short time ($\sim$0.2 ps). However, the quantities involved in the relaxation times (110, 127) measured in fluorescence depolarization (trigonometric functions of the angles) decay much more slowly. For the tyrosine rings in PTI there is rapid partial decay in less than a picosecond to a plateau value equal to about 75% of the initial value; this behavior was recently confirmed by fluorescent depolarization measurements (128). Corresponding calculations (130) for the fluorescent depolarization of the tryptophan residues in lysozyme based on a molecular dynamics simulation (106) indicate a wide range of variation in the depolarization behavior. Since there are six tryptophans in a variety of environments, their behavior is expected to correspond to that which occurs more generally in proteins (129). Certain interior tryptophans have almost no decay over the time scale of the simulation while one in the active site (Trp 62) has its anisotropy reduced to 0.6 after 5 ps.

Tyrosine and phenylalanine ring rotations by 180° were studied by NMR in proteins (40, 43, 44, 56). Such ring "flips" occur very infrequently because of the large energy barrier due to steric hindrance (57–59). The long time intervals separating flips preclude systematic study by conventional molecular-dynamics methods. A modified molecular-dynamics method was recently developed to handle such local activated processes (59). This method is similar to adiabatic mapping in that one starts with an assumed "reaction coordinate" that defines the fundamental structural changes involved. It differs from the adiabatic method in that it involves consideration of all thermally accessible configurations and not just the minimum energy one for each value of the reaction coordinate. Also it provides a detailed description of the structural and dynamical features of the process. In this method, one calculates separately the factors in the rate constant expression (82, 131):

$$k = \frac{1}{2}\kappa < |\dot{\xi}| > [\rho(\xi^{\dagger})/\textstyle\int_i \rho(\xi) d\xi]. \qquad 5.$$

Here, ξ is the reaction coordinate, $\dot{\xi} = d\xi/dt$, and $\xi^{\dagger}$ is the value of ξ in the transition state region for the process. The factor in square brackets is the probability that the system will be in the transition state region, relative to the probability that it is in the initial stable state. This quantity corresponds roughly to the term $\exp(-\Delta G^{\dagger}/RT)$ in more familiar expres-

sions for rate constants; it can be calculated by carrying out a sequence of simulations in which the system is constrained to stay near particular values of ξ. The remaining factors can be evaluated by analysis of trajectories initiated in the transition state region (59, 85, 86). The transmission coefficient κ is equal to one in ideal transition state theory (equilibrium populations maintained in the stable states and uninterrupted crossings through the transition state region); for real systems κ is less than one.

Application of this modified molecular dynamics method to the flipping of a tyrosine ring in PTI shows that the rotations themselves required only 0.5–1.0 ps (85, 86). At the microscopic level, the processes responsible for flipping are the same as those responsible for the smaller amplitude librations. The ring goes over the barrier not as the result of a particularly energetic collision with some cage atom, but as the result of a transient decrease in frequency and intensity of collisions that would drive the ring away from the barrier. These alterations of the collision frequency are caused by small, transient packing defects (86). The packing defects help to initiate ring rotation, but they are much too small to allow free rotation of the ring by a simple vacancy or free-volume mechanism (86, 132). The ring tends to be tightly encaged even in the transition state orientation. Collisions with cage atoms in the transition state produce frictional forces similar to those that occur in the stable state librations; these frictional effects reduce the transition rate to about 20% of the ideal transition-state theory value (59). As to the free energy of activation, the calculations suggest that the activation enthalpy contribution is similar to that found by adiabatic mapping techniques (57, 58) and that the activation entropy is small.

Although no enzyme has yet been studied by the techniques applied to the tyrosine ring flips, the methodology is applicable to the activated processes central to most enzymatic reactions. Further, many of the qualitative features found for the tyrosines (e.g. lowering of the potential of mean force by cage relaxation, alteration of the rate by frictional effects) should be present in general.

Ligand-Protein Interaction in Myoglobin

A biological problem where sidechain fluctuations are important concerns the manner in which ligands like carbon monoxide and oxygen are able to get from the solution through the protein matrix to the heme group in myoglobin and hemoglobin and then out again. The high-resolution X-ray structure of myoglobin (8, 9, 133, 134) does not reveal any path by which ligands such as O_2 or CO can move between the heme-binding site and the outside of the protein. Since this holds true both for the unliganded and

liganded protein, i.e. myoglobin (133) and oxymyoglobin (134), structural fluctuation must be involved in the entrance and exit of the ligands. Empirical energy function calculations (96) showed that the rigid protein would have barriers on the order of 100 kcal/mol; such high barriers would make the transitions infinitely long on a biological time scale. Figure 5, panel I gives the nonbonded potential contour lines seen by a test particle representing an O_2 molecule in a plane (xy) parallel to the heme and displaced 3.2 Å from it in the direction of the distal histidine; the coordinate system in this and related figures has the iron at the origin and the z-axis normal to the heme plane. The low potential-energy minimum corresponds to the observed position of the distal O atom of an O_2 molecule forming a bent Fe–O–O bond (134). The shortest path for a ligand from the heme pocket to the exterior (the low energy region in the upper left of the figure) is between His E7 and Val E11. However, this path is not open in the X-ray geometry because the energy barriers due to the surrounding residues indicated in the figure are greater than 90 kcal/mol.

To analyse pathways available in the thermally fluctuating protein, ligand trajectories were calculated with a test molecule of reduced effective diameter to compensate for the use of the rigid protein structure (96). A trajectory was determined by releasing the test molecule with substantial kinetic energy (15 kcal/mol) in the heme pocket and following its classical motion for a suitable length of time. A total of 80 such trajectories were computed; a given trajectory was terminated after 3.75 ps if the test molecule had not escaped from the protein. Slightly more than half the test molecules failed to escape from the protein in the allowed time; 25 molecules remained trapped near the heme-binding site, while another 21 were trapped in two cavities accessible from the heme pocket. Most of the molecules that escaped did so between the distal histidine (E7) and the sidechains of Thr E10 and Val E11 (see Figure 5, panel I) A secondary pathway was also found; this involves a more complicated motion along an extension of the heme pocket into a space between Leu B10, Leu E4, and Phe B14, followed by squeezing out between Leu E4 and Phe B14. Figure 6 shows a typical model trajectory following this path. Additional, more complicated pathways also exist, as indicated by the range of motions observed in the trial trajectories.

In the rigid X-ray structure, the two major pathways have very high barriers for a thermalized ligand of normal size. Thus, it was necessary to study the energetics of barrier relaxation to determine whether either of the pathways had acceptable activation enthalpies. Local dihedral rotations of key sidechains, analogous to the tyrosine sidechain oscillations described above, were investigated; it was found that the bottleneck on the primary pathway could be relieved at the expense of modest strain in the protein by rigid rotations of the sidechains of His E7, Val E11, and Thr E10. The reorientation of these three sidechains and the resultant opening of the

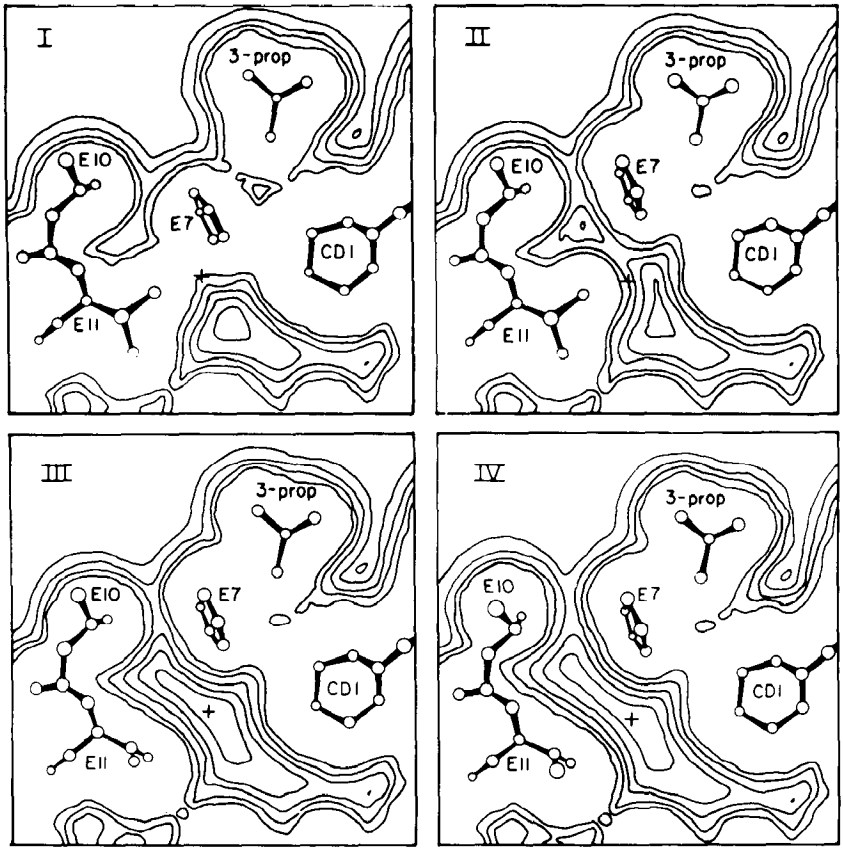

Figure 5 Myoglobin-ligand interaction contour maps in the heme (x,y) plane at $z = 3.2$ Å (the iron is at the origin) showing protein relaxation; a cross marks the iron atom projection onto the plane. Distances are in Å and contours in kcal; the values shown correspond to 90, 45, 10, 0, and –3 kcal/mol relative to the ligand at infinity. The highest contours are closest to the atoms whose projections onto the plane of the figure are denoted by circles. *Panel I:* X-ray structure; *panels II–IV:* sidechain rotations discussed in the text.

pathway to the exterior is illustrated schematically in Figure 5; Panel I shows the X-ray structure; in Panel II the distal histidine (E7) was rotated to $\chi_1 = 220°$ at an energy cost of 3 kcal/mol; in Panel III, Val E11 was also rotated to $\chi_1 = 60°$ ($\sim$5 kcal/mol); and Panel IV has the additional rotation of Thr E10 to $\chi_1 \cong 305°$ ($<$1 kcal/mol). In this manner a direct path to the exterior was created with a barrier of $\sim$5 kcal/mol at an energy cost to the protein of $\sim$8.5 kcal/mol, as compared with the X-ray structure value of nearly 100 kcal/mol. On the secondary path, however, no simple torsional motions reduced the barrier due to Leu E4 and Phe B14, since the

necessary rotations led to larger strain energies. A test sphere was fixed at each of the bottlenecks and the protein was allowed to relax by energy minimization (adiabatic limit), in the presence of the ligand (57, 96).

Approximate values for the relaxed barrier heights were 13 kcal/mol and 6 kcal/mol for the two primary path positions and 18 kcal/mol for the secondary path position. These barriers are on the order of those estimated in the photolysis, rebinding studies for CO myoglobin by Frauenfelder et al (45, 135, 136). Further, a path suggested by the energy calculations was found to correspond to a high mobility region in the protein as determined by X-ray temperature factors (102).

The type of ligand motion expected for such a several-barrier problem can be determined from the trajectory studies mentioned earlier. What happens is that the ligand spends a long time in a given well, moving around in and undergoing collisions with the protein walls of the well (see Figure 6). When there occurs a protein fluctuation sufficient to significantly lower

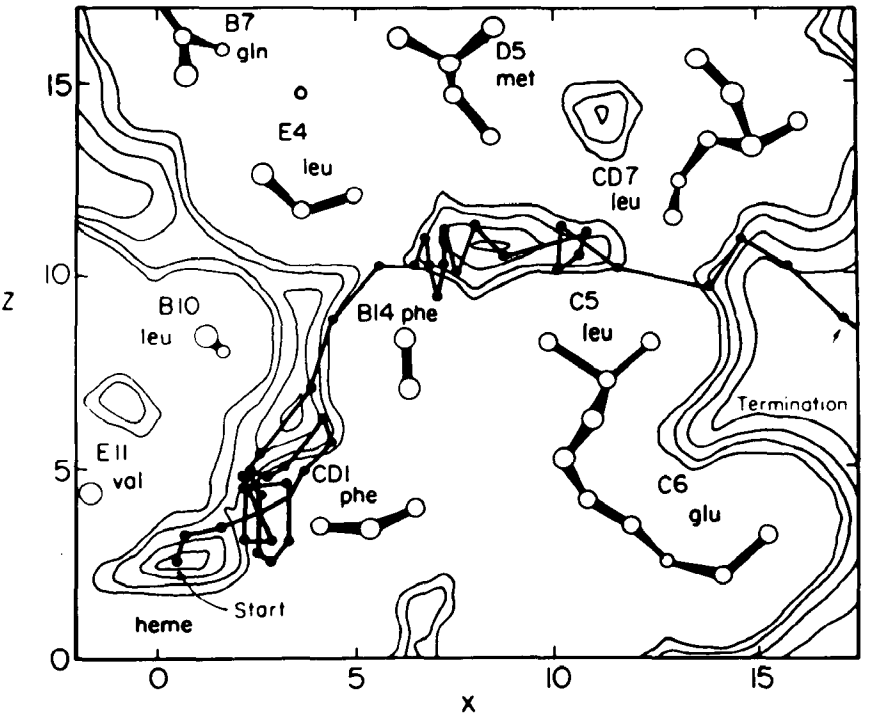

Figure 6 Diabatic-ligand trajectory following a secondary pathway (see text); a projection of the trajectory on the plane of the figure [(x,z) plane at $y = 0.5$ Å)] is shown with the dots at 0.15 ps intervals. The start of the trajectory at the heme iron and the termination point exterior to the protein are indicated by arrows.

the barrier, or the ligand gains sufficient excess energy from collisions with the protein, or more likely both at the same time, the ligand moves rapidly over the barrier and into the next well where the process is repeated. In a completely realistic trajectory involving a fluctuating protein and ligand-protein energy exchange, the time spent in the wells would be much longer than that found in the diabatic model calculations (Figure 6). Further, from the complexity of the range of pathways in the protein interior, it is likely that the motion of the ligand will have a diffusive character.

The analysis of myoglobin suggests that the native structure of a protein is often such that the small molecules that interact with the protein cannot enter or leave if the atoms are constrained to their average positions. Consequently, sidechain and other fluctuations may be required for ligand binding by proteins and for the entrance of substrates and exit of products from enzymes. Some analyses of the effects of such "gated" accessibility on the observed kinetics were made (137–139).

Exterior Sidechain and Loop Motions

In several enzymes, a displacement of surface sidechains or entire loops on substrate binding occurs. In carboxypepdidase A (140), for example, when the substrate binds, the structural changes include a large displacement of the sidechain of Tyr 248, which moves through more than 10 Å toward the active site. Another example is provided by an external loop in triophosphate isomerase, which was shown by X-ray diffraction to fold over the substrate when it is bound (141). If surface residues are involved, as is often the case, the motion is best treated by stochastic dynamics.

To study the motion of aliphatic sidechains in solution (76), the end of the chain attached to the macromolecule is held fixed and the Langevin equations of motion (Equation 3) for the atoms of the chain are solved simultaneously for periods of up to a microsecond. The methyl and methylene groups of the chain are treated as single extended atoms with a friction coefficient corresponding to methane in water and a generalized empirical potential energy function is used to represent the intramolecular interactions (nonbonded and torsional) in the usual way, except that they are slightly modified to take into account the presence of solvent; that is, a potential of a mean force replaces the isolated molecule potential function (142). It is found that the motion with respect to a given torsion angle separates into two time scales (76). The shorter time motion, on the order of tenths of picoseconds, corresponds to torsional oscillations within a potential well, and the longer, on the order of two hundred picoseconds, corresponds to transitions from one potential well to another; the torsional barrier used in the potential function is ~ 2.8 kcal/mol. Thus, analogous to the above description for an oxygen molecule moving through myoglobin,

the sidechain spends most of the time oscillating about a single conformation (i.e. with each dihedral angle remaining in a given well) and only rarely makes a transition from one conformation to another. To test the validity of this type of calculation, comparisons of the stochastic trajectory results with NMR relaxation measurements (e.g. ^{13}C NMR) were made (122).

RIGID BODY MOTIONS

A type of motion that plays an important role in proteins is referred to as a rigid-body motion (Table I). It involves the displacement of one part of a protein relative to another such that each moving portion can be approximated as a rigid body. However, smaller fluctuations must accompany the rigid-body motions to reduce the required energy and permit them to proceed at a sufficiently rapid rate.

Hinge Bending

Many enzymes (23–25) and other protein molecules (e.g. immunoglobulins) consist of two or more distinct domains connected by a few strands of polypeptide chain that may be viewed as "hinges." In lysozyme, for example, it was noted in the X-ray structure (143) that when an active-site inhibitor is bound, the cleft closes down somewhat as a result of relative displacements of the two globular domains that surround the cleft. Other classes of proteins (kinases, dehydrogenases, citrate synthase) have considerably larger displacements of the two lobes on substrate binding than does lysozyme (23–25).

In the theoretical analysis of lysozyme (88), the stiffness of the hinge was evaluated by the use of an empirical energy function (66, 88). An angle-bending potential was obtained by rigidly rotating one of the globular domains relative to a bending axis which passes through the hinge and calculating the changes in the protein conformational energy. This procedure overestimates the bending potential, since no allowance is made for the relaxation of the unfavorable contacts between atoms generated by the rotation. To take account of the relaxation, an adiabatic potential was calculated by holding the bending angle fixed at various values and permitting the positions of atoms in the hinge and adjacent regions of the two globular domains to adjust themselves so as to minimize the total potential energy. As in a previous adiabatic ring rotation calculation (57), only small (<0.3 Å) atomic displacements occurred in the relaxation process. Localized motions involving bond angle and local dihedral angle deformations occur. The frequencies associated with them (>100 cm^{-1}) are much greater than the hinge-bending frequency (≈ 5 cm^{-1}), so that the use of the adiabatic-bending potential is appropriate.

The bending potentials were found to be approximately parabolic, with the restoring force constant for the adiabatic potential about an order of magnitude smaller than that for the rigid potential (see Figure 7). However, even in the adiabatic case, the effective force constant is about 20 times as large as the bond-angle bending force constant of an α carbon (i.e. N–C_α–C); the dominant contributions to the force constant come from repulsive nonbonded interactions involving on the order of fifty contacts. If the adiabatic potential is used and the relative motion is treated as an angular harmonic oscillator composed of two rigid spheres, a vibrational frequency of about 5 cm^{-1} is obtained. This is a consequence of the fact that, although the force constant is large, the moments of inertia of the two lobes are also large.

Although fluctuations in the interior of the protein, such as those considered in myoglobin, may be insensitive to the solvent (because the protein matrix acts as its own solvent), the domain motion in lysozyme involves two lobes that are surrounded by the solvent. To take account of the solvent effect in the simplest possible way, the Langevin equation (Equation 3) for a damped harmonic oscillator was used. The friction coefficient for the solvent damping term was evaluated by modeling the two globular domains as spheres (144). From the adiabatic estimate of the hinge potential and the magnitude of the solvent damping, it was found that the relative motion of the two globular domains in lysozyme is overdamped; i.e. in the absence of

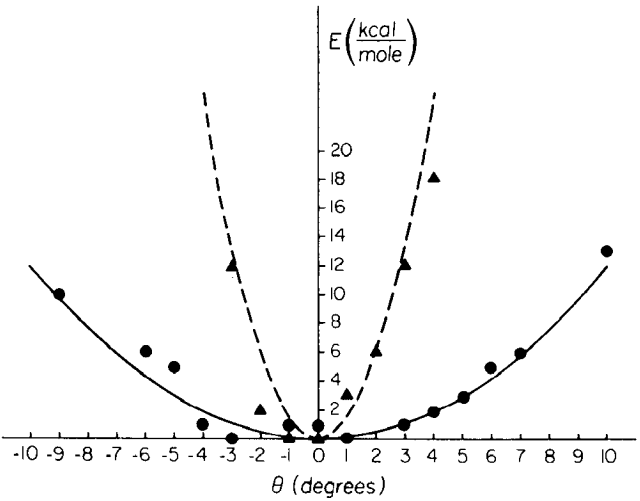

Figure 7 Change of conformational energy produced by opening ($\theta<0$) and closing ($\theta>0$) the lysozyme cleft; calculated values are for the rigid bending potential (triangles) and for the adiabatic-bending potential (circles); the origins for the two calculations are superposed.

driving forces the domains would relax to their equilibrium positions without oscillating. The decay time for this relaxation was estimated to be about 2×10^{-11}s. Actually, the lysozyme molecule experiences a randomly fluctuating driving force owing to collisions with the solvent molecules, so that the distance between the globular domains fluctuates in a Brownian manner over a range limited by the bending potential; a typical fluctuation opens the binding cleft by 1 Å and lasts for 20 ps.

The methodology developed in the lysozyme study is now being applied to a number of other proteins; they include antibody molecules (145), L-arabinose binding protein (146), and liver alcohol dehydrogenase (147). For the L-arabinose binding protein, calculations and experiment both suggest that the binding site is open in the unliganded protein but is induced to close by a hinge-bending motion upon ligation (146, 148). In the case of liver alcohol dehydrogenase, the open structure is stable in the crystal for the apoenzyme (149). Adiabatic energy-minimization calculations (147) suggest that the apoenzyme is highly flexible as far as its hinge-bending mode (rotation of the catalytic relative to the coenzyme binding domain) is concerned and that normal thermal fluctuations would lead to a closed structure (rotation of $\sim 10°$) similar to that found in the holoenzyme (150).

Since the hinge-bending motion in lysozyme and in other enzymes involves the active-site cleft, it is likely to play a role in the enzymatic activity of these systems. In addition to the possible difference in the binding equilibrium and solvent environment in the open and closed state, the motion itself could result in a coupling between the entrance and exit of the substrate and the opening and closing of the cleft (137–139). The interdomain mobility in immunoglobins may be involved in adapting the structure to bind different macromolecular antigens and, more generally, it may play a role in the cross-linking and other interactions required for antibody function. In the coat protein of tomato bushy stunt virus, a two-domain structure with a hinge peptide was identified from the X-ray structure (29, 151) and rotations about the hinge were shown to be involved in establishing different subunit interactions for copies of the same protein involved in the assembly of the complete viral protein shell.

Quaternary Structural Change

A classic case where large-scale motion plays an essential role is the allosteric transition in the hemoglobin tetramer (152–154). It is clear from the X-ray data that the subunits move relative to each other (quaternary change) and that more localized atomic displacements occur within each subunit (tertiary change). The coupling between these two types of structural changes is an essential factor in the cooperative mechanism of hemoglobin. As a first step in unraveling the nature of the motions involved, a

reaction path for the tertiary structure change induced by ligand binding within a subunit was worked out by the use of empirical energy calculations (155, 156). The results are in good agreement with limited structural data available for intermediates in the ligation reaction (157). The calculations show how the perturbation introduced in the heme by the binding of ligand leads to displacements in the protein atoms, so that alterations appear in surface regions in contact with other subunits. This provides a basis for the coupling between tertiary and quaternary structural change, although the details of the motions leading from the unliganded to the liganded quaternary structure have yet to be worked out.

As is clear from the above, most of the information available on rigid motions comes from high-resolution crystal structures of proteins. Low angle X-ray scattering analyses of solutions provided evidence for radius of gyration changes that are in accord with the crystal results where available (158) or provide evidence for structural changes in cases where only one structure is known (148, 159). However, there is almost no experimental evidence on the time scale of the rigid-body motions. Fluorescence depolarization studies of labeled antibody molecules show that the time scale for internal motions is consistent with the diffusional displacements of flexibly hinged domains (145, 160). It would be of great interest to have corresponding data on the domain motions of enzymes.

α-HELIX MOTION: HARMONIC AND SIMPLIFIED MODEL DYNAMICS

Early evidence for motion in the interior of proteins or their fragments comes from analyzing vibrational spectroscopic studies. It is generally assumed in interpreting such data that a harmonic potential and the resulting normal-mode description of the motions is adequate. This approximation is most likely correct for the tightly bonded secondary structural elements, like α-helices and β-sheets. The fluctuations of a finite α-helix (hexadecaglycine) were determined from the normal modes of the system (63). At 300°K, the rms fluctuations of mainchain dihedral angles (ϕ and ψ) about their equilibrium values are equal to $\sim$12° in the middle of the helix and somewhat larger near the ends. The dihedral angle fluctuations are significantly correlated over two neighboring residues; these correlations tend to localize the fluctuations (63, 71, 79, 80). Fluctuations in the lengths between adjacent residues (defined as the projection onto the helix axis of the vector connecting the centers of mass of adjacent residues) ranged from about 0.15 Å in the middle of the helix to about 0.25 Å at the ends. These length fluctuations are negatively correlated for residue pairs $(i-1,i)$ and $(i,i+1)$ so as to preserve the overall length of the helix; positive correlations are observed

for the pairs (4, 5), (8, 9) and (8, 9), (12, 13), suggesting that the motion of residue 8 is coupled to the motions of residues 4 and 12 to retain optimal hydrogen bonding.

Recently a full molecular-dynamics calculation was performed (81) for a decaglycine helix as a function of temperature between 5°–300°K and the results compared with those obtained in the harmonic approximation (63). For the mean-square positional fluctuations, $<\Delta r^2>$, of the atoms, the harmonic approximation is valid in the classical limit below 100°K, but there are significant deviations above that temperature; e.g. at 300°K, the average value of $<\Delta r^2>$ obtained for the α-carbons from the full dynamics is more than twice that found in the harmonic model. Quantum effects on the fluctuations are found to be significant only below 50°K. The temperature dependence of the fluctuations in the simulations is similar to that observed for α-helices in myoglobin between 80–300°K by X-ray diffraction (161).

As an approach to the helix-coil transition in α-helices, a simplified model for the polypeptide chain was introduced to permit a dynamic simulation on the submicrosecond time scale appropriate for this phenomenon (78): each residue is represented by a single interaction center ("atom") located at the centroid of the corresponding sidechain and the residues are linked by virtual bonds (97), as described earlier in the section on simplified model dynamics. The diffusional motion of the chain "atoms" expected in water was simulated by using a stochastic dynamics algorithm based on the Langevin equation with a generalized force term, Equation 3. Starting from an all-helical conformation, the dynamics of several residues at the end of a 15-residue chain were monitored in several independent 12.5-ns simulations at 298°K. The mobility of the terminal residue was quite large, with a rate constant $\approx 10^9\ s^{-1}$ for the transitions between coil and helix states. This mobility decreased for residues further into the chain; unwinding of an interior residue required simultaneous displacements of residues in the coil, so that larger solvent frictional forces were involved. The coil region did not move as a rigid body, however; the torsional motions of the chain were correlated so as to minimize dissipative effects. Such concerted transitions are not consistent with the conventional idea that successive transitions occur independently. Analysis of the chain diffusion tensor showed that the frequent occurrence of the correlated transition results from the relatively small frictional forces associated with these motions (100).

PERSPECTIVE

Theoretical protein dynamics did not exist before 1977 when the first paper presenting a detailed molecular-dynamics simulation of a small protein was published (71). In the next five years more than 50 theoretical papers

appeared. They explored dynamic phenomena in depth for a variety of proteins (protein inhibitors, transport and storage proteins, enzymes). The magnitudes and time scales of the motions were delineated and related to a variety of experimental measurements, including NMR, X-ray diffraction, fluorescent depolarization, infra-red spectroscopy and Raman scattering. It was shown how to extend dynamical methods from the subnanosecond time range accessible to standard molecular-dynamics simulations to much longer time scales for certain processes by the use of activated, harmonic and simplified model dynamics. Further, the effect of solvent was introduced by stochastic dynamic techniques or accounted for in full dynamic simulations including also the crystal environment. Concomitantly, a wealth of experimental information on the motions appeared. The interplay between theory and experiment provides a basis for the present vitality of the field of protein dynamics.

What is known and what remains to be done? On the subnanosecond time scale our basic knowledge of protein motions is essentially complete; that is, the types of motion that occur have been clearly presented, their characteristics evaluated and the important factors determining their properties delineated. Simulation methods have shown that the structural fluctuations in proteins are sizable; particularly large fluctuations are found where steric constraints due to molecular packing are small (e.g. in the exposed side chains and external loops), but substantial mobility is also found in the protein interior. Local atomic displacements in the interior of the protein are correlated in a manner that tends to minimize disturbances of the global structure of the protein. This leads to fluctuations larger than would be permitted in a rigid polypeptide matrix.

For motions on a longer time scale, our understanding is more limited. When the motion of interest can be described in terms of a reaction path (e.g. hinge-bending, local-activated event), methods exist for determining the nature and rate of the process. However, for the motions that are slow owing to their complexity and involve large-scale structural changes, extensions of the approaches described in this review are required. Harmonic and simplified model dynamics, as well as reaction-path calculations, can provide information on slower processes, such as opening fluctuations and helix-coil transitions, but a detailed treatment of protein folding is beyond the reach of present methods.

In the theory of protein dynamics there are two directions where active study and significant progress can be expected in the near future. One concerns the more detailed examination of processes of biological interest and the other, an improvement in approaches to longer-time dynamics. As to the latter, a variety of extensions of the methodology described in this review, as well as the availability of faster computers, may yield the necessary insights. As to the former, there are many biological problems to which

current dynamical methods can be applied and for which a knowledge of the dynamics is essential for a complete understanding. Some of these are listed below.

For the transport protein hemoglobin, there is more evidence concerning the role of motion than for any other protein. The tertiary and quaternary structural changes that occur on ligand binding and their relation to the allosteric mechanism are well documented. An important role of the quaternary structural change is to transmit information over a longer distance than could take place by tertiary structural changes alone; the latter are generally damped out over rather short distances unless amplified by the displacement of secondary structural elements or domains. The detailed dynamics of the allosteric mechanism has yet to be investigated; in particular, the barriers along the reaction path from the deoxy to the oxy structure have not been analyzed, nor has the importance of the fluctuations for the activated processes involved been determined. For the related storage protein, myoglobin, fluctuations in the globin are essential to the binding process; that is, the protein matrix in the X-ray structure is so tightly packed that there is no sufficiently low energy path for the ligand to enter or leave the heme pocket. Only through structural fluctuations in certain bottleneck regions can the barriers be lowered sufficiently to obtain the observed rates of ligand binding and release. Although energy minimization was used to investigate the displacements involved and the resulting barrier magnitudes, activated dynamic studies are needed to analyze the activation entropies and rates of ligand motion across the barriers.

In many proteins and peptides, the transport of substances is through the molecule rather than via overall translation as in hemoglobin. The most obvious cases are membrane systems, in which fluctuations are likely to be of great importance in determining the kinetics of transport. For channels that open and close (e.g. gramicidin) as well as for active transport involving enzymes (e.g. ATPases), fluctuations, in some cases highly correlated ones, must be involved. At present, structural details and studies of the motions are lacking, but this is an area where dynamic analyses are likely to be made in the near future.

In electron-transport proteins, such as cytochrome c, protein flexibility is likely to play two roles in the electron transfer. Evidence now favors a vibronic-coupled tunneling mechanism for transfer between cytochrome c and other proteins, although outer-sphere mechanisms are not fully excluded. In the vibronic-coupled tunneling theory, processes which would be energetically forbidden for rigid proteins become allowed if the appropriate energies for conformational distortions are available. Experimental data indicate that the important fluctuations are characterized by an average frequency on the order of 250 cm^{-1}, close to that associated with the

collective modes of proteins. Also, the transfer rate is a sensitive function of donor-acceptor distance and may be greatly increased by surface side-chain displacements that allow for the closer approach of the interacting proteins.

For proteins involved in binding, flexibility and fluctuations enter into both the thermodynamics and the kinetics of the reactions. For the rate of binding of two macromolecules (protein-antigen and antibody, protein-inhibitor and enzyme), as well as for smaller multisite ligands, structural fluctuations involving side chains, hydrogen-bonding groups, etc, can lead to lowering of the free energy barriers. Dividing the binding process into successive steps for which flexibility may be needed can increase the rate. The required fluctuations are likely to be sufficiently small and local that they will be fast relative to the binding and therefore not rate limiting.

The relative flexibility of the free and bound ligand, as well as changes in the binding protein, must be considered in the overall thermodynamics of the binding reaction. If the free species have considerable flexibility and fluctuations are involved in the binding step as described above, it is likely that the bound species will be less flexible and a significant entropic destabilization will result. Thus, for strong binding in cases where the rate is not important, relatively rigid species are desirable. This would reduce the conformational entropy decrease and could lead to a very favorable enthalpy of binding if there is high complementarity in the two binding sites. However, some flexibility and an increase in the conformational space available to the bound species has a stabilizing effect that partly compensates for the loss of translational and rotational entropy on binding. Conversely, the entropy loss of binding a flexible substrate or the rigidification of a protein on substrate binding can be used to modulate the binding constant even when strong, highly specific enthalpic interactions are present. The required balance between flexibility and rigidity will be determined by the function of the binding in each case. Dynamical techniques can be employed to determine the entropy differences for such systems. Further, the mechanism and rates of the binding processes, itself, are an ideal subject for dynamical analysis.

In the function of proteins as catalysts, there is the greatest possibility of contributions from motional phenomena. The role of flexibility per se has often been discussed, particularly from the viewpoint of structural changes induced by the binding of the substrate. In addition to cooperative effects caused by quaternary alterations, a variety of results can arise from the perturbation of the tertiary structure. One example is the ordered binding of several substrates (or effectors and substrates), with the first molecule to bind altering the local conformation so as to increase or decrease the subsequent binding of other molecules. The occurrence of large-scale changes,

such as the closing of active-site clefts by substrate binding, as in certain kinases, has been interpreted in terms of catalytic specificity, alteration of the solvent environment of the substrate, and exclusion of water that could compete with the enzymatic reaction. In large enzymes with more than one catalytic site or in coupled enzyme systems, conformational freedom may be important in moving the substrate along its route from one site to the next. Many of these processes are ready for the application of dynamical methods, particularly in cases where structural data are available.

The flexibility of the substrate-binding site in enzymes can result in effects corresponding to those already considered in receptor binding. In the enzyme case there exists the often-discussed possibility of enhanced binding of a substrate with its geometry and electron distribution close to the transition state; for this to occur, conformation fluctuations are essential. Entropic effects also are likely to be of significance, both with respect to solvent release on substrate binding and possible changes in vibrational frequencies that alter the vibrational entropy of the bound system in the enzyme-substrate complex or in the transition state. There are also indications that the inactivity of enzyme precursors can result from the presence of conformational freedom in residues involved in the active site. The entropic cost of constraining them in the proper geometry for interacting with the substrate may be so high that the activity is significantly reduced relative to that of the normal enzyme where the same residues are held in place more rigidly. Such a control mechanism was suggested for the trypsin, trypsinogen system, and for other proteins. As to the time dependence of fluctuations and structural alternations, there are a variety of possibilities to be considered. In the binding of reactants and release of products, the time course of fluctuations in the enzyme could interact with the motion of the substrate. The opening and closing fluctuations of active-site clefts may be modified by interactions with the substrate as it enters or leaves the binding site.

Fluctuations could play an essential role in determining the effective barriers for the catalyzed reactions. If the substrate is relatively tightly bound, local fluctuation in the enzyme could couple to the substrate in such a way as to significantly reduce the barriers. If such coupling effects exist, specific structures could have developed through evolutionary pressure to introduce directionality and enhance the required fluctuations. Frictional effects that occur in the crossing of barriers in the interior of the protein could act to increase the transition state lifetime and so alter the reaction rates relative to those predicted by conventional rate theory. Energy released locally in substrate binding may be utilized directly for catalyzing its reaction, perhaps by inducing certain fluctuations. Whether such an effect occurs would depend on the rate of dissipation of the (mainly) vibrational

energy and the existence of patterns of atoms and interactions to channel the energy appropriately. It will be of great interest to determine whether any of the rather speculative possibilities outlined here for the role of the energy and directionality of structural fluctuations in enzymatic reactions can be documented theoretically or experimentally for specific systems.

A wide range of biological problems involving proteins, not to mention nucleic acids and membrane lipids, are ready for study and exciting new results can be expected as dynamical methods are applied to them. In the coming years, we shall learn how to calculate meaningful rate constants for enzymatic reactions, ligand binding and many of the other biologically important processes mentioned above. The role of flexibility and fluctuations will be understood in much greater detail. It should become possible to determine the effects upon the dynamics of changes in solvent conditions and protein amino acid sequence. As the predictive powers of the theoretical approaches increase, applications will be made to practical problems arising in areas such as genetic engineering and industrial enzyme technology.

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