

Energetics and Dynamics of Deterministic Protein Motion

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A number of important biological processes involve structural changes which modify the chemical behavior or function of the active molecular agents (e.g., allosteric regulation of substrate binding). These structural transitions generally involve a highly directed process in which small amounts of input energy can lead to correlated displacements involving thousands of atoms. The vectorizing force for the motion presumably originates from the three-dimensional structure of the molecule in question. In this sense, there is a deterministic aspect to the motion, superimposed on top of the random thermal fluctuations.

This Account will focus on recent developments in our understanding of functionally important protein motions from a dynamics point of view. The emphasis will be on the use of optical methods to probe the protein motion directly in the time domain. In this application, phase grating spectroscopy has proven to be extremely sensitive to energy and structural relaxation processes.^{1,2} This spectroscopy is an extension of transient grating methods^{3–5} in which the protein's motion is holographically recorded in real time to give a direct probe of the protein strain and the accompanying energetics, two fundamental parameters needed to understand the mechanics of the motion.

In the following discussion, oxygen-carrying heme proteins will serve as the model systems. These proteins undergo structural changes at both the tertiary and quaternary levels which act concertedly to control oxygen binding at the heme iron site and form the basis of the oxygen transport system in the body. The synergistic coupling between the tertiary structure of the adjacent subunits and the quaternary structure of hemoglobin is one of the hallmark examples of molecular cooperativity and serves as the basis for understanding allosteric regulation.^{6–8} From an experimental standpoint, heme proteins have nearly ideal optical properties in that it is possible to optically trigger the structural changes by photodissociation of the iron ligand, which makes these systems amenable to optical probes.⁹

The three-dimensional structure of hemoglobin (Hb) is shown schematically in Figure 1, in which the interconversion between the high oxygen affinity R and low-affinity T quaternary structure is depicted. The most important point to note from this figure is the degree of structural changes involved in modifying the activation barriers to Fe–O₂ bond formation. The large-

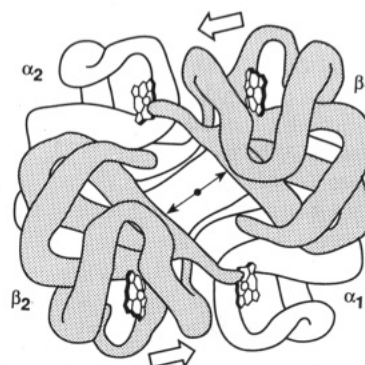


Figure 1. Three-dimensional structure of hemoglobin (MW = 64 500) showing the four heme protein subunits, each of which consists of ~1000 atoms (adapted from ref 7). The oxygen-binding heme is in the center of the helical sections of the globin, which are sketched showing only the effective radius of the helix. The arrows indicate the relative motions of the subunits in undergoing the R to T quaternary structure change. The whole system performs a kind of “ball-in-socket” motion with a 12–15° rotation and as much as a 6-Å relative translation between contacts in the interface (refs 6 and 7).

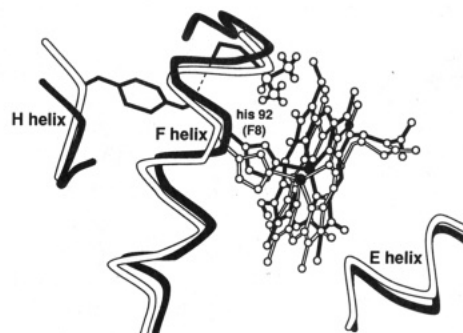


Figure 2. Close-up view of the tertiary structural changes at the heme site. The black outlined structure corresponds to the ligated oxy tertiary structure, and the white is the deoxy structure. The puckering of the heme ring, motion of the iron out of plane, and rotation of the proximal histidine are key motions. These displacements ultimately couple to the helical sections and communicate the state of occupancy at the heme site to adjacent subunits (adapted from ref 7).

amplitude motion at the quaternary level is driven by the changes in tertiary structure upon ligation, which are shown in Figure 2. These tertiary motions ultimately propagate to the $\alpha\beta$ subunit interface and form the communication link between subunits that directs the R to T switch for oxygen binding.^{6–13} It is this aspect

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of the protein dynamics that needs to be understood first. The tertiary displacements are themselves powered by the potential energy gradients that develop at the saddle point in the reaction coordinate for the Fe-O₂ bond formation. This complex potential energy surface is outside the realm of a detailed determination. However, it is still possible to determine general features of the structure/reactivity relationship. Information on the length scale of the acting forces and relaxation energetics is needed in order to understand the ensuing structural changes. Since the protein's potential energy surface is nested within a myriad of nearly degenerate surfaces, the multiplicity of structural relaxation pathways also needs to be considered.¹⁴ Finally, how energy is exchanged among the different mechanical degrees of freedom needs to be determined. This latter aspect of the problem is related to the relaxation dynamics and forms the guidelines for thinking about protein motions as a "collection of balls and springs" that are weakly interacting (nearly harmonic) or highly damped (nearly diffusive) motions. These different aspects of the problem will be discussed below.

Energy Exchange and Relaxation Processes in Proteins

At any particular instant in time, one part of the protein will have excess energy relative to an adjacent point in space. There are potential energy fluctuations related to thermal sampling of different conformations which can be long-lived. The energy currency which is rapidly exchanged is the kinetic and potential energy associated with the vibrational, (hindered) translational, and (hindered) rotational internal nuclear degrees of freedom of the protein. The dynamics of energy exchange between these different degrees of freedom determines the fluctuation and dissipation processes that govern the protein's motion and is central to understanding how energy is transduced from a stimulus into motion.

This problem is ideally suited to time domain measurements in which the energy redistribution can be followed by monitoring both the decay of the initially excited mode(s) and the buildup of energy in accepting modes. With this approach it is possible to spatially and temporally map the energy relaxation pathways. By taking advantage of the short nonradiative excited-state lifetime of deoxyheme proteins ($\leq 2-3$ ps),¹⁵ it is possible to optically deposit $10\,000-20\,000\text{ cm}^{-1}$ of excess vibrational energy selectively at the heme site fast enough to resolve the subsequent energy redistribution processes. For this initial condition, the vibrational modes of the isolated heme porphyrin, the surrounding protein matrix, and highly damped collisional modes

of the water can be used as a basis set to discuss the energy flow into and out of the protein.^{1,16}

Time-resolved Raman studies^{17,18} and power dependencies of Stokes and anti-Stokes modes of the heme group¹⁹ have determined that the transfer of energy from the vibrationally excited heme to the surrounding protein occurs with time constants on the order of 5 ps. Our approach to the problem has been to follow the relaxation of this excess energy into translational energy of the aqueous bath using thermal phase grating spectroscopy.^{20,21} By adopting counterpropagating beam geometries to monitor the highest possible frequency acoustic component to the thermally driven expansion, the grating studies determined that most of the energy is dissipated from the protein into the aqueous bath is less than 22 ps. This observation also indicated that the excess vibrational energy leaves the initial heme site to the surrounding protein in less than 22 ps and becomes highly dispersed throughout the protein. This latter conclusion is based on collisional exchange limits for transferring such large amounts of excess energy to the water layer.²² Additional evidence for this fast energy dissipation to the aqueous bath has been attained using time-resolved infrared studies which monitor the heating of the water through thermal broadening effects.²³ The exact form of the relaxation is better fitted to a Gaussian distribution of components (11 ps FWHM) which is consistent with a rapidly attained statistical distribution of energy within the protein that undergoes thermal diffusion to the water layer.

These studies give direct experimental insight into how energy *sloshes* about in biological systems. The main conclusion to be drawn is that the vibrational energy exchange and redistribution within the protein and the exchange with the aqueous interface are extremely efficient. The energy becomes statistically redistributed on a 1-10-ps time scale, in qualitative agreement with molecular dynamics simulations.²⁴ The observation that energy exchange occurs within this time scale means that the spatially extended low-frequency modes of the protein ($\leq 50\text{ cm}^{-1}$) are strongly damped, i.e., the periods of these modes are comparable to the time for energy redistribution. This aspect of the fluctuation and dissipation processes in proteins will be an important concept in the following discussion.

Protein Structural Relaxation: Force Distribution

There have been numerous models proposed for the mechanics of the heme protein structural changes. Fundamentally, these models all rely on the forces that

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develop at the saddle point in the potential energy surface for the Fe-O₂ bond formation.⁶⁻¹⁴ In this picture, the heme ligation site is viewed as the focal point for the interconversion of stored potential energy into motion. The mechanical response function of the protein to these forces will have a certain frequency dependence. Thus, there is also a time scale issue concerned with the protein structural changes along this reaction coordinate.

On time scales long compared to the damping of strongly coupled modes (preceding section), the atomic displacements should be considered diffusive in nature. The protein motion between local minima in its structure (conformational substates) may still involve abrupt changes; but averaged over some longer time interval, the net atomic displacements along the reaction coordinate would appear as a thermally activated Brownian process. Stated differently, in the limit of rapid energy exchange between the different degrees of freedom, there are many scattering events in the structural relaxation such that it would occur diffusively. This is the prevalent view in which the time-weighted protein motions involve diffusive displacements over dimensions of approximately one amino acid residue, i.e., localized strain.⁶ The overall structural relaxation along the reaction coordinate is constrained by the general three-dimensional structure of the protein. However, the large number of nearly degenerate conformational substates leads to a distribution of barrier heights and pathways such that the structural relaxation is highly nonexponential.^{14,25,26}

At the opposite extreme, on short time scales, the protein may respond as a semirigid body in which the motions are nondiffusive. In this limit, the reaction forces are redistributed over the entire protein structure. The nascent potential energy gradients that develop during ligand dissociation would lead to the collective displacement of a large number of atoms. In this event, the propagation of the structure changes would best be described by the displacement of a superposition of low-frequency collective modes of the protein.

This latter mechanism was proposed as an explanation of the extremely fast changes in acoustic strain (volume changes) observed for heme proteins following ligand dissociation.^{1,27,28} Using phase grating spectroscopy to monitor the protein-driven acoustics, this aspect of the protein motion was found to occur on picosecond time scales, which is consistent with highly damped motion of spatially extended modes of the globin. One of the observations that prompted this model for the protein structural relaxation is shown in Figure 3. This study shows the observed acoustic waveform for carboxymyoglobin (MbCO). (MbCO is a monomeric heme protein and is used as a model system for the tertiary structure changes.) By using CO as the ligand, the structural changes can be photoinitiated with 100% quantum yield (within ~50 fs) and with minimal

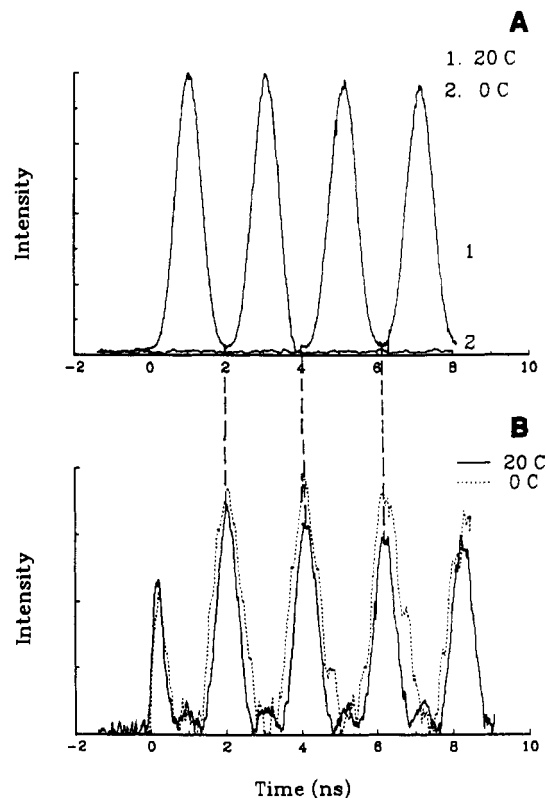


Figure 3. Picosecond phase grating studies of myoglobin. The grating was written with 532-nm pulses and probed off-resonance at 1.064 μm (100-ps pulses). (A) Deoxymyoglobin. The unligated protein does not undergo a structural change and serves as a control for a purely thermally driven acoustic response. Note the reduction of the photoacoustic waveform at 0 °C. (B) Carboxymyoglobin. The protein-driven acoustics appear 180° phase shifted relative to deoxymyoglobin, and the amplitude remains constant near the zero thermal expansion point, which demonstrates the nonthermal nature of the acoustics.

recombination to complicate the kinetics.^{9,15,23,29} The observed acoustic response to ligand dissociation is referenced to deoxymyoglobin which does not undergo structural changes and serves as a control for a pure thermally driven acoustic wave. For MbCO, there is a very rapid rise in the diffracted signal relative to the pure thermal case with nearly complete modulation of the signal. The beat frequency is the grating acoustic period which corresponds to the speed of sound in water.

This form of the acoustic signal was only observed for carboxyheme proteins undergoing structural changes. The implications were that the optically triggered tertiary structural changes were impulsively coupling to the fluid hydrodynamics. In this case, the acoustics are generated by the protein's motion and not by thermal heating. This feature of the protein-driven acoustics was verified by studies near the zero thermal expansion point of water, which eliminates acoustic generation through thermal expansion. As seen in Figure 3A, the thermally driven acoustic signal from the deoxymyoglobin control has been reduced by a factor of 140 and is barely discernible near 0 °C. In contrast, the acoustic signal generated from MbCO is essentially identical under the same conditions. This study demonstrated that the acoustics generated by the excitation of MbCO are nonthermal in nature.

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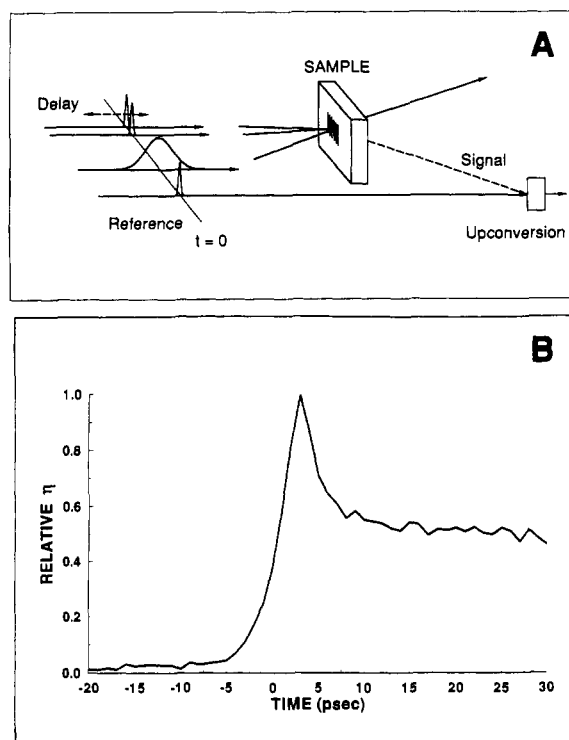


Figure 4. (A) The four-pulse grating sequence, illustrating the upconversion of the diffracted $1.053\text{-}\mu\text{m}$ probe with 580-nm pulses to attain picosecond time resolution to the grating formation dynamics seen in Figure 3. (B) MbCO dynamics. The sharp peak in the signal is due to a Kerr effect and short-lived excited-state components. The underlying rise time of the phase grating component associated with the tertiary structural changes coupling to the fluid hydrodynamics is on the order of 10 ps or less.

Additional control studies of the signal as a function of protein structure and probe wavelength also indicate that the signal originates from changes in strain of the surrounding globin which is part of the tertiary structural relaxation of the protein.²⁸

The tertiary structural changes initiate motion along the quaternary structure coordinate. One of the key questions in understanding the mechanics of this coupling is the time scale on which the tertiary structural changes are transmitted to the quaternary interface. This aspect of the protein response is related to the rise time for the protein's strain through stress/strain relationships. With the grating technique, this question can be addressed directly. Relaxation times shorter than one-tenth of an acoustic period can be sensitively detected as phase shifts in the acoustics.^{2,21} From the rise time and position of the acoustic maxima in Figure 3 and other studies, the protein strain is found to develop in less than 30 ps.²⁸ More recently, picosecond time resolution was attained by using a signal upconversion method.³⁰ This study (Figure 4) found that the phase grating component associated with the development of the protein strain has a rise time of less than 5 ps. However, there are other contributions to the signal at early times (≤ 10 ps) such that the best estimate from this study, and the phase of the acoustics, is that the strain is developing within 10 ps. This motion is

coupling to the water acoustics and would be transmitted to the quaternary interface on this same time scale.

The coupling of this motion to the fluid hydrodynamics requires a change in the protein volume (strain), i.e., a net displacement of the exterior residues of the protein relative to the residues in the interior. This motion is global in nature in the sense that the displacement must involve motion of one part of the protein relative to another to effect a volume change. Similar observations and arguments for global conformational relaxation on longer time scales have been made based on viscosity and pressure dependencies which are related to protein volume changes. These volume changes have been likened to "protein quakes".²⁶ One of the main conclusions from this work is that the largest change in protein strain occurs during the initial picosecond relaxation phase. There are also slower nanosecond relaxation components attributed to conformational relaxation which are in agreement with other studies.³¹⁻³³ However, these changes are much smaller in strain amplitude ($\leq 10\%$). The observed dynamics, for the dominant relaxation component to the protein strain is consistent with nuclear motion along the reaction coordinate occurring through the displacement of "acoustic" modes of the protein structure. These modes involve the hindered translational motion of a large number of atoms in a collective fashion.

The dynamics of the protein strain should be compared to the displacements occurring at the heme site which is the focal point for the forces driving the structural changes. This comparison is important in establishing whether or not the protein strain changes observed on the picosecond time scale are related to the primary oxy to deoxy tertiary structural changes. In this regard, transient Raman studies of the proximal histidine are key. This residue is the closest point of the protein to the heme and experiences the largest displacement with changes in tertiary structure. For myoglobin, Friedman and co-workers have shown that the proximal histidine moves to its relaxed deoxy tertiary position on at least a 10-ps time scale.¹³ The other important spectral marker at the heme site is the charge-transfer transition of the deoxyheme at 760 nm (band III). The spectral position of this band is sensitive to the displacement of the iron out of the heme plane.³⁴ The recent work of Lim et al. has shown that the motion of the iron is coupled to the protein relaxation leading to highly multiexponential dynamics.^{33,35} The largest displacement occurs within 2-4 ps, and this relaxation component is significantly reduced in amplitude (hindered) when the protein was embedded in higher viscosity glycerol/water solutions.³⁵ This condition can only occur if the iron motion is coupled to a coordinate involving translational motion of the exterior protein atoms with the displacement of solvent, i.e., a volume change. Similar viscosity dependencies have been

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observed for CO recombination on longer time scales in which Ansari et al. have discussed the protein motion as being "stuck" or hindered by the solvent coordinate.³⁶ These studies, taken together, illustrate that the protein strain and tertiary structural changes are interrelated.

The important new information from the phase grating and band III studies is that even the picosecond relaxation components involve volume changes. These observations are consistent with an *initial* collective mode response to the reaction forces. This point can be further clarified by comparing the dynamics of the proximal histidine and band III transitions to the phase grating dynamics. The probes of the proximal histidine and the iron displacement are most sensitive to changes in the vicinity of the heme site (short length scale probes), whereas the phase grating studies are sensitive to the relative motions of the exterior with respect to the interior of the protein that lead to a volume change (long length scale probe). In all cases, the time scales for these different length scales of motion are approximately the same. In order for the dynamics to be so well correlated, the atomic displacements at the exterior must be occurring at the same time as those in the interior of the protein which are at the focal point of the reaction forces. Rather than a series of sequential deformations (local strain) in a diffusive fashion, the atoms are moving together as a unit, i.e., collectively.

Further support for this concept comes from the normal mode analysis of the oxy and deoxy tertiary structures of myoglobin by Seno and Gō. This analysis found that more than 57% of the tertiary structural changes could be accounted for by the displacement of five spatially extended modes with frequencies ranging from 5 to 12 cm^{-1} .³⁷ Normal mode analyses are only relevant to short time dynamics.^{38,39} However, the important point is that the highly damped displacement of modes in the 10- cm^{-1} range would lead to correlated atomic motions on picosecond time scales. Thus, both the observed magnitude of the protein strain changes and the dynamics are consistent with this analysis.

A highly simplified diagram for the coupling of the reaction coordinate to the collective modes of the protein is shown in Figure 5 to illustrate this concept. At the reaction saddle point, the more rigid helical sections act to spatially redistribute the forces at the heme site. Those sections most strongly coupled to the heme undergo the greatest displacement (F, and to a lesser extent the A, C, E, and H helices)³⁷ relative to the less rigid corners which would serve as hinges for the motion. This net motion could be described as the superposition of spatially extended modes of the protein structure. The highly damped segmental displacement of the helical sections of the protein would act as a very efficient conduit for transferring forces from the heme site to the quaternary interface and may be a general feature in protein architecture.^{1,27,37}

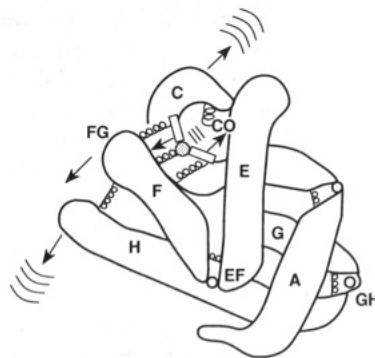


Figure 5. Schematic representation of the mechanics of the initial collective phase of the protein response. The dissociation of the CO leads to distributed forces that displace the more rigid helical sections of the surrounding globin that are most strongly interacting with the heme site. These damped collective motions would act as a very efficient mechanism for propagating the system toward the global minimum of the deoxy tertiary structure. This nondiffusive motion would be similar for all conformational substates at the barrier crossing point. Subsequent conformational relaxation leads to the fully equilibrated distribution.

Energetics: The Driving Force for Protein Motions

From the above discussion, the nuclear motion along the reaction coordinate toward the deoxy tertiary structure involves both coupling to low-frequency modes of the protein and conformational redistribution. Relative to the reaction saddle point, each different process has a certain amount of relaxation energy associated with it. The interconversion of stored potential energy in the protein structure in going from the oxy to deoxy tertiary structure is an important factor in the mechanism of allosteric regulation of hemoglobin. The question is, On what time scale does the protein access this stored potential energy? This question can be addressed by directly determining the dynamics of the energy relaxation process.

For the optically prepared reaction conditions, the nuclear configuration of the protein system is displaced from the thermally accessed transition-state region. However, the dynamics of the protein response will mimic the thermally sampled reaction coordinate.^{38,39} The relaxation energy from this nonequilibrium configuration can be determined for each component to determine the relative importance of the different phases in the protein motion. In the energy balance of the absorbed photon energy, the dissipated energy from the heme protein will involve the energy in excess of the Fe-CO bond energy and the relaxation energy of the protein structure in going from the oxy to deoxy tertiary structure. In order to be credited to a relaxation process, the energy must be transferred to the aqueous bath. This dissipation process takes on the order of 10–20 ps (see above), which represents the upper limit to following bioenergetics. Any slower relaxation processes involving the protein structural changes can be resolved within this time resolution.

The phase grating method is capable of following the energetics with high time resolution by monitoring the thermal component to the acoustics. However, as can be appreciated from Figure 3, the protein-driven acoustic waves dominate the signal. When the solvent system is changed to 75% glycerol/water, the thermal

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expansion coefficient increases by approximately an order of magnitude and the thermal component can be made to dominate the signal. Using the deoxyheme protein as a reference for complete nonradiative relaxation, thermal phase grating measurements indicated that the photodissociation of CO is endothermic by 19 ± 2 kcal/mol and 14 ± 3 kcal/mol for MbCO and HbCO, respectively. These determinations include a correction factor for the protein acoustic contributions.⁴⁰

These numbers should be compared to determinations made using conventional calorimetry for the reaction enthalpies. Values for the reaction of MbCO to the fully relaxed Mb range between 16 and 18 kcal/mol (with aqueous CO solvation correction).^{41,42} Studies of the enthalpy change from the fully ligated HbCO(R) to fully relaxed deoxy-Hb(T) found enthalpy changes of approximately 15 kcal/mol.⁴² These values are within experimental error of the picosecond determinations.²⁸ The importance of this comparison is that within the sensitivity of the thermal phase grating studies the protein appears to have fully relaxed energetically within 200 ps. These results support the concept that the protein accesses most of the stored potential energy in its structure on a very fast time scale. These values are also similar to those of related photoacoustic studies, but there are differences that may reflect energetically significant slower relaxation processes that are related to ligand escape.^{43,44}

The above thermal grating studies give the time dependence of the reaction enthalpy. Overall, one has to consider the reaction free energy as the driving force for the protein motion. Within the present resolution, it appears that the dominant enthalpic contributions to the reaction coordinate are accessed during the initial phase of the protein relaxation. In this case, the slower conformational relaxation processes contribute primarily through the entropic terms to the reaction free energy as predicted earlier by Austin et al.⁴⁵

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Summary

Proteins are the medium for chemical reactions in biological systems. With this view, the nuclear motion along a reaction coordinate depends on the frequency distribution of the nuclear degrees of freedom coupled to the reaction coordinate and also the energy relaxation processes of the surrounding medium. For highly associated systems such as proteins, this response function is highly nonexponential with relaxation times ranging from picoseconds to microseconds and longer. This review has focused on the initial motions along the reaction coordinate. The correlation in dynamics for probes sensitive to different length scales of protein motion, as well as the acoustic nature of the protein response to ligand dissociation, provides evidence that the *initial* picosecond components to the motion involve the displacement of the low-frequency skeletal modes of the protein structure. This is an interesting observation as a damped collective displacement of atoms represents the most efficient mechanism possible for propagating functionally important motions of the protein. This mechanism would have the effect of minimizing the total configuration phase space the system would have to sample to relax toward the final equilibrium structure.

In terms of making a connection between the tertiary structural changes and the quaternary changes, i.e., protein functionality, the coupling of the reaction forces to the spatially extended modes of the protein would transfer the forces from the heme site to the interface at the speed of sound. This represents a very efficient mechanism in which the tertiary structural changes and state of ligation modulate the force balance at the quaternary interface. The subsequent development of strain along the quaternary coordinate in response to these forces occurs on much longer time scales^{29,46} such that this phase of the protein motion is just one facet of the overall structural relaxation. The evidence for the coupling of the protein's low-frequency collective modes to the reaction coordinate, however, provides new insight into understanding how the action of a stimulus can direct functionally relevant motions at remote sites within the protein.

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