The Dynamic Picture of Protein Structure

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An important change is occurring in our picture of globular proteins. These molecules have traditionally been described in static terms. The high specificity of enzymes for their substrates has, for example, been likened to the complementarity of two pieces of a jigsaw puzzle. The static view of protein structure is now being replaced by a dynamic picture. It is recognized that the protein atoms are in a state of constant motion. The average positions correspond to what may be seen in an X-ray structure, but the atoms exhibit fluidlike motions of sizable amplitude around these average positions. The new dynamic picture subsumes the static picture in that the average positions allow for interpretation of many aspects of protein function in the classical language of structural chemistry. The recognition of the importance of fluctuations opens the way for more sophisticated and accurate discussions of protein function.

This Account deals primarily with theoretical studies of protein dynamics, which have played an essential role in changing our fundamental view of proteins. Protein dynamics holds great promise for interpreting experimental results and calculating rates of biochemical processes from first principles. This rapidly developing field is founded on efforts to supplement protein structural theory with concepts and techniques from modern statistical mechanics. In statistical mechanics, fundamental data on atomic properties and interactions are used to calculate the thermodynamic characteristics and the rates of processes in systems such as liquids that are composed of many atoms.[1-4] The application of such methods to proteins is natural in that proteins contain many atoms, are densely packed,[5] and typically function in liquid environments.

Although the dynamic picture is inevitably more complicated than its static predecessor, the results of recent studies can be qualitatively understood in terms of a few simple concepts. It should be recalled at the outset that polypeptide chains, like other polymer chains, are intrinsically flexible. The single bonds that link the side chains to the main chain and connect the successive amide groups within the main chain are rotationally permissive. For times up to a few tenths of a picosecond, the motion of individual groups in a compactly folded protein is therefore similar to that of small molecules in a liquid. The groups jiggle about as a result of frequent collisions with the surrounding "cage" atoms. Over longer times, the displacements are limited by the forces that maintain the native folding pattern of the protein. The net motion of a typical group is therefore a superposition of the rapid, local jiggling and slower, elastic movements that involve larger regions in the protein. The folding forces are sufficiently soft that the large-scale or "collective" motions may have amplitudes substantially in excess of 1 Å. Such motions involve displacements of the protein surface and therefore display solvent damping effects.

Theoretical protein dynamics is still in its infancy. Nevertheless, quantitative results have been obtained for a wide variety of specific processes in proteins.[6,7] These have been used successfully to predict such experimental results as X-ray diffraction temperature factors,[8,9] NMR relaxation times,[10,11] and fluorescence depolarization rates;[12,13] in a number of cases, the theoretical predictions have preceded the experimental measurements.[8,9] Theoretical studies have also helped to clarify the mechanisms of ligand-binding, allosteric transitions, and other functional events in biochemistry. As has been the case with the dynamics of small molecules and liquids, the detailed application of physical theory to proteins will display increasing sophistication, accuracy, and importance with continued effort.

Although experimental studies will be considered only incidentally, it should be noted that they have made an important contribution to the dynamic picture of proteins.[14-19] In fact, hydrogen-exchange studies indicated

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that occurrence of extensive structural fluctuations in proteins more than 25 years ago.[16] These and other early experiments were less influential than they might have been because the experiments did not admit of a unique structural interpretation of the motions. More recent spectroscopic studies have provided a wealth of data on the rates of protein internal motions; in favorable case, correlations with structure can be drawn.[17,18]

Methodology

To study theoretically the microscopic structure and dynamics of any molecular system, it is necessary to be able to calculate its potential energy as a function of the atomic positions. The relative populations of the different possible structures of the system at a given temperature are given in terms of the potential by the Boltzmann distribution law.[1] The mechanical forces acting on the atoms are related to the slope of the potential. These forces can be used to determine dynamical properties of the system by solving Newton's equations of motion to determine how the atomic positions change with time.[1,2]

The potential energy functions used for proteins and their solvent surroundings are empirical in character.[20-22] One starts with a parametrized form for the function and chooses values for the parameters on the basis of experimental and quantum mechanical studies of small components of the whole system. The functions used for proteins are of the valence force-field type that has long been used in the analysis of molecular vibrational spectra.[23] Such functions are sums of terms associated with bond lengths, bond angles, dihedral angles, and nonbonded atom pair separations (van der Waals, electrostatic, and hydrogen-bond interactions). The nonbonded interactions play an important role in globular proteins because of the dense packing of the atoms in these molecules. The resulting expressions are similar to the molecular mechanics functions introduced by Westheimer[24] and Kitaigorodskii[25] for analysis of conformational effects in organic reactions and crystallography. The protein potentials were, in fact, originally developed to deal with analogous problems, such as conformational transitions in polypeptides[20] and the energy refinement of protein crystal structures.[21]

The energy functions presently used for proteins are simpler than those used in modern physical organic chemistry.[26] That these relatively crude functions have been used successfully is due in part to the close-packed nature of globular proteins. In other dense systems such as simple liquids, it is known that many features of the structure and dynamics are determined largely by the shape of the component molecules or, more exactly, by the steeply repulsive part of their intermolecular potentials.[2,3] The properties of the system are similar to those of one in which the molecules are composed of hard sphere atoms; that is, the structure is one that optimizes the packing of such molecules, and the molecular motion reflects the frequency of collisions between them. The usefulness of such ideas for the structural analysis of proteins was demonstrated some time ago by Ramachandran and co-workers.[5,27]

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 calculations, in which one uses a computer to solve the Newtonian equations of motion for the atoms of the protein and any surrounding solvent.[22,28,29] With current computers, it is possible to simulate the dynamics of small proteins for periods of up to a few hundred picoseconds. 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 other simulation methods. A variety of dynamical approaches, such as stochastic dynamics,[30-34] harmonic dynamics,[22,23,35-38] and activated dynamics,[39-42] can be introduced to study particular problems. Stochastic dynamics, based on the Langevin equation of motion (see below), is perhaps of the greatest utility both for the simulation of complex processes[31-34] and for the analysis of the molecular dynamics results themselves.[22,43,44] In such an analysis of, for example, a group of side-chain atoms in a protein, it is presumed that the displacement of the group relative to its neighbors is 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.[1,22] This potential is the free energy of relative displacement of the group. It is therefore a temperature-dependent quantity and typically becomes softer or more permissive as the mobility of neighboring groups increases. The dynamics of the group motion within its allowed range is determined largely by the time variation of its

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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 modeled by a Langevin equation of motion, such as[22]

$$m\frac{d^2x}{dt^2} = F(x) - f\frac{dx}{dt} + R(t) \quad (1)$$

Here, m and x are the mass and position of the group, respectively, and t is the time; thus, the term on the left is simply the mass times the acceleration of the group. The term $F(x)$ represents the systematic force on the group derived from the potential of mean force. The terms $-f dx/dt$ and $R(t)$ represent the direct effects of the varying forces acting on the group; the first term is the average frictional force due to the relative motion of the group through its surrounding (f is the friction coefficient), and $R(t)$ represents the remaining randomly fluctuating force.

The Langevin equation also provides a useful focal point in the discussion of large-scale motions.[45,46] For displacements of whole sections of polypeptide chain away from the protein surface (local denaturation), the terms corresponding to the one on the left of eq 1 are typically negligible in comparison to the others.[33] 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 shapes, the potential of mean force may have a simple Hooke's law or springlike character.[45] Such deformations are of functional importance in proteins that contain globular lobes linked by flexible hinges; these hinge-bending motions typically have Brownian character.[45] 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.[47,48]

For processes that are intrinsically fast but rare, such as the activated events involved in enzyme reactions, alternative dynamical methods can be employed. It is necessary to identify the particular character of the structural change involved (e.g., to determine 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.[49,50] 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,[41] 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 familar transition-state theory.[49] A more detailed understanding of the process can be obtained by analyzing trajectories chosen to sample the barrier region.[39-42] 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.

Local Motions

Molecular dynamics studies have provided an abundance of data on typical thermal motions of the atoms in two proteins, bovine pancreatic trypsin inhibitor (BPTI)[22,28,29,44,51-53] and cytochrome c,[8,9,54-57] and in an isolated section of α helix.[38] Analysis of this data has revealed some of the general characteristics of these motions, although much work remains to be done. Modified molecular dynamics techniques have provided data on an activated rotational isomerization reaction in BPTI,[39-42] but the study of such infrequent processes is even less developed than that of the typical thermal motions.

The time-average structures from the existing molecular dynamics simulations are similar to the X-ray crystal structures. The differences that occur are primarily due to differences in the protein environment (vacuum or a simplified model solvent[52]) from that in a crystal. The average root-mean-square (rms) deviations of the atom positions from those in the crystal structure are less than 2 Å in recent simulations; the largest deviations occur near the protein surface. Smaller deviations occur in simulations of a protein in its hydrated crystal ennvironment.[53]

The time-dependent fluctuations of the atom positions around their averages are comparable in magnitude to the atomic radii. The average rms fluctuations are typically in the range 0.4–0.6 Å for atoms near the protein center but in fluctuations increase markedly near the protein surface.[9] Backbone atoms have somewhat smaller fluctuations than side-chain atoms.[9,29] The fluctuations are generally anisotropic.[9,51] 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. 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).[9] In most cases, however, the directional preferences appear to be determined by larger scale collective motions involving the atom and its neighbors.[9,57]

The properties described above are time averages. To begin a discussion of actual time dependence of the atom motions, it is natural to consider how the magnitude and anisotropy of the atom position fluctuations depend on the observation period (Figure 1).[57] In cytochrome c and BPTI, the rms position fluctuations typically attain one-fourth to one-half their long-time-average values in only 0.2–0.5 ps.[44,57] This time interval

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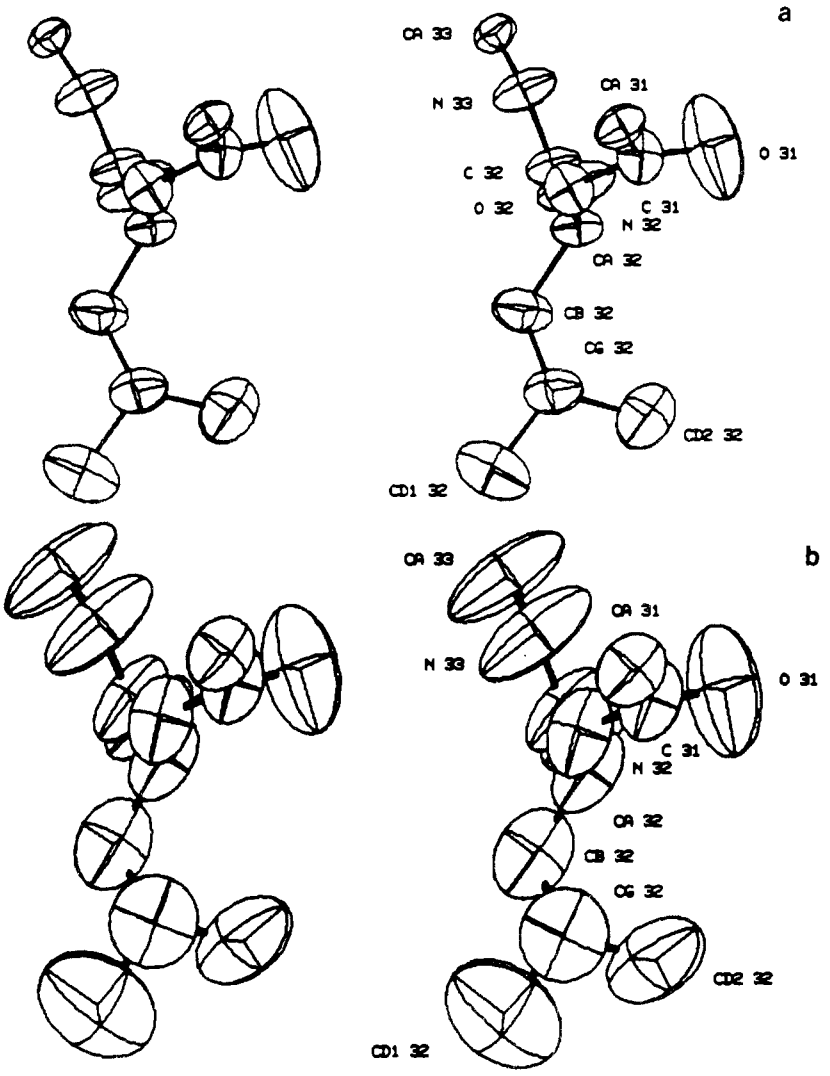

Figure 1. Steroviews of atomic thermal ellipsoids for leucine-32 of ferrocytochrome *c*.[57] The atomic thermal displacements are from a molecular dynamics computer simulation.[9,56] (a) Displacements observed during 160 intervals of 0.2 ps. (b) Displacements observed during one interval of 32 ps. The axes of the ellipsoids in (a) are magnified by a factor of 2 relative to those in (b).

is long enough to include an average over localized vibrational modes with frequencies greater than about 150 cm^{-1}. The anisotropy of the atomic displacements for 0.2-ps periods correlates well with the pattern expected for torsional librations about single bonds. The limiting values of the rms position fluctuations are typically approached over a period on the order of 10 ps. Thus, the largest contribution to the rms position fluctuation of an atom is due to slow collective motions of the atom and its neighbors. The correlations of anisotropy with local bonding are often washed out by these large-amplitude collective motions. For certain proteins in solution (e.g., the "hinged" proteins described in the next section) the collective contributions to the rms position fluctuations may converge more slowly.

The time dependence of atom and group motions in proteins can be characterized more fully by calculating appropriate time correlation functions.[22,28,29,44] The time correlation function of a fluctuating quantity describes the average manner in which a typical fluctuation decays.[1,2,58] Time correlation functions for fluctuations of individual atom positions in the protein interior (or of the associated dihedral angles) often decay in nearly monotonic fashion on two distinct time scales, although the slow component has significant oscillations in many cases. A partial loss of amplitude occurs within the first 0.2 ps, followed by much slower decay on a time scale of several picoseconds. The decay times of the position correlation functions are increased by including external solvent in the dynamic simulation; this effect is most pronounced for atoms at the protein surface.[52] An approximate analysis of the relaxation times for the atoms in BPTI plus solvent yields a wide range (0.45–8 ps); in vacuum, the times shift to somewhat shorter values (0.2–6 ps).

The torsional librations of buried tyrosine rings in BPTI have been studied in some detail.[22] The librations examined are those about axes along the single bonds by which the rings are linked to the protein. The motion is analogous to rotation of a benzene molecule about an axis passing through two atoms at opposite vertices of the ring. The rms amplitude of libration in the protein ($\sim 12°$) is limited by steric hindrance between atoms in the ring and those in the cage surrounding the ring. The time correlation functions for the torsional fluctuations decay to small values in a short time (~ 0.2 ps); subsequent decay occurs on a time

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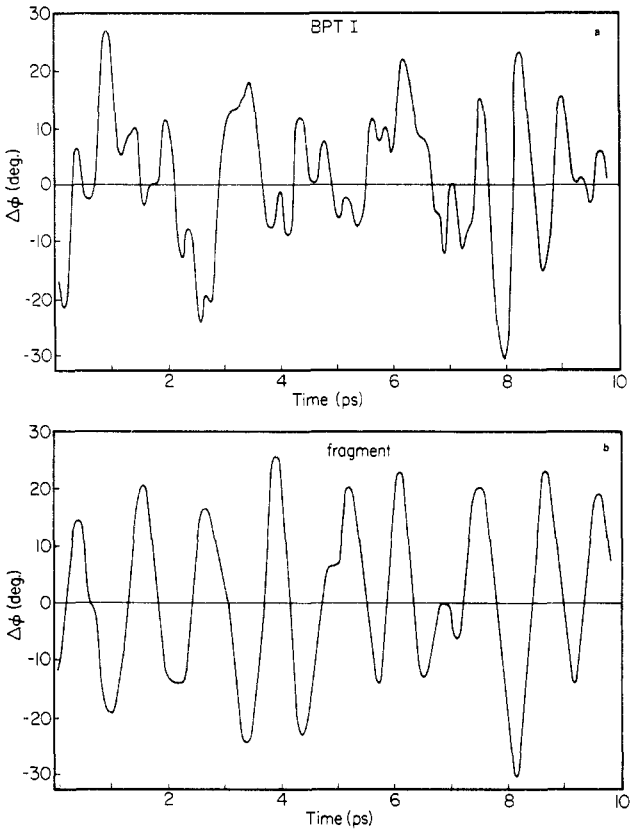

Figure 2. Evolution of the ring torsion angle for tyrosine-21 of BPTI during two molecular dynamics computer simulations.[22] (a) Displacements observed during a dynamical simulation of the whole protein. (b) Displacements observed during a dynamical simulation of tyrosine-21 and several backbone atoms from the adjacent residnes.

scale of several picoseconds. This result indicates that the motion is predominantly local in character; the ring exhibits torsional rattling in its cage. On the longer time scale, large-scale distortions of the protein produce small changes in the overall shape of the cage with a concomitant alteration of the ring orientation. These properties of the ring motion are evident in a plot of the time history of the torsional fluctuations (Figure 2a). The short-time rattling due to collisions between ring and cage atoms is superimposed on a slower component arising from the larger scale motions. The randomizing effects of such interactions are markedly diminished in a dynamical simulation in which most of the cage atoms were omitted (Figure 2b). The short-time, local motion in the protein is consistent with a torsional Langevin equation that contains a harmonic restoring force.[22] The frictional and 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.

Torsional fluctuations of tyrosine and phenylalanine rings in which the ring rotates by 180° are known to occur in proteins.[14,17] Such rotational isomerizations or ring "flips" occur very infrequently because of the large energy barrier due to steric hindrance.[22,41,49] The long time intervals separating flips preclude systematic study by conventional molecular dynamics methods. A modified molecular dynamics method has recently been developed to handle such local activated processes.[39-42] This method is similar to adiabatic mapping in that one starts with an assumed "reaction coordinate" that defines the fundamental structure 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, and also in that it provides a detailed description of the structural and dynamical features of the process. In this method, one calculates separately the factors in the following expression[59] for the rate constant of the process:

$$k = (1/2)\kappa\langle|\dot{\zeta}|\rangle\left[\rho(\zeta^{\ddagger})/\int_i \rho(\zeta)\mathrm{d}\zeta\right] \qquad (2)$$

Here, ζ is the reaction coordinate, $\dot{\zeta} = \mathrm{d}\zeta/\mathrm{d}t$, and $\zeta^{\ddagger}$ is the value of ζ in the transition-state region of 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. The factor corresponds roughly to the $\exp(-\Delta G^{\ddagger}/RT)$ factor in more familar expressions 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 ζ.[41] The remaining factors can be calculated by analysis of trajectories initiated in the transition-state region.[39-41] 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 always less than 1. Analysis of the trajectories also provides a wealth of information on the mechanistic details of the process.[39,40,42]

Application of this modified molecular dynamics method to the flipping of a tyrosine ring in BPTI shows that the rotations themselves require only 0.5–1.0 ps.[39-42] At the microscopic level, the processes responsible for flipping are the same as those responsible for the smaller amplitude librations. The ring is driven over the barrier not as the result of a particularly energetic collisions 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 due to small, transient packing defects.[42] The packing defects help to initiate ring rotation, but they are too small to allow free rotation of the ring by a simple vacancy or free-volume mechanism.[42,60] The ring, in fact, 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 librations; these frictional effects reduce the transition rate to about 20% of the ideal transition state theory value.[41]

Large-Scale Motion

Large-scale motions involve collective displacement of some or all of the residues in a protein. Some such motions could make significant contributions to the rms atomic fluctuations, but others (e.g., partial unfolding) are expected to be too rare to be involved. Here, we consider only one type of large-scale motion, namely, elastic deformations of the native globular structure. Dynamical simulations related to local unfolding have also been carried out.[33,34,61]

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In deformations of the overall shape of a protein, the relative displacements of neighboring groups within the molecule may be relative small. The protein is therefore expected to behave as an elastic material under such deformations. Before more detailed models were available, large-scale motions of globular proteins were, in fact, analyzed in terms of the vibrational modes of a homogeneous elastic sphere.[62,63] The amplitudes of these spherical motions are small at normal temperatures, but more substantial elastic motions do occur in a large class of proteins that contain globular domains linked by flexible "hinges".[64] Such hinge-bending motions are of special interest because they may modulate the accessibility of binding sites for ligands or substrate molecules.

Hinge bending was first studied theoretically in the enzyme lysozyme, which contains two globular domains linked by a single hinge.[45,46] The effective energy cost for hinge bending was estimated by an adiabatic mapping technique in which the protein was relaxed by energy minimization while the hinge angle was held at different values. The adiabatic energy approximates the potential of mean force for bending because relaxation involves local motions (e.g., bond-angle deformations) that are fast compared to the overall bending motion. Since this energy has an approximately parabolic form, the bending motion can be described by the Langevin equation with a harmonic potential. The frictional effects are larger than inertial effects so that the motion has a Brownian character. A typical fluctuation opens the binding cleft by 1 Å and lasts for 20 ps. The ideas developed in the lysozyme study have been applied to other biopolymers, including antibody molecules,[65] DNA,[66] t-RNA,[67] and the L-arabinose-binding protein (ABP).[68]

Experimental Studies

The rapid development of the field of protein dynamics is partly due to a robust synergism between theory and experiment. Particularly important is the high correlation ($R = 0.73$) found between rms atomic displacements in molecular dynamics simulations and the atomic temperature factors in X-ray diffraction studies of the same protein (Figure 3).[8,9] This correlation confirms the reliability of both the simulation results and the temperature factors[69-71] as detailed measures of the internal mobility of proteins. It has also been shown that there is some correspondence between the rms fluctuations of hydrogen-bond lengths in a dynamical simulation of BPTI and the relative stability of the hydrogens involved toward exchange with solvent hydrogens.[72] These correlations between time-average

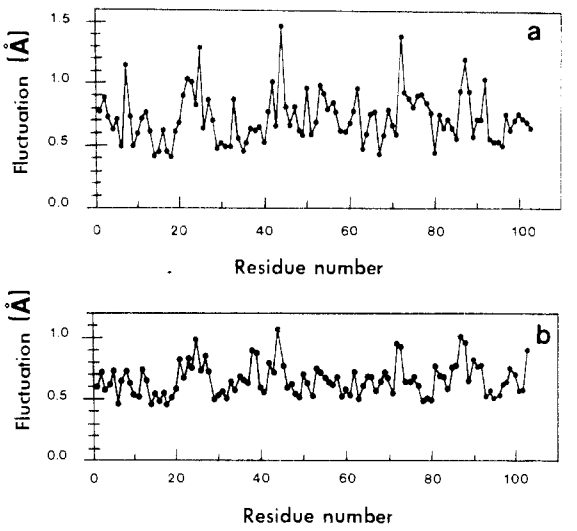

Figure 3. Average rms atomic position fluctuation for each amino acid residue in cytochrome c.[8] (a) Simulation result. (b) X-ray result. The experimental values have been corrected uniformly for lattice disorder.

results from dynamical simulations and experimental data are complemented by an increasing number of results that bear more directly on the time dependence of the motions. NMR relaxation[10,11] and fluorescence depolarization[12,13,73] times have been shown to reflect the picosecond time scale motions seen in the simulations.

With respect to large-scale motions, the flexibility of hinged proteins[74,75] (and t-RNA[76]) has been demonstrated in crystallographic and low-angle X-ray scattering studies. Fluorescence depolarization studies of antibody molecules show that the time scale for internal motions is consistent with the diffusional motion of flexibly hinged domains.[77]

Biological Implications

The dynamical nature of protein molecules is essential to much of their biological activity. Indeed, it is reasonable to suppose that the internal flexibility of proteins and some of their dynamical characteristics have been shaped by evolution to allow efficient expression of protein function. We can expect to see an increasing number of correlations between dynamics and function as different proteins are studied in detail, but a number of suggestive examples are already available.

For local motions, a clasic example was recognized soon after the crystal structure of the oxygen-binding protein myoglobin was determined.[78] The protein forms a dense wall between the solvent and the internal binding site. No channel for oxygen entry is apparent in the structure of either the liganded or unliganded species. Thus, ligand penetration is dependent on transient structural fluctuations in the protein. Adiabatic mapping calculations have shown that protein

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structural fluctuations reduce the energy barriers found in the static structure for ligand insertion to realistic values.[50] Further, a path suggested by the energy calculations has been found to correspond to a high-mobility region in the protein as determined by X-ray temperature factors.[69] Detailed analyses of ligand flash photolysis experiments suggest barriers in accord with the energy calculations.[79]

Local motions are also involved in enzymic activity, both in the binding of ligands and the catalytic steps. Although detailed dynamical studies of enzymes are only now beginning, the theoretical analysis of tyrosine rotational isomerization dynamics indicates some of the factors involved.[39-42] Displacements of sizable groups are often involved in the mutual accommodation of enzyme and ligands upon substrate binding and product release and between the intervening changes of local covalent bonding. Such displacements will have an erratic character due to frequent collisions of the groups with neighboring atoms of the protein and solvent. These effects will cause a significant slowing of the net displacements. The relative rate constants for transitions from one configuration to other configurations will generally be influenced by such effects; this phenomenon has been observed in other Brownian processes in solution.[34,61,80] In addition to these direct dynamical effects, the mobility of the atoms that surround a group will permit some amount of local structural relaxation correlated with the group displacements. The relaxation will result in a lowering of effective energy barriers that may be surmounted during the displacements.[42]

Large-scale motions are also involved in biological function. Motions of a polypeptide segment at the protein surface appear to play a role in the activity of a number of enzymes. X-ray studies of penicillopepsin, for example, indicate that a loop in the chain over the active site is disordered in the free enzyme but is fixed like a cap on this site when substrate is bound.[81] Hinge-bending motions are now implicated in the function of a variety of proteins, including enzymes,[64,74] antibody molecules,[82] and binding proteins.[68,75] For the L-arabinose-binding protein, calculation 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.[68,75] This "Venus's flytrap" behavior is well-suited to the function of the protein. The open binding site allows a high binding rate, while the conformation change traps the ligand and prepares the protein for interaction with other components of the transport apparatus.

The fluctuations in proteins and their changes on ligand and substrate binding can have thermodynamic as well as dynamic consequences. Clearly the configurational entropy of the system will change if the fluctuation magnitudes are altered; an experimental analysis of this possibility has been given.[83] It has recently been shown how to use the results of molecular dynamics simulations to evaluate changes in the configurational entropy;[84] applications to a number of systems are in progress (e.g., the effect of ligand binding on the configurational entropy in myoglobin).

An important functional aspect of protein dynamics that is just now being addressed is the effect of binding-site dynamics on the rates of ligand binding and release. In proteins such as myoglobin, lysozyme, and penicillopepsin, the accessibility of the binding site is modulated by internal rearrangements of the protein. A first attempt has recently been made to assess the effects of such "gated" accessibility on the observed kinetics of ligand binding.[85-87] The results show that in simple cases of slow gate dynamics, the rate is just the ungated rate multiplied by the fraction of the time that the gate is open. In other cases, substantial deviations from this intuitive result may occur.

The Future

Recent work has led to the introduction of a variety of theoretical tools for the study of protein dynamics. The application of these tools has helped to establish many fundamental characteristics of the internal motions of proteins. However, the most dramatic developments clearly lie in the future. Before the end of this century, we will almost surely be able to calculate meaningful rate constants for some enzymatic reactions and ligand-binding processes. Motion pictures of the corresponding atomic trajectories will be in common use as teaching aids and as guides for the researcher's intuition. It will be possible to predict the effects upon such processes of changes in solvent conditions and protein amino acid sequence. The predictive powder of this theoretical work will ultimately be of practical use in connection with genetic engineering and industrial enzyme technology.

From a more basic standpoint, the important developments will revolve around the new questions raised by continuing efforts in this area. The diversity of these will require multidisciplinary responses. Some typical questions that are already apparent include the following. Can the refinement of finite-temperature X-ray diffraction structures be improved by using molecular dynamics simulations to provide input information on the atomic displacement? Can one construct optimal reaction coordinates for multidimensional problems in which frictional forces compete with those derived from the potential surface? How does the displacement of solvent molecules from a binding site influence the kinetics of ligand binding? Clearly there are many more interesting questions than convincing answers in this area.

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