Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5908–5912, August 1988
Biophysics

Protein dynamics and reaction rates: Mode-specific chemistry in large molecules?

WILLIAM BIALEK* AND JOSÉ NELSON ONUCHIC†‡

*Departments of Physics and Biophysics, University of California at Berkeley, Berkeley, CA 94720; and †Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125

Communicated by John J. Hopfield, February 16, 1988

ABSTRACT **Reactive events in proteins may be strongly coupled to a few specific modes of protein motion or they may couple nonspecifically to the dense continuum of protein and solvent modes. We summarize the evidence that at least some biologically important reactions can be described in terms of a few specific modes, and we propose experiments to quantify the strength of coupling to the continuum. We also show that large entropic effects—solvent ordering, for example—can be rigorously incorporated in few-mode models without losing mode specificity. Within our description, the dynamics that determine chemical reaction rates can be summarized by a small number of parameters directly related to spectroscopic and thermodynamic data. Mode specificity allows protein dynamics to contribute directly to the control and specificity of biochemical reaction rates.**

Proteins are complicated systems with thousands of interacting degrees of freedom; still more degrees of freedom describe the solvent that surrounds the protein. Are the dynamics of a biochemical reaction in the interior of such a molecule shared more or less equally among these many degrees of freedom, or is the reactive event dominated by a few well-defined modes of motion? If only a few modes are important, it is likely that we can construct a relatively simple dynamical theory of reaction rates in proteins. More importantly, if reaction rates are determined by the properties of particular modes of motion in the protein, then substantial control over reaction rates may be achieved through relatively small changes in these dynamical properties.

In the past 15 years, a number of experiments on biomolecules—particularly the temperature dependence of photosynthetic electron transfer reactions—have been successfully interpreted in terms of simple dynamical models, and to some extent predictions from these models have been verified by independent experiments (1). A key feature of these models is in fact the assumption that reaction dynamics are dominated by a small number of vibrational modes in the protein and only weakly coupled to the continuous smear of remaining modes in the protein and solvent. In view of these results, the hypothesis of mode-specific chemistry in proteins seems especially attractive. There are, however, two very serious objections to this idea: (*i*) Numerical simulations (2) and inelastic neutron scattering experiments (3) both indicate that the modes of a protein as a whole form a dense continuous spectrum; (*ii*) biochemical reactions are often accompanied by large entropy changes that cannot be accounted for by changes in the dynamics of just a few modes (4).

In this paper, we show that the relative importance of specific modes and the dense continuum can be directly measured both in kinetic experiments and in spectroscopies directed at the protein active site, and that large entropic effects can be rigorously incorporated in simple models without sacrificing the idea that a few specific modes dominate the reactive dynamics. We also consider the ways in which the hypothesis of mode specificity could be tested in molecular dynamics simulations.

Looking for the Continuum

Much of the interest in dynamical models for reaction rates in proteins was sparked by the observation of DeVault and Chance (5) that one of the electron transfer reactions in the photosynthetic bacterium *Chromatium vinosum* exhibits classical Arrhenius behavior around room temperature but the transfer rate becomes temperature independent below 200 K. Similar behavior has been observed in the semibiological electron transfer reactions of Zn-substituted hemoglobin (6), and also in the binding of small ligands to heme proteins (7), where the crossover is at much lower temperatures; none of the primary electron transfer reactions in bacterial photosynthesis display Arrhenius behavior even near 300 K (8–10). The explanation (11) of temperature-independent reaction rates is based on the idea that motion from reactants to products occurs along a specific vibrational mode of the molecule with frequency Ω. For temperatures $T << \hbar\Omega/k_B$, this mode is frozen in its quantum mechanical ground state and the reaction occurs only by tunneling out of this state, so that there is no temperature dependence; the rate becomes thermally activated only once the temperature is high enough to populate the first excited vibrational level. To account for the DeVault–Chance data, one requires $\hbar\Omega \approx 200\ \text{cm}^{-1}$, for example, which is a reasonable value for iron–histidine vibrational frequencies in the cytochrome *c* molecule, which acts as donor in this reaction.

It is clear that if a reaction is coupled uniformly to a dense continuum of modes then an abrupt crossover to temperature independence at low T will not be observed. Quantitatively, if we have a specific mode then the rate varies as $k(T) = k(0) + B\exp\{-\hbar\Omega/k_BT\}$ at low temperatures, so the corrections to T independence are exponentially small. We shall see that with a continuum $k(T) = k(0) + C(k_BT/\hbar\Omega)^n$, so that the approach to T independence is only gradual. What is important is that C measures the strength of coupling to the continuum and n measures the effective density of continuum modes at low frequencies. By searching for this power-law behavior, one can thus quantify the extent to which a given reaction is affected by the continuum (12, 13).

To make these considerations precise, we study the model schematized in Fig. 1. We have two electronic states representing reactants and products and a single vibrational mode of frequency Ω. This mode interacts with all the other modes in the system and thus mixes with the continuum. The vibra-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‡On leave of absence from the Instituto de Física e Química de São Carlos, Universidade de São Paulo, 13560, São Carlos, SP, Brazil.

tional energy of the specific mode (coordinate Q) and the continuum (coordinates x_i) together is

$$H_{vib} = \frac{1}{2}\left[\left(\frac{dQ}{dt}\right)^2 + \Omega^2(Q - Q_0)^2\right] + \frac{1}{2}\sum_i \left[\left(\frac{dx_i}{dt}\right)^2 + \omega_i^2(x_i - g_iQ)^2\right], \quad [1]$$

where Q_0 is the equilibrium position of the specific mode—which will be different in the reactant and product states—and g_i measures the amount by which continuum mode i "wants" to move in response to displacements of the specific mode; g_i can also be viewed as determining the force the continuum modes apply to the specific mode. It is clear that Eq. 1 is not the most general possible description of interaction with the continuum; in particular, it must be extended if we are to account for entropic effects, as discussed below. In the limit that interactions between the specific mode and any single mode of the continuum are small, however, Eq. 1 gives a correct description of the dynamics even when the total mode–continuum interaction is strong (14).

If we were to apply a time-dependent force $F(t)$ to the molecular coordinate Q we would find that $Q(t)$ obeys a classical equation of motion

$$\frac{d^2Q(t)}{dt^2} + \int dt' \int \frac{d\omega}{2\pi} e^{-i\omega(t-t')}\gamma(\omega)\frac{dQ(t')}{dt'} + \Omega^2[Q(t) - Q_0] = F(t), \quad [2]$$

where $\gamma(\omega) = (\pi/2)\Sigma\,(g_i\omega_i)^2\delta(\omega - \omega_i)$, which is a smooth function of ω in the limit that the modes ω_i form a dense continuum. Eq. 2 is just the equation of motion for a damped harmonic oscillator with frequency-dependent damping $\gamma(\omega)$. The familiar example on which we will concentrate is the "Ohmic" limit of $\gamma(\omega) \approx \gamma$, a constant, that describes damping proportional to the instantaneous velocity $dQ(t)/dt$ and corresponds to a continuum with a nonzero density of states at zero frequency.

To complete our model we must add to H_{vib} the energy of the electronic states. These states are separated by an energy gap or driving force ε for the reaction and connected by a small matrix element V, which, for example, measures the frequency at which an electron can hop between donor and acceptor sites in an electron transfer reaction. This model is the simplest member of a whole family of models whose dynamics have been studied by a variety of techniques (15–17). Here we phrase our arguments in semiclassical terms, and although this is only an approximation to the exact reaction rates predicted by the model, it is sufficient to capture the important effects of coupling to the continuum. An important caveat is that some reactions may be dominated by coupling to high frequency localized vibrational modes, such as the carbonyl stretching modes of quinones in the photosynthetic reaction center (9). In these cases, a semiclassical approach is not sufficient, and the temperature dependence of the rate constant may present anomalies distinct from the behavior discussed here. These issues will be discussed elsewhere.

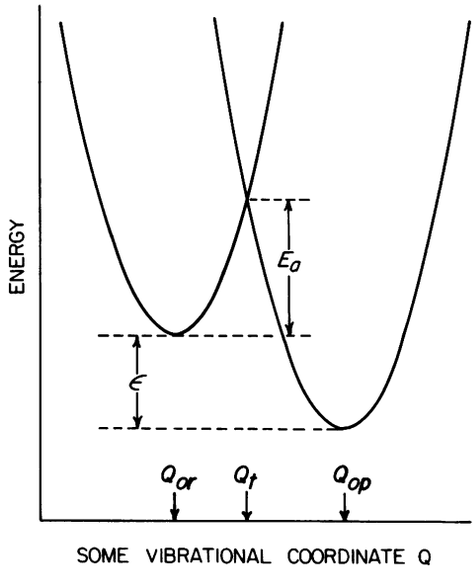

FIG. 1. Potential energy surfaces for the reactant and product states. Q_{0r} and Q_{0p} are the equilibrium positions for the reactants and products, respectively. E_a is the classical activation energy, and ε is the energy gap or driving force for the reaction.

In a semiclassical picture, the transition between reactant and product states can occur only at those instants in time when the potential energies of the two states are equal: In terms of Fig. 1, only when the vibrational coordinate Q is at the point Q_t, where the energy surfaces cross. But for the energy function in Eq. 1, the probability distribution of Q is predicted—both in classical mechanics and in quantum mechanics—to be Gaussian. Thus, we expect that the rate of the reaction is proportional to the probability that $Q = Q_t$, or

$$k(T) = \frac{stuff}{\sqrt{2\pi\langle(\delta Q)^2\rangle}} \exp[-(Q_t - Q_{0r})^2/2\langle(\delta Q)^2\rangle], \quad [3]$$

where Q_{0r} is the equilibrium position of coordinate Q in the reactants state, *stuff* is temperature independent, and $\langle(\delta Q)^2\rangle$ is the temperature-dependent variance in the coordinate Q. These mean-square fluctuations are determined from statistical mechanics (18) to be

$$\langle(\delta Q)^2\rangle = \hbar\int \frac{d\omega}{2\pi}\coth(\hbar\omega/2k_BT)\,\frac{\gamma\omega}{(\omega^2 - \Omega^2)^2 + (\gamma\omega)^2}. \quad [4]$$

At high temperatures, $T >> \hbar\Omega/k_B$, we have $\langle(\delta Q)^2\rangle = k_BT/\Omega^2$ and we recover the Arrhenius law $k(T) = A \exp\{-E_a/k_BT\}$, with the activation energy $E_a = \Omega^2(Q_t - Q_0)^2/2$. At low temperatures we find, after some calculation,

$$\langle(\delta Q)^2\rangle = \frac{\hbar}{2\Omega}\left[1 + 2e^{-\hbar\Omega/k_BT} - \frac{1}{\pi}\cdot\frac{\gamma}{\Omega} + \frac{2\pi}{3}\cdot\frac{\gamma}{\Omega}\cdot\left(\frac{k_BT}{\hbar\Omega}\right)^2 + \ldots\right], \quad [5]$$

where we are neglecting terms with higher powers of T and γ. Here the leading term is proportional to $(k_BT/\hbar\Omega)^2$ because we are using an Ohmic form for $\gamma(\omega)$; more generally, we find $(k_BT/\hbar\Omega)^{n+2}$ if $\gamma(\omega)$ is proportional to ω^n at low frequencies.

To obtain the temperature dependence of the rate at low temperatures, we simply combine Eqs. 3 and 5 and identify the activation energy as above:

$$\ln k(T << \hbar\Omega/k_B) = \ln k(T = 0) + \left[\frac{2E_a}{\hbar\Omega} - \frac{1}{2}\right]\left[\frac{2\pi}{3}\cdot\frac{\gamma}{\Omega}\cdot\left(\frac{k_BT}{\hbar\Omega}\right)^2 + 2e^{-\hbar\Omega/k_BT} + \ldots\right]. \quad [6]$$

The magnitude of the power-law correction to T independence can be estimated for the DeVault–Chance reaction, where (1) $E_a = 0.18$ eV and $\hbar\Omega \approx 200$ cm^{-1}. This gives $\ln[k(T)/k(T = 0)] \approx 4(\gamma/\Omega)(T/100\text{ K})^2$, which means that even if $\gamma/\Omega = 0.01$—so that the coordinate Q can undergo $\Omega/2\pi\gamma \approx 15$ oscillations at frequency Ω before equilibrating with the continuum—one should be able to observe a 4%

effect on $k(T)$. Recent experiments on photosynthetic electron transfer demonstrate that few percent effects can be measured with remarkable accuracy (19).

In some cases, it is possible to directly measure $\langle(\delta Q)^2\rangle$, with Q the position of an atom in the active site. If some isotope of this atom exhibits the Mössbauer effect, then the integrated intensity of the Mössbauer spectrum determines the mean-square displacement of this atom through the Debye–Waller factor. Wise *et al.* (20) have found that the temperature dependence of $\langle(\delta Q)^2\rangle$ for the iron atom at the active site of carbon monoxide myoglobin is described from 2 to 180 K by coupling to a single mode at 25 cm^{-1}. Measurements of the mean-square velocity of the iron atom are also possible with the Mössbauer technique, and these data (taken up to 250 K) reveal an additional mode at 190 cm^{-1}; similar results are obtained for oxymyoglobin. From these data and the expressions above, one finds that power-law deviations from the predictions for an undamped 25-cm^{-1} mode are $20(\gamma/\Omega)$ % at 10 K. Certainly the qualitative appearance of the data demand $\gamma/\Omega < 1$, and it should be possible to provide a more stringent bound.

Just as the Mössbauer effect is sensitive only to the motion of a single atom in the active site, extended x-ray absorption fine structure spectra are sensitive only to relative motions of a single atom and its near-neighbors. Ordinarily, estimates of Debye–Waller factors from such data are confounded by energy-dependent scattering amplitudes, which can only be approximately determined from model compounds. As emphasized by Stern and co-workers (21), measurements of the temperature dependence of the Debye–Waller factors do not suffer from this problem, since the $T = 0$ spectrum serves as the standard for interpreting the spectra at $T \neq 0$. This approach has been used to detect large changes in mean-square displacements with temperature and ligation of hemerythrin (21), and it may also be possible to detect the smaller effects discussed here, or at least to place significant bounds on the strength of coupling to the continuum.

Let us suppose that the specific mode Q is not quite a simple harmonic oscillator, as in Eq. 1, but rather has some mild anharmonicities. Then as the temperature is increased the mean value of the coordinate will change—thermal expansion or contraction—and the fluctuation in Q will occur not at the "bare" frequency Ω but at some effective T-dependent frequency $\Omega_{eff}(T)$. This latter effect is observable as T-dependent shifts in infrared or Raman spectral lines. For any weakly anharmonic potential, it can be shown that

$$\Omega_{eff}(T) = \Omega[1 - (\Omega^2/4V_0)\langle(\delta Q)^2\rangle + \ldots], \qquad [7]$$

where V_0 measures the energy scale on which the potential becomes significantly anharmonic (the dissociation energy of a Morse potential, for example), and . . . denotes terms of higher order in the strength of the anharmonicity and/or in powers of γ/Ω. As before, if the mode Q were undamped this would result in exponentially small T dependence of $\Omega_{eff}(T)$ at low T, but coupling to the continuum produces power-law corrections determined by $\langle(\delta Q)^2\rangle$.

For the iron–histidine stretching mode of myoglobin ($\hbar\Omega$ = 220 cm^{-1} at 300 K), J. Friedman (personal communication) has found that the effective frequency shifts by ≈5% between 2 and 300 K. This determines $V_0 \approx 1000$ cm^{-1}, so that power-law corrections are $\approx(\gamma/\Omega)(T/100\ K)^2$ cm^{-1}. Careful Raman-difference measurements can reveal frequency shifts much smaller than 1 cm^{-1} (22), which would translate into significant bounds if not actually a direct estimate of γ/Ω. Note that by detecting temperature-dependent shifts of the spectrum, one can infer values for the natural linewidth γ that are much smaller than the inhomogeneous width of the observed spectral line.

Entropy

Our discussion thus far has emphasized effects of the continuum that can be seen at very low temperatures where we expect that entropic effects are negligible. At room temperature, however, we know that many biochemical reactions are accompanied by large entropy changes. In many discussions of reaction rate theory (ref. 23, for example), one finds that entropy has been included by drawing surfaces as in Fig. 1, but with the assumption that these are free energy surfaces rather than potential energy surfaces. The problem with this idea is that the free energy is an average thermodynamic property of an ensemble of molecules, and hence it does not determine the forces acting on a single molecule in the sense of ordinary mechanics. Molecular dynamics are determined by the potential energy surface, and the free energy is a quantity one can calculate by averaging over many dynamical trajectories.

The notion of free energy surfaces can be made precise by fixing our specific mode at some coordinate Q, allowing all other degrees of freedom to equilibrate, then changing Q and repeating the procedure. In this way, one traces out surfaces of free energy $F(Q)$ for the "other" degrees of freedom as a function of the specific mode coordinate, or "reaction coordinate" in conventional rate theories. This procedure is essentially a description of the "umbrella sampling" strategy in molecular dynamics simulations, and indeed this approach has been used to determine effective free energy surfaces in proteins (ref. 24, for example). The free energy surface obtained in this way is well-defined, but what we need to know is whether this picture has anything to do with the *dynamics* of the reaction.

Intuitively, we expect that if the dynamics of the specific mode are very slow, then the other degrees of freedom will actually have time to equilibrate before Q changes significantly. This means that at least a subset of the many degrees of freedom in the problem can be characterized by a free energy that varies in time as Q varies. This is the analog of the usual Born–Oppenheimer approximation in molecules—the time scales of motion for the electrons are much faster than those for motion of the atoms, and hence we describe atomic motion as occurring on a potential surface that includes the instantaneous energy of the electrons. What is different from the Born–Oppenheimer case is that we usually talk about electrons in a particular quantum state, so that there is no entropy and no real distinction between energy and free energy.

If we have a slow specific mode interacting with a collection of fast modes, then at zero temperature we can perform the standard Born–Oppenheimer approximation to show that the slow mode sees an effective potential energy determined in part by the properties of the fast modes. This contribution to the effective potential is exactly that obtained by calculating the ground-state energy of the fast degrees of freedom while treating the slow coordinate as a parameter. If one carries through the same calculation at nonzero temperature, one finds again that the slow mode moves in an effective potential, but now this effective potential is determined by the free energy of the fast modes at each value of the slow coordinate. The condition for the validity of this generalized Born–Oppenheimer approximation is that the fast modes come to thermal equilibrium with their environment on a time scale faster than that characteristic of motion along the specific slow mode. These results are formalized in the *Appendix*.

The notion of dynamics on a free energy surface is familiar in several contexts. In ordinary macroscopic physics, we are accustomed to defining an effective potential energy for stretching a rubber band, which is in fact a free energy, dominated by entropic effects; this is a case where the separation

of time scales referred to above is obvious—the specific mode (stretching of the band) is orders of magnitude slower than any internal relaxation of the rubber polymers. In molecular dynamics, one makes use of the "potential of mean force," which is essentially equivalent to effective potential defined here, although the conditions for applicability of this concept are sometimes not made clear. Finally, in quantum field theory (particularly as applied to cosmology), one studies temperature-dependent effective potentials for slowly varying fields (25), although the fact that these potentials include entropic components is usually not emphasized.

For our purposes, there are two crucial points to be taken from this theoretical discussion and its formalization. First, if there is no separation of time scales then there is no justifiable notion of dynamics on a free energy surface, and hence there is no rigorous one-dimensional picture one can draw of free energy vs. reaction coordinate, no matter how complex one allows this picture to become. Second, if there is a separation of time scales—that is, if there are specific slow protein modes of interest—then one can think about the dynamics on the slow time scale in terms of motion on a possibly rather simple effective potential surface without regard for the complex dynamics on shorter time scales, which determine the shape of this surface.

To emphasize the second, more positive, point, consider making a microscopic model that expresses these ideas. As an example, one could couple the specific mode Q to a collection of rigid rotors—perhaps modeling the tumbling of solvent molecules—so that as the protein breathes, changing Q, the barrier to rotation changes. This corresponds to our usual notion of changes in the degree of solvent ordering in response to structural changes of the protein, and if the rotational relaxation time of the relevant solvent molecules is faster than the frequency of motion along Q then our "generalized Born–Oppenheimer" approximation will give a correct description of the dynamics. We emphasize that such models are necessarily more complex than Eq. **1**, but unless one actually wants to explain the origin of the entropic effects it is not necessary to keep track of all this complexity.

The fact that the dynamics of a slow specific coordinate can be rigorously described as motion on a free energy surface obtained by averaging over rapidly relaxing degrees of freedom in the protein and solvent means that a model for reaction dynamics with even one slow specific mode can nonetheless include all of the entropic effects associated with faster motions. As a result, the energy gap of Fig. 1 can be taken as a free energy gap, the spectroscopically observable vibrational frequencies and structural changes of the coordinate Q are related to changes in free energy, and so on. We thus conclude that there is no conceptual barrier to describing complex reactive events in proteins in terms of a few critical degrees of freedom, as is in fact suggested by the experiments discussed above.

Conclusions

The basic ideas of this paper are summarized schematically in Fig. 2. The dynamics of a protein that are relevant in determining chemical reaction rates can be divided into groups based on time scales. (*i*) There are specific low frequency motions of the protein, whose coupling to reactive events can apparently be resolved above the continuum background. (*ii*) The continuous background of modes provided by the vast majority of protein and solvent motion can be divided into slow degrees of freedom, which damp the specific protein modes, and intermediate modes, which convert the potential energy surface of the specific modes into a free energy surface. (*iii*) There are very high frequency, localized motions, such as stretching of a bond between two light atoms, which are almost certainly well isolated from the continuum and must be described in quantum mechanical terms.

From a conceptual standpoint, the greatest simplification we have introduced is the rigorous incorporation of entropic effects without having to make an explicit model of the rather complex many-body dynamics that give rise to these effects. It is precisely because the origins of entropic effects are complex that Fig. 2 can only be schematic. In particular, the frequencies assigned to the "intermediate modes" are intended only as qualitative indicators of the time scales for motion in these modes—there is nothing about our arguments that assumes true vibrational (harmonic or quasi-harmonic) motion in this intermediate regime. From an experimental standpoint, we have shown how the degree of specificity in protein dynamics can be measured by several techniques, all of which focus on the dynamics of the protein active site and on those dynamics that actually couple to the reactive event.

It would clearly be very attractive if we could test the schematic Fig. 2 by direct molecular dynamics simulation. In principle this is straightforward: We study the dynamics along some important reaction coordinate and examine carefully the time dependencies to see whether specific low frequency modes emerge. There are certainly examples in the literature where the spectral properties of different generalized coordinates have been measured in molecular dynamics simulations, and there is at least one case in which the presence of a resolved specific mode is sensitive to the functional state of the molecule (26). It is more difficult, of course, to choose a coordinate that is clearly the reaction coordinate for some interesting chemical event. In the case of electron transfer, one can meaningfully look at the energy difference between oxidized and reduced species, and measurements of the spectral characteristics of this quantity in molecular dynamics simulations of cytochrome *c* gave results remarkably similar to our Fig. 2 (27). All of these studies sample only configurations that are easily accessible through thermal fluctuations on the picosecond time scale, so we do not know whether these apparently resolvable modes retain their identity as the molecule evolves from reactant to product. To conclusively test the hypothesis of mode specificity in molecular dynamics simulations would require a new technique that combines the advantages of conventional molecular dynamics (temporal/spectral information) with those of umbrella sampling (access to unlikely configurations).

Finally, we consider the relationship of mode specificity to the control of biochemical reaction rates. We emphasize that mode specificity does not necessarily imply some exotic dynamical effects on the rate constant; in particular, it may still be possible to think about reaction rates in a semiclassical transition state picture even with such specificity. There are, however, two important consequences of mode specificity in protein-mediated reactions. First, the importance of

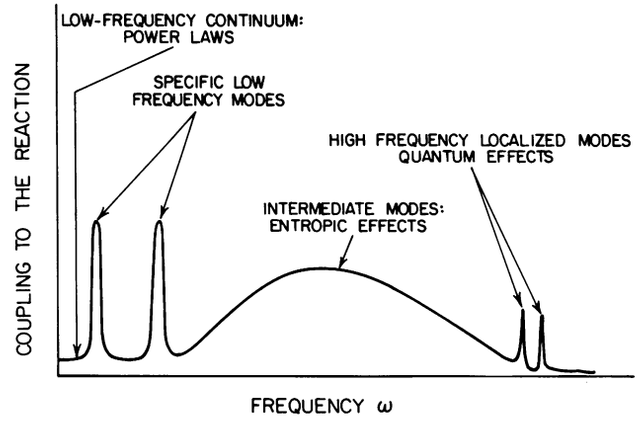

FIG. 2. Schematic representation of protein and solvent degrees of freedom as they couple to reaction dynamics.

a few specific modes makes it possible to construct relatively simple dynamical models for the reactive event within which different approximate concepts (such as the transition state) can be more rigorously tested, as we have considered in some detail for the case of electron transfer (W.B., R. F. Goldstein, and J.N.O., unpublished work). Second, even in the absence of a detailed dynamical theory of the rate constant, mode specificity implies a correspondence between spectroscopically observable dynamics of the protein as a whole and the dynamics along some "reaction coordinate" for a biochemical event of interest. This possibility of thinking intuitively about the effects of the protein on a reaction in terms of just a few spectrally identified degrees of freedom is to us the most appealing consequence of mode specificity.

Appendix

We are interested in studying the dynamics of a single coordinate Q interacting with a large number of other coordinates $\{y_a\}$, which we shall assume relax rapidly compared to any motion of Q. All of the dynamics of Q at nonzero temperature can be reconstructed (28) from the imaginary-time correlation functions, which are in turn determined by the generating functional ($\hbar = 1$)

$$Z[J(\tau)] = \int DQ \prod_\alpha Dy_\alpha \exp\left\{-S[Q(\tau); \{y_\alpha(\tau)\}] - \int_0^\beta d\tau\, J(\tau)Q(\tau)\right\}, \quad \text{[A1]}$$

with the action

$$S[Q(\tau); \{y_\alpha(\tau)\}] = S_0[Q(\tau)] + S_{\text{bath}}[\{y_\alpha(\tau)\}] + \int_0^\beta d\tau V_{\text{int}}[Q(\tau); \{y_\alpha(\tau)\}], \quad \text{[A2]}$$

where we have included some arbitrary interaction potential V_{int} between Q and the bath; note that the functional $S_0[Q(\tau)]$ depends on both Q and its time derivatives, while the interaction potential depends only on Q—actions can be nonlocal in time, but potentials are local in time. We can formally integrate over the functions $y_\alpha(\tau)$ to find that the remaining integral over $Q(\tau)$ is determined by an effective action

$$S_{\text{eff}}[Q(\tau)] = S_0[Q(\tau)] + \int_0^\beta d\tau \langle V_{\text{int}}[Q(\tau); \{y_\alpha(\tau)\}]\rangle^{\text{bath}} - \int_0^\beta d\tau \int_0^\beta d\tau' \langle V_{\text{int}}[Q(\tau); \{y_\alpha(\tau)\}] V_{\text{int}}[Q(\tau'); \{y_\alpha(\tau')\}]\rangle_c^{\text{bath}} + \ldots, \quad \text{[A3]}$$

where $\langle \ldots \rangle^{\text{bath}}$ means that we average over bath coordinates $\{y_\alpha(\tau)\}$ as if they did not interact with $Q(\tau)$, and $\langle \ldots \rangle_c$ means that we keep only connected averages, or cumulants of V_{int} rather than moments. The assumption that $Q(\tau)$ moves slowly is equivalent to the statement that $Q(\tau)$ may be taken as a constant in each of the time integrals of Eq. A3. The result is that

$$S_{\text{eff}}[Q(\tau)] = S_0[Q(\tau)] + \int_0^\beta d\tau V_1[Q(\tau)] + \int_0^\beta d\tau V_2[Q(\tau)] + \ldots, \quad \text{[A4]}$$

with the effective potentials

$$V_1[Q] = \langle V_{\text{int}}[Q; \{y_\alpha(\tau)\}]\rangle^{\text{bath}},$$

$$V_2[Q] = -\int_0^\beta d\tau \langle V_{\text{int}}[Q; \{y_\alpha(\tau)\}] V_{\text{int}}[Q; \{y_\alpha(0)\}]\rangle_c^{\text{bath}}, \quad \text{[A5]}$$

and so on. Note that $V_1[Q] + V_2[Q] + \ldots$ acts exactly as a term in the potential energy for the coordinate Q. These terms can be compared with those in the corresponding perturbation series for the free energy of the bath at fixed Q, which is determined from the functional

$$e^{-\beta F(Q)} = \int \prod_\alpha Dy_\alpha(\tau) \exp\left\{-S_{\text{bath}}[\{y_\alpha(\tau)\}] - \int_0^\beta d\tau V_{\text{int}}[Q; \{y_\alpha(\tau)\}]\right\}. \quad \text{[A6]}$$

Expanding as before, we have

$$\beta F(Q) = \int_0^\beta d\tau \langle V_{\text{int}}[Q; \{y_\alpha(\tau)\}]\rangle^{\text{bath}} - \int_0^\beta d\tau \int_0^\beta d\tau' \langle V_{\text{int}}[Q; \{y_\alpha(\tau)\}] V_{\text{int}}[Q; \{y_\alpha(\tau')\}]\rangle_c^{\text{bath}} + \ldots, \quad \text{[A7]}$$

where we neglect Q-independent terms. We now note that all equilibrium correlation functions of the $\{y_\alpha(\tau)\}$ must be stationary, so that they are functions only of time differences. This allows a factor of β to be removed from each of the terms in Eq. A7, and we are left with $F(Q) = V_1[Q] + V_2[Q] + \ldots$, as promised.

We thank P. Debrunner, J. Friedman, and E. Stern for teaching us about the experiments discussed above, and J. J. Hopfield for his comments on the manuscript. This work was supported in part by the Miller Institute for Basic Research, by the U.S. Public Health Service through a Biomedical Research Support Grant, by the Brazilian agency CNPq, and by the National Science Foundation through Grant PCM84-06049 and a Presidential Young Investigator Award.

1. Goldstein, R. F. & Bialek, W. (1986) *Comments Mol. Cell. Biophys.* **5**, 407–438.
2. Brooks, B. & Karplus, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6571–6575.
3. Middendorf, H. D. (1984) *Annu. Rev. Biophys. Bioeng.* **13**, 425–451.
4. Sturtevant, J. M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2236–2240.
5. DeVault, D. & Chance, B. (1966) *Biophys. J.* **6**, 825–847.
6. Peterson-Kennedy, S. E., McGourty, J. L., Kalweit, G. A. & Hoffman, B. M. (1986) *J. Am. Chem. Soc.* **108**, 1739–1746.
7. Alberding, N., Beeson, K. W., Chan, S. S., Eisenstein, L., Frauenfelder, H. & Nordlund, T. M. (1976) *Science* **192**, 1002–1004.
8. Kiramaier, C., Holton, D. & Parson, W. W. (1985) *Biochim. Biophys. Acta* **810**, 33–48.
9. Gunner, M. R., Robertson, D. E. & Dutton, P. L. (1986) *J. Phys. Chem.* **90**, 3783–3795.
10. Blankenship, R. E. & Parson, W. W. (1979) in *Photosynthesis in Relation to Model Systems*, ed. Barber, J. (Elsevier, Amsterdam), pp. 71–114.
11. Hopfield, J. J. (1974) *Proc. Natl. Acad. Sci USA* **71**, 3640–3644.
12. Larkin, A. I. & Ovchinikov, Yu. N. (1983) *J. Exp. Theor. Phys. Lett.* **37**, 382–385.
13. Grabert, H., Weiss, U. & Hanggi, P. (1984) *Phys. Rev. Lett.* **52**, 2193–2196.
14. Caldeira, A. O. & Leggett, A. J. (1983) *Ann. Phys. (Paris)* **149**, 374–456.
15. Leggett, A. J., Chakravarty, S., Dorsey, A., Fisher, M. P. A., Garg, A. & Zwerger, W. (1987) *Rev. Mod. Phys.* **59**, 1–86.
16. Bialek, W. & Goldstein, R. F. (1986) *Phys. Scr.* **34**, 273–282.
17. Garg, A., Onuchic, J. N. & Ambegaokar, V. (1985) *J. Chem. Phys.* **83**, 4491–4503.
18. Landau, L. D. & Lifshitz, E. M. (1977) *Statistical Physics* (Pergamon, Oxford).
19. Okamura, M. Y. & Feher, G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8152–8156.
20. Wise, W. W., Wagner, G. C. & Debrunner, P. G. (1987) in *Protein Structure: Molecular and Electronic Reactivity*, eds. Austin, R., Buhks, E., Chance, B., DeVault, D., Dutton, P. L., Frauenfelder, H. & Gol'danskii, V. I. (Springer, Berlin), pp. 509–510.
21. Elam, W. T., Stern, E. A., McCallum, J. D. & Sanders-Loehr, J. (1983) *J. Am. Chem. Soc.* **105**, 1919–1923.
22. Rousseau, D. L. (1981) *J. Raman Spectrosc.* **10**, 94–99.
23. Marcus, R. A. (1984) *J. Chem. Phys.* **81**, 4494–4500.
24. Hwang, J. & Warshel, A. (1987) *J. Am. Chem. Soc.* **109**, 715–720.
25. Brandenberger, R. H. (1985) *Rev. Mod. Phys.* **57**, 1–60.
26. Aqvist, J., Sandblom, P., Jones, T. A., Newcomer, M. E., van Gunsteren, W. F. & Tapia, O. (1986) *J. Mol. Biol.* **192**, 593–604.
27. Churg, A. K. & Warshel, A. (1985) in *Structure and Motion, Membranes, Nucleic Acids and Proteins*, eds. Clementi, E., Corongiu, G., Sarma, M. H. & Sarma, R. H. (Adenine, New York), pp. 361–374.
28. Doniach, S. & Sondheimer, E. (1974) *Green's Functions for Solid State Physicists* (Benjamin, Reading, MA).