Electrostatic Basis of Structure–Function Correlation in Proteins

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The interest in the structural basis for the action of proteins dates back to 1894 when Emil Fischer[1] introduced the idea that an enzyme and its substrate have a structural complementarity analogous to that of a "lock and key". In 1959 after the elucidation of the first X-ray structures of proteins by John Kendrew[2] and Max Perutz,[3] it seemed that this problem was destined to be solved in a short time. This expectation was partially fulfilled in 1965 when David Phillips and his co-workers[4] determined the first X-ray structure of an enzyme–substrate complex, revealing the details of a reacting biological system and locating the positions of the important catalytic group. However, even now, more than 20 years after the beginning of the X-ray structural revolution in biochemical studies, we still do not have a unique explanation for the relation between the structure of enzyme active sites and their catalytic role. The rate of an enzymatic reaction is determined by the energy of interaction between the active site and the substrate, but the available experimental information does not discriminate between the various components of these interactions and assess their relative importance. This challenges the theoretician to provide a reliable correlation between the X-ray structures of proteins and their biological action.

This Account describes our studies aimed at developing practical and reliable methods for studying chemical processes in proteins. These studies point toward electrostatic interactions as the common denominator and probably the most important element in structure–function correlation in biological systems.

Energetics of Enzymatic Reactions

Enzymes can accelerate chemical reactions by more then 10 orders of magnitudes relative to the corresponding rates in solution. The key question in this respect is how the interaction between the active site and the substrate decreases the activation energy, $\Delta G^{\ddagger}_{cat}$ of an enyzmatic reaction relative to the activation energy, $\Delta G^{\ddagger}_{cage}$, of the corresponding reference reaction in a solvent cage (Figure 1). At the beginning of 1974, we began a naive attempt of evaluating the energetics of enzymatic reactions. At that time, it was clear from the pioneering study of Levitt and Lifson[5] that force field approaches[6,7] could handle the possible effects of strain in proteins,[8] but that the description of the "chemistry" of enzymatic reactions required more elaborate methods. Our first step involved designing a new analytical all-valence-electron approach based on hybrid orbitals that allowed us to "fuse" a quantum mechanical description of the reacting region with a classical description of the rest of the enzyme.[9] After calibrating the model on gas-phase reactions, we started in the ambitious attempt to reproduce the activation energy of a saccharide bond-breaking reaction in lysozyme. Several failures had taught us that ours or any other properly calibrated quantum mechanical model must overestimate this energy by more than 30 kcal/mol, regardless of complete minimization and concerted pathways.

At this point, it became apparent that understanding chemistry in solution is a prerequisite for any meaningful theoretical study of reactions in enzymes. To our disappointment, we found no complete quantum mechanical study of even the simplest solution reaction: dissociation of H_2O to H^+ and OH^-. Examination of the experimental information about the energetics of simple dissociation reactions in solutions[9] indicated that quantum mechanical calculations of reactions in solutions are rather meaningless unless they include the effect of the solvation energy (this point is demonstrated in Figure 2 and discussed in detail below). Thus, the first problem in treating reactions in solutions (or in enzymes) was obtaining an accurate estimate of solvation energies. Examination of the literature indicated that the available macroscopic dielectric models[10,11] could not be expected to provide reliable estimates and new microscopic approaches that include the solvent were needed. The microscopic models derived for this task.[9,12] are described in Figure 3. These models simulate the most important effect of solvation free energy, the interaction between the solvent dipoles and the solute charges. With the inclusion of this effect it was possible to calibrate the models and to obtain quite accurate estimates of solvation free energies.[12]

Incorporating the calculated solvation energies in quantum mechanical calculations[9] began to give reasonable reaction energies and allowed us to obtain a preliminary assessment of the importance of electro-

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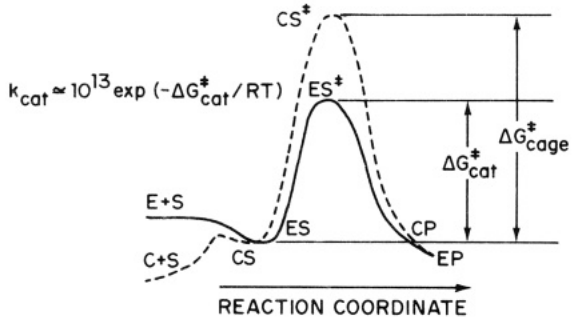

Figure 1. Activation free energies in enzyme and water environments. E, S, and C are, respectively, the enzyme, the substrate, and the necessary catalytic group (e.g., an acid in general acid catalysis).

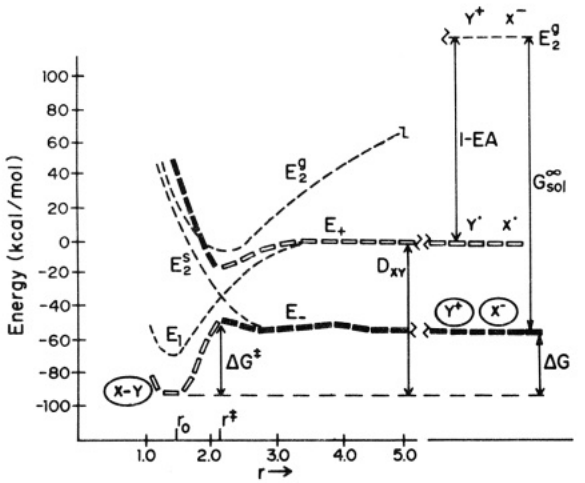

Figure 2. Ionic bond cleavage in solution. The figure shows how the gas-phase ionic state X^-Y^+ becomes a ground state due to the contribution of the solvation energy, G_{sol}. The region of the ground-state surface with more than 50% ionic character is shown as ■■ and that with more then 50% covalent state as □□. E_+ and E_- are the potential surfaces obtained from mixing of the covalent and ionic resonance forms (E_1 and E_2). g and s indicate gas phase and solution, respectively.

static interactions in enzyme catalysis.[9] However, it had appeared that our all valence electron approach[9] as well as other available quantum mechanical approaches (which could, in principle, be modified to include solvent effects) were not likely to provide quantitative tools for comparison of activation energies of reactions in solutions and in enzymes in the near future. Exploring different alternatives, we examined the valence bond (VB) approach (see, for example, ref 13–15), which was abandoned 20 years ago when molecular orbital methods became popular. It appeared that incorporation of the solvation energies of the ionic states in the diagonal matrix elements of the VB Hamiltonian could provide the simplest, and probably the most reliable, approach for treating chemical reactions in solution. This modification, which is called the empirical valence bond (EVB) method,[16] is outlined below.

The EVB method represents the reacting system as a superposition of ionic and covalent resonance forms closely related to bonding diagrams frequently used in

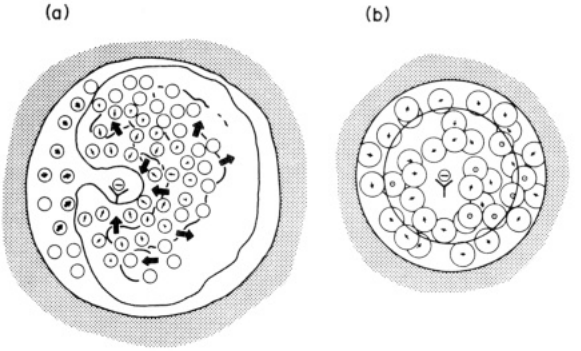

Figure 3. Microscopic dielectric models for calculating solvation energies in (a) proteins and (b) aqueous solutions. The protein environment is simulated by its permanent and induced dipoles and the surrounding water molecules (see refs 9 and 22). The microscopic model for solvation in aqueous solution represents the water molecules by soft-sphere point dipoles surrounded by a surface of randomly oriented dipoles (see ref 12).

organic chemistry text books. For example, in the case of the bond-breaking reaction $X–Y \rightarrow X^- + Y^+$ the relevant resonance forms are

$$\psi_1 = (X–Y)$$
$$\psi_2 = (X^-Y^+) \quad (1)$$

The basic task of the EVB is to evaluate empirically the matrix elements (energies and interaction terms) of these resonance forms and to solve the corresponding secular equation. We adopt the gas-phase matrix elements from the "covalent–ionic" resonance treatment[13,14] and modify them by incorporating the effect of the solvent in the diagonal element of the ionic resonance forms. Thus, for the example of eq 1, we obtain

$$E_1^s = E_1^g = M(r)$$
$$E_2^s = E_2^g + G_{sol}^s(r) = (\Delta^{(2)} - e^2/r + V_{nb}) + G_{sol}^s(r) \quad (2)$$

where g and s indicate gas and solution, $M(r)$ is a Morse potential for the purely covalent X–Y bond, V_{nb} is the repulsive nonbonded interaction between the ions, $\Delta^{(2)}$ is the energy of formation of X^-Y^+ from X·Y· at infinite separation, and G_{sol} is the solvation energy of the X^-Y^+ ion pair. The interaction between the resonance forms is obtained by requiring that the gas-phase ground-state potential, obtained by mixing ψ_1 and ψ_2, reproduce the observed gas-phase Morse potential.[14]

A solution potential surface obtained by this approach is described in Figure 2. This surface is calibrated by requiring that the calculated reaction free energy, ΔG, reproduce the corresponding observed value. The potential surface for the enzymatic reaction is obtained by retaining all the terms of the calibrated solution secular equation except the energy of the ionic resonance form. This energy is modified by simply replacing the solvation energy, G_{sol}^s, by the corresponding electrostatic interactions, G_{sol}^p, between the ionic resonance form and the protein. That is, E_2 in the example of eq 2 will be given by

$$E_2^p = E_2^s + (G_{sol}^p - G_{sol}^s) \quad (3)$$

Thus, if the enzyme gives more solvation energy than water, it will reduce the energy of E_2 in Figure 2 and reduce $\Delta G^{\ddagger}$ to a similar extent. This point is demonstrated in Figure 4, which describes a simplified version

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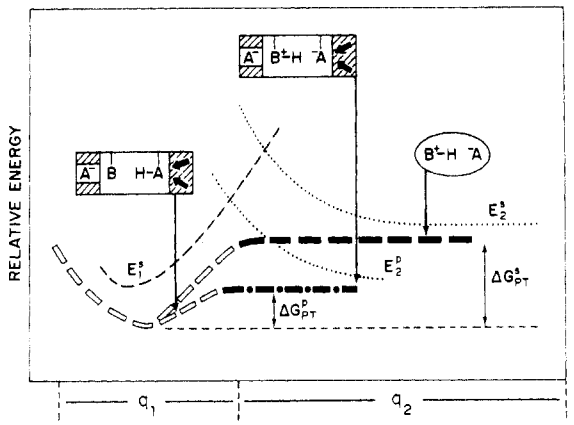

Figure 4. Comparing potential surfaces for proton transfer reactions in solution (■■) and in an enzyme (■•■). The figure considers the energy of the covalent (BH–A) and ionic (BH^+A^-) resonance forms. The energy of the covalent resonance form is the same in solution and in the enzyme, while the ionic resonance form is strongly stabilized by the enzyme. This makes the free energy of proton transfer in the enzyme, ΔG_{PT}^{p}, much smaller than the corresponding free energy in solution, ΔG_{PT}^{s}.

of the EVB potential surface for a typical proton transfer reaction ($AH + B \rightarrow A^- + BH^+$) in solution and in an enzyme. The figure considers the covalent (A–H B) and the ionic (A^-H–B^+) resonance forms. The solution surface is calibrated by requiring that the calculated proton transfer energy, ΔG_{PT}^{s}, at infinite separation between A and B reproduce the observed free energy (obtained from the pK_a difference between A and B).[16] The surface for the enzymatic reaction is obtained from the calibrated secular equation by replacing G_{sol}^{s} in the energy of the ionic resonance form by G_{sol}^{p}. As demonstrated in Figure 4, there is an almost linear correlation between the stabilization of the ionic state and the reduction of the activation energy for the reaction.

The EVB approach provides a tool for quantitative studies of enzymatic reactions. This point will be demonstrated in the next section, which summarizes some of our recent studies on the origin of enzyme catalysis.

Other approaches for studies of enzymatic reactions are described in ref 17–21. Although some of these approaches are promising, they do not give quantitative results, since they fail to include the dielectric effect of the protein induced dipoles. (This gives, for example, ~30 kcal/mol error in evaluating the energy of a charged group in the protein.[22])

Steric Strain and Adiabatic Mapping

Steric strain is considered a crucial catalytic factor in many biochemical textbooks. It is argued that if the structures of the ground state and transition states are different, then the enzyme may be built so that the steric interaction with the substrate will "squeeze" the ground state into a geometry close to that of the transition state. This can result in a reduction of $\Delta G^{\ddagger}_{cat}$.

Force field studies are the simplest way to examine the strain mechanism. This is quite analogous to representing the enzyme–substrate complex by many flexible springs rather than by a rigid framework used in building protein models. Such studies for the most frequently quoted case of lysozyme[9] have demonstrated quite conclusively that the proteins cannot impose sufficient strain to change the ground-state geometry of the substrate. Yet, it is still possible that the enzyme (rather than the substrate) is being deformed by binding the ground-state substrate and this "deformation energy" is released in the transition state leading to a reduction in $\Delta G^{\ddagger}_{cat}$.

This can be examined by the "adiabatic mapping" procedure[6,23] where one of the substrate internal coordinates is fixed at various points along the reaction coordinate, while the energy of the enzyme–substrate complex is minimized with respect to all its Cartesian coordinates. This provides the least energy pathway for the reaction and reflects properly the flexibility of the enzyme. Comparing the steric energy of the system for the adiabatic mappings with and without the protein gives the strain energy contribution to $\Delta G^{\ddagger}_{cat}$. Such calculations have been performed in the case of lysozyme.[8,9] It was found that the substrate geometry changes during the reaction involve shifts no greater than 0.4 Å for any atom.[9] The protein can relax to accommodate such motions by small displacements of its many atoms; this increases the ground-state energy by no more than 1 kcal/mol and decreases the activation energy by the same amount. Related calculations for hemoglobin,[24] where ligand binding leads to geometry changes of no more than 0.8 Å, have indicated that the strain energy stored in the protein is less than 2 kcal/mol. Thus, it seems that strain is unlikely to account for a significant part of the reduction of $\Delta G^{\ddagger}_{cat}$ relative to $\Delta G^{\ddagger}_{cage}$ (which can amount to as much as 10 kcal/mol).

Electrostatic Stabilization of Ionic Resonance Forms

If the enzymatic reaction involves large contributions from ionic resonance forms, then the enzyme may reduce $\Delta G^{\ddagger}_{cat}$ by interacting in an optimal way with the charge distribution of the ionic resonance forms at the transition state. This electrostatic contribution to catalysis can be studied quantitatively by the EVB approach as demonstrated in Figure 5. The figure describes the potential surface for general acid catalysis for sugar hydroylsis in solution and in the active site of lysozyme. The energies of the most important resonance forms and the ground state obtained from their mixing are described for both the solution and the enzyme cases. As the figure shows, the enzyme reduces the energies of the ionic resonance forms relative to their energy in solution, thereby reducing $\Delta G^{\ddagger}_{cat}$ relative to $\Delta G^{\ddagger}_{cage}$. Similar conclusions have emerged from preliminary studies of the catalysis of peptide hydrolysis by serine proteases where the enzyme reduces the energy of the ionic resonance form [His^+tet^-] of the ionized histidine and the negatively charged tetrahedral intermediate relative to their energy in aqueous solution.

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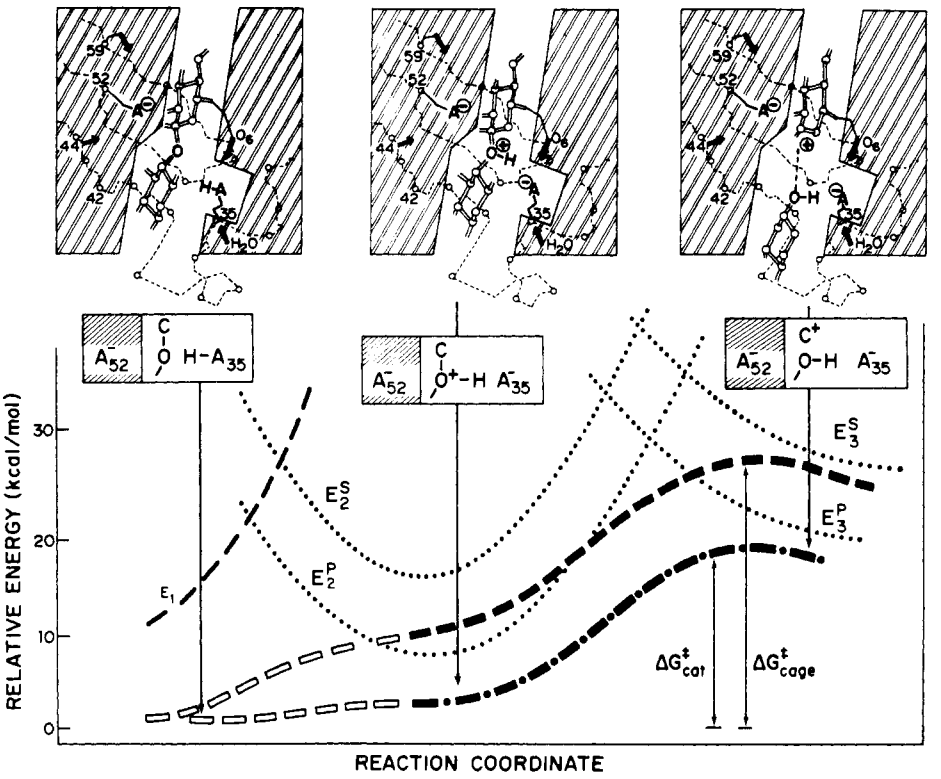

Figure 5. Comparison of the potential surfaces for general acid catalysis in lysozyme (■•■) and in solution (■■). The figure describes the energetics of the reaction as crossing between the indicated resonance forms. The extra stabilization of the ionic resonance forms in the enzyme relative to solution is almost equal to the reduction of $\Delta G^{\ddagger}_{cat}$ relative to $\Delta G^{\ddagger}_{cage}$.

Some important points emerge from Figure 5 and related calculations: (i) Enzymatic reactions can be represented as a simple crossing between resonance forms. (ii) The reduction of the activation energy $\Delta G^{\ddagger}$ is almost linearly related to the stabilization of the ionic resonance forms. Thus, it seems that the key point in looking for structure–function correlation in enzymes is the structural basis for the electrostatic stabilization of the ionic transition states. Frequently asked questions about the degree of mixing of the ionic and covalent states and the concerted nature of the reaction appear to be of secondary importance.

Examination of active sites of several enzymes indicates that the active sites include structural motifs that evolve to stabilize the ionic resonance forms in the transition state (e.g., Asp-52 in lysozyme, Asp-102 and the oxyanion hole in chymotrypsin). We predict that this type of effect will be the most common structural element in related enzymes. The reader can check this point by simply taking his favorite enzyme, drawing the important resonance forms of the substrate, and looking in the X-ray structure of the active site for complementary ionized groups and permanent dipoles.

The idea that enzymes can change the energies of ionic transition states requires further consideration in view of its possible importance. Consider, for example, a dipole, μ, that corresponds to ψ_2 in eq 1. The solvation of such a dipole in a medium with a dielectric constant ϵ_3 is given roughly by[10]

$$G_{sol} \simeq -166(\mu^2/\bar{a}^3)(2\epsilon_0 - 2)/(2\epsilon_0 + 1) \qquad (4)$$

where $\bar{a}$ is the radius of the solvent cavity around the solute. G_{sol} is given in kcal/mol for $\bar{a}$ in Å and μ in units of Å times electron charge. The experimental estimate of the solvation energy of a dipole involving COO^- and NH_3^+ (or other charged groups of similar size) 3 Å apart in aqueous solution ($\epsilon_0 \sim 80$) is $\sim$−70 kcal/mol (Figure 2 in ref 12). A completely hydrophobic environment ($\epsilon \sim 2$) would give (according to eq 4) about half this stabilization, and an ionic transition state with $r^* \simeq 3$ Å would be $\sim$70/2 kcal/mol less stable in the nonpolar solvent than in water (see Figure 6). Thus, the enzyme could, in principle, slow reactions drastically by surrounding the substrate with a nonpolar environment. Since enzymes are designed to accelerate reactions, rather than to slow them down, we conclude that an important feature of an enzyme is a polar active site that stabilizes an ionic transition state better than does water.

Previous work[25] has demonstrated that enzymes can stabilize ionic transition states by comparing the energetics of charge separation in water and in a hypothetical enzyme. It was also shown that a (− + −) resonance form can be more stable in an enzyme than in water.[25] This point was examined by comparing the stabilization energies of the ionic transition state [Asp^- carbonium$^+$ Glu^-] in the active site of lysozyme and in an aqueous solvent cage. It was found that the interaction energy of the surrounding dipoles of the enzyme (particularly those of Asn-59, Asn-44, O_6 of the D sugar, and a bound water molecule) with the transition state was larger than the comparable solvation energy in aqueous solution (Figure 12 of ref 22). Similar conclusions emerged from studies of the reaction of serine proteases where the enzyme dipoles stabilized an ionic state [Asp^- His^+ tet^-] more effectively than does water.

We generalize and describe an enzyme active site as a "supersolvent" for a particular transition state, with

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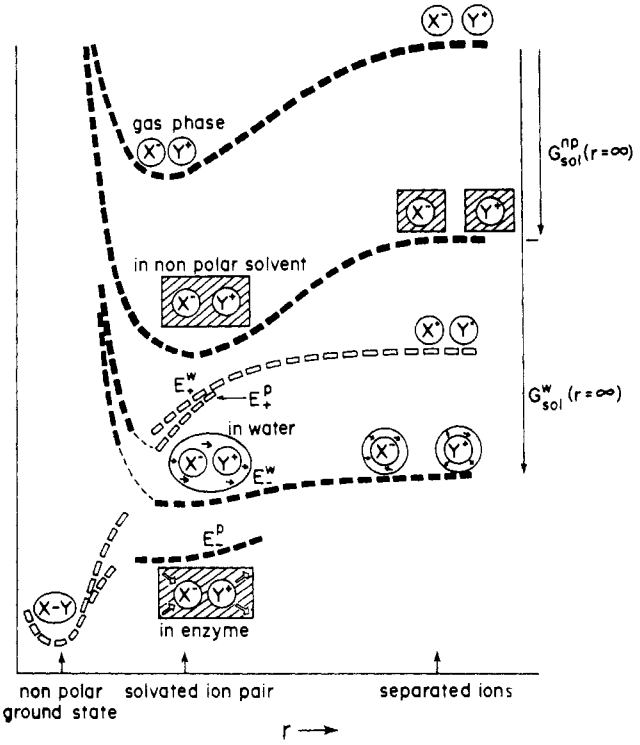

Figure 6. Comparison of the energies of ion-pair type transition states in different environments. The figure demonstrates how the solvation energy makes ionic transition state more stable in polar environments than in non polar environments.

enzyme dipoles preoriented to create an electric field complementary to the charge distribution of the transition state. It seems now that the "lock and key" idea[1] needs modification in view of the fact that enzymes are flexible (steric complementary does not seem to be a major catalytic factor). However, the electrostatic interaction between the charge distribution of enzymes and the ionic resonance forms of the transition states might qualify for the lock and key analogy.

Electrostatic Interactions in Other Systems

Electrostatic interaction seems to be a major factor in structure–function correlation in other processes in addition to enzyme catalysis. For example, in studying the control of redox potentials in proteins, we calculated the electrostatic interaction between the protein and the heme in cytochrome *c* and metmyoglobin.[26] These calculations indicated that the differences in electrostatic stabilization of the oxidized heme by the protein dipoles can be correlated with the corresponding differences in redox potentials. Systematic studies of this effect within mutants of the same protein are now under way.

Another interesting point that was examined recently is the relationship between the rate of electron transfer in proteins and their structures. The activation energy of electron transfer between a donor (D) and acceptor (A) of equal potential is given by $\Delta G^{\ddagger} = (E_2(r_1^{eq}) - E_2(r_2^{eq}))/4$ where 1 and 2 indicate DA^- and D^-A respectively, and $\mathbf{r}^{eq}$ is the equilibrium geometry of the indicated system (see ref 26–28 for discussion). Electron transfer in solution involves a large energy relaxation ($E_2(r_1^{eq}) - E_2(r_2^{eq})$) of the solvent dipoles which result in an activation barrier of ~15 kcal/mol and a slow rate. To examine the corresponding activation barrier in proteins, we studied the recent X-ray structures of ferri- and ferrocytochrome *c*.[29] Converting the observed changes in the orientation of the protein dipoles to energy relaxation gave an activation barrier of only ~2 kcal/mol.[26] It seems that the protein helps to control the rate of electron transfer by holding its dipoles in a relatively fixed orientation and preventing large energy relaxation upon electron transfer.

Ionization of functional groups in proteins play a major role in controlling various processes such as enzymatic reactions, action of allosteric systems, subunit assembly, and probably most processes that convert a pH gradient to chemical energy. In order to analyze the energetics involved in such processes, we calculated the pK_a's of groups in the active sites of lysozyme[22] and chymotrypsin[30]. The calculations reproduced, to within 3 pK_a units, the observed pK_a's. This result is quite encouraging since alternative conventional approaches that view the protein active sites as hydrophobic regions of low dielectric constant give errors of ~20 pK_a units. Considerations of only the protein permanent dipoles give similar errors (see discussion in ref 22). Only the contribution of both the permanent and induced dipoles of the protein can account for the observed pK_a's. Our calculations have indicated that enzymes stabilize ionized groups in their active sites by oriented permament dipoles so that the local environment resembles a crown ether more closely than an "oil drop". In the absence of permanent dipoles, an ionized acidic group in the protein interior will become unstable upon increase of the external pH, leading to partial unfolding. Such pH-induced conformational changes can play an important role in energy transduction in biological systems.

Examination of other processes where electrostatic interactions play an important role are under way. This includes the energetics of salt bridges in various systems, ion binding to proteins, and allosteric effects.

It should be noted that the importance of electrostatic effects in proteins has been considered before (see, for example, the excellent discussion of Max Perutz[31] and the important work of C. A. Vernon[32]). However, only the application of high-speed computers to microscopic analysis of the protein dielectric effect made it possible to evaluate quantitatively the importance of electrostatic interactions in proteins.

Electrostatic Aspects of Storage of Light Energy

The concepts considered above seem to be of direct relevance to fundamental problems in photobiology associated with light energy transduction. We will consider here the energy-converting pigments rhodospin and bacteriorhodopsin and the energetics of light-induced electron transport in photosynthetic systems.

The first intermediate formed after absorption of light by a rhodospin molecule stores more than 30

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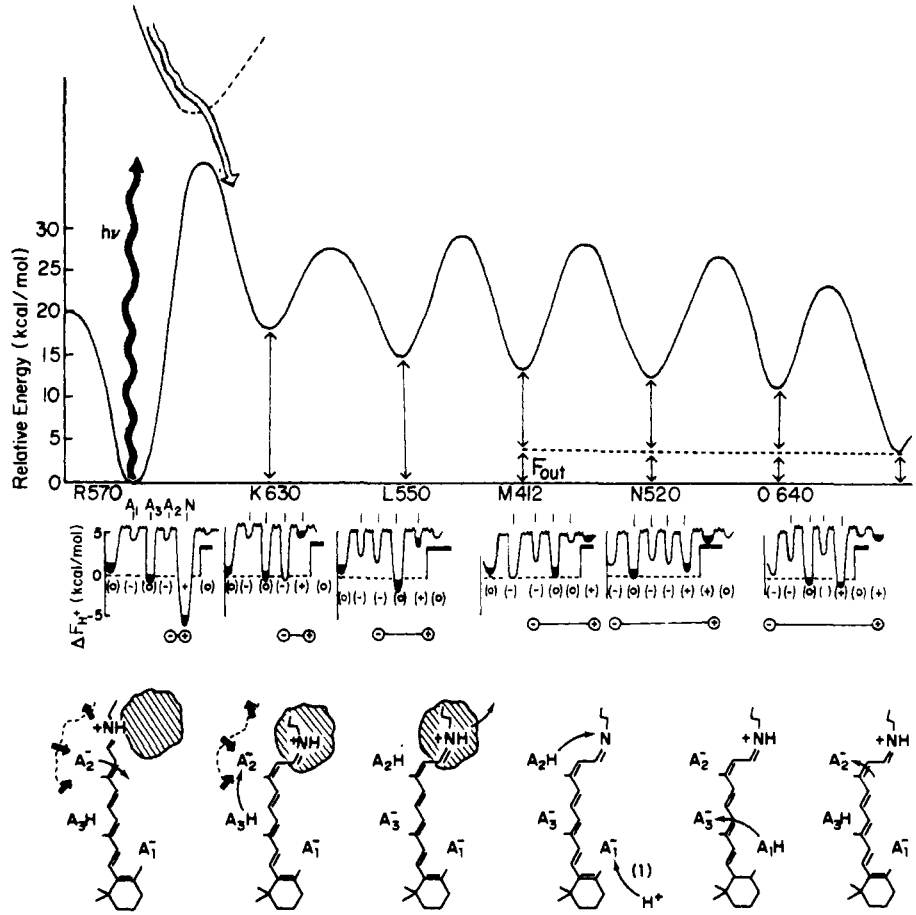

Figure 7. Conversion of light energy to pH gradient in bacteriorhodopsin. The figure relates sequential changes in pK_a's in a model of the light cycle of bacteriorhodopsin to the total energy stored in the system. The initial changes in pK's are due to light-induced isomerization that moves the NH^+ edge of the chromophore from a polar to a nonpolar (cross-hatched) environment. The changes in pK_a's (which are given in the middle diagram) can be related to the total energy of each state by eq 5. See ref 36 for details.

kcal/mol[33] of the light energy. A significant amount of energy is also stored in the first intermediate of the light cycle of bacteriorhodopsin. Recent studies of these phenomena[34-36] have suggested that both involve through-space light-induced charge separation as a result of photoisomerization that moves the protonated Schiff base of the chromopore away from an ionized acidic group of the protein. Explaining such a large energy storage involves the following problem: if the protonated Schiff base and its counterion are in a low dielectric environment, then it is easy to account for a large energy storage[35] using Coulomb's law and a small dielectric constant. However, such an oversimplified explanation is inconsistent with the fact (see Figure 6) that the (COO^-, NH^+) ion pair (bottom of Figure 7) cannot exist in a low dielectric medium where it would form the neutral (N---COOH) configuration. If, on the other hand, the protonated Schiff base is placed in a high dielectric region of the protein, then only a small amount of energy would be stored by charge separation. The resolution of this problem involves the interesting concept of moving the positively charged Schiff base from a polar region to a nonpolar region of the protein (see the lower part of Figure 7). The energy stored in moving charged groups between a polar and nonpolar region can amount to about 30 kcal/mol.[36]

The bacteriorhodopsin molecule provides a unique example of a light-driven proton pump coupled to ATP synthesis,[37] giving strong support to Mitchell's chemisomotic hypothesis.[38] Understanding the overall energetics of this system on a molecular level is one of the great challenges in bioenergetics. In an attempt to attack this problem, we explored ways of expressing the total energy stored in the system on a microscopic level. A clue to this problem comes from our studies of electrostatic energy in proteins and solutions. We realized that the total change of the energy of the protein upon ionization of a given group can be evaluated by considering the protein as a solvent for its charged groups. Relating the solvation free energies of the ionized groups of the protein to their local pK_a's, we derived[36] a general formula that expresses the total free energy of the *m*th configuration of the protein ionizable groups (relative to a reference state where all the protein groups are neutral) by

$$\Delta G^{(m)} = \sum_i -2.3RT[q_i^{(m)}(pK_a^{(m,i)} - pH_0)] \quad (5)$$

where $q_i^{(m)}$ is the charge of the ith protein group (in units of +1, 0, and −1) at the mth configuration and $-2.3RT(pK_a^{(m,i)} - pH_0)$ is the free energy of bringing a

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proton from water at pH = pH_0 to the ith group of the protein in the mth configuration. Each $pK_a^{(m,i)}$ includes half of the contribution of electrostatic interaction with other charged groups (see ref 36 for more details).

Using eq 5 we were able to relate light-induced sequential changes in pK_a's to total energy and describe, for the first time, the overall energetics of driving protons against a pH gradient (see Figure 7 and ref 36).

The derivation of eq 5 considered the protein as a solvent with a slow relaxation time for refolding its dipoles parallel to the field at their locations. Thus, the microscopic dielectric that determines the $pK_a^{(m,i)}$ appears to be time dependent.[36] This concept of "dielectric relaxation" of the protein dipoles provides an interesting insight into what is probably the most important aspects of protein dynamics; it appears that the protein cannot store large amounts of energy as a change in local pK_a's for a time longer than its dielectric relaxation time. If a transition between two states in a charge-separation conduction chain (e.g., K630 to L550 in Figure 7) takes longer than the dielectric relaxation time, then the electrostatic energy will be wasted. Thus, efficient reactions of charge separation across membranes should occur at times shorter than the relaxation time of the protein dipoles.[36]

Equation 5 seems to provide a promising way to analyze other biological systems such as enzymatic reactions[22] or coupling of the action of allosteric systems with pH gradients.

Biological photosynthetic systems convert light energy to electrostatic energy by electron transfer across membranes. Understanding the factors that control the efficiency of such systems is important, both for better understanding of the fundamental aspects of photobiology and for designing artificial energy conversion devices. After preliminary studies of the dynamics of the primary event in bacterial photosynthesis,[28] we became interested in the microscopic factors that determine the overall efficiency. Here, again, it appears that the only simple way to comprehend such a complicated system is to consider its electrostatic aspects.

Considering a conduction chain of stacked donors and acceptors that span the width of a membrane (upper part of Figure 8),[39] we describe the electron-transfer process as a crossing between the relevant ionic valence bond states. Determining the electrostatic energy of the ionic states by an expression similar to eq 5 (with redox potentials instead of pK_a's), we were able to determine the overall barrier for the forward and backward reactions in terms of the membrane dielectric and the relaxation of the dielectric at the sites of the electron donors and acceptors.[39] Our approach allows one to assess the efficiency of photosynthetic systems in terms of their molecular components. This point is demonstrated in Figure 8 for a conduction chain of identical acceptors immersed in a membrane of a low dielectric. In this case, the charge-separation process is shown to be inefficient since it involves transferring a charge through a low dielectric region. Consideration of the electrostatic factors that govern the efficient "downhill" charge separation in biological systems are given in ref 39 and 40.

(39) Warshel, A.; Schlosser, D. W. *Proc. Natl. Acad. Sci. U.S.A.*, in press.

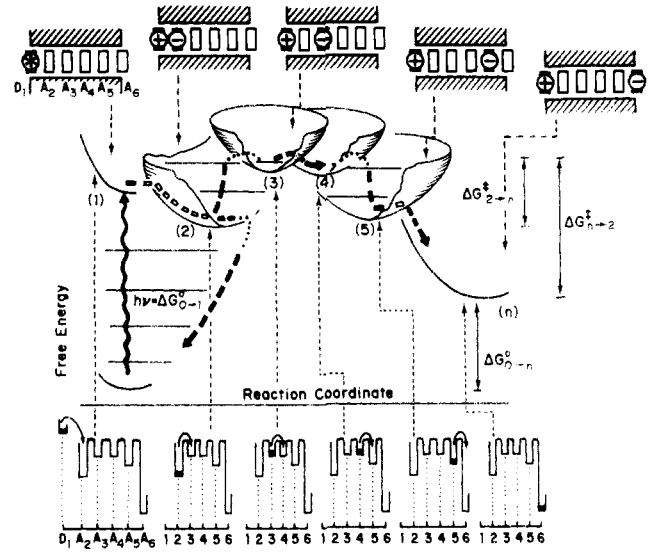

Figure 8. Energetics and dynamics of light-induced electron transfer across membranes. The figure considers a conduction chain of donor, D, on the membrane–solution boundary and acceptors that span the width of the membrane. The charge separation process involves the indicated charge-transfer ionic states. The energies of these states are simply determined by their electrostatic stabilization (see ref 39 for details). The energies of the ionic states and their intersections which are determined by the dielectric relaxation at the sites of the donors and acceptors[39] govern the overall activation energies for the forward ($2 \rightarrow n$) and the backward ($2 \rightarrow 0$) reactions. The figure uses the same type of diagrams developed for treating proton pump systems (Figure 7) to relate the electrostatic potential of an electron at each acceptor to the total energy of the system.

Concluding Remarks

X-ray studies have provided in recent years more and more direct structural information about active sites of proteins. This opens up the exciting possibility of correlating the action of proteins with their observed structures. Detailed analysis is expected to reveal in the future many different contributions to the energetics of biological processes. However, this account argues that the most important single element in structure–function correlation in proteins is associated with electrostatic interactions. This is demonstrated in studies of enzyme catalysis, electron-transfer reactions, control of pK_a's, and light-induced charge separation across membranes. Although much more research is needed before we have a clear quantitative idea about the role of electrostatic effects, we can already locate clear structural motifs associated with electrostatic stabilization of transition states.

We believe that eventually electrostatic interactions will be established as the key factor in bioenergetics. This point was already alluded to in considering the microscopic nature of coupling between pH and energetics of biological processes (eq 5) and in examining the molecular basis of efficiency in photosynthetic systems. Similar considerations are expected to be useful in describing the energetics of ATP synthesis and other major processes in bioenergetics.

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