on the imidazolyl ring enhances the catalytic activity of the central metal ion. This is related to the carboxylate–imidazole–Zn(II) triad[103] of many Zn(II)-metalloenzymes, which suggests that the properties of the Zn(II) ion are modified through interaction of the imidazole ligand with the distant carboxylate anion. The reason why the increase in the electron density of the imidazole ligand raises reactivity in both the metalloenzymes and the models is not understood. Many novel aspects of catalysis by metal ions as Lewis acids, especially the effects of the nature of metal ions and their ligands, therefore, will be revealed as investigation progresses further in this area.

Incorporation of many catalytic features together in small molecules is needed to improve models of specific target enzymes. As for the models of CPA, the next goal is to raise the reaction rate of the models to the level exhibited by CPA. In addition, reproduction of other characteristics of CPA such as enantioselectivity and ability to form complexes with substrates will be pursued. Both CPA and CPA models are investigated in this laboratory. Such a dual mechanistic approach will provide valuable clues to elucidation of the details of CPA action.

Reproduction of the major characteristics of enzyme catalysis such as complexation, acceleration, and specificity is pursued intensively by using synthetic or semisynthetic molecules and antibodies.[104-107] In the design of catalysts mimicking metalloenzymes, knowledge of the catalytic roles of metal ions and the ability to combine the catalytic features of metal ions with those of organic catalytic groups are also needed. Artificial metalloenzymes based on PEI may be improved by introducing additional catalytic or binding sites in planned positions close to the macrocyclic centers. This might be achieved by the site-directed modification of PEI backbones using the macrocyclic centers as anchors.

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On the Molecular Mechanisms of the Solar to Electric Energy Conversion by the Other Photosynthetic System in Nature, Bacteriorhodopsin

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Natural Photosynthesis: Chlorophyll vs Bacteriorhodopsin Photosynthesis

Photosynthesis is a process by which nature converts solar energy into the chemical energy that is required for fueling the different living processes on earth. There are two main different photosynthetic systems in nature: the older (~3 billion years) and much more developed chlorophyll system present in green plants, and the much younger (millions of years) bacteriorhodopsin (bR) system present in *Halobacterium halobium*.

As complex chemical changes usually occur on a long time scale, it is fortunate that nature converts solar energy first into electric energy. In order to store most

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of the photon energy, the initial process occurs extremely rapidly (much faster than 10^{-9} s, the time it takes excited electrons to re-emit the photon energy), thus insuring that the solar energy captured by the absorption process can be stored for later conversion from electric to chemical energy. In both the chlorophyll and bR systems, the solar to electric energy conversion is completed in a few steps, involving charge separation and leading to the creation of proton gradients. The proton gradients (electrochemical gradients) live sufficiently long to drive the metabolic process which converts this form of electric energy into chemical energy in the form of adenosine triphosphate (ATP).

The first and fastest step of the charge-separation process occurs extremely rapidly (on the picosecond time scale). In chlorophyll, the first step involves an electron transfer between one chlorophyll molecule and the other within the special pair in the reaction center. This leads to the formation of an ion pair. This is followed by further charge separation by transferring the electron from the anion of the ion pair to a pheophytin that is distant from the initially formed ion pair
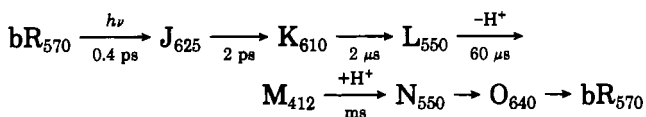
(in 2.8–25 ps). Further electron transfer from the negatively charged pheophytin to a distant quinone takes place on the 100 picosecond time scale. On much longer time scales, the negatively charged quinone initiates the building up of the proton gradient, which is the final step in the solar to electric energy conversion in chlorophyll.

In bR, on the other hand, the initial storage process involves rapid retinal isomerization, leading to a separation of a protonated Schiff base (PSB) from its counterion(s). Following the creation of this electric-induced strain within the protein, relaxation processes occur via protein conformation changes, which lead to the deprotonation of PSB. As a result, proton pumping occurs, leading to the creation of the proton (electrochemical) gradients required for the relatively slow conversion of the electric energy into chemical energy.

From the above, one can see that the formation of proton gradients involves an electron pump in chlorophyll but a proton pump in bR. Thus nature seems to have utilized the two lightest charged particles, the electron and the proton, in providing for life on earth (and maybe somewhere else) from its solar energy.

Bacteriorhodopsin and Its Photocycle

bR is the only protein found in the purple membrane of *Halobacterium*[1] *halobium*. This is a one-cell (few micrometers in length) light-utilizing bacterium that was first discovered by Oesterhelt and Stoeckenius[2] in 1971. It has a sensory system and demonstrated memory in addition to its photosynthetic function. It contains retinal as a chromophore, which is covalently bound via a protonated Schiff base (PSB) linkage (–C–NH–) to the ϵ-amino group of a lysine residue in the protein.[1,3] Upon absorbing a photon, it goes through[1,4] a number of intermediates occurring on different time scales before returning to its original form, bR_{570} (i.e., having its retinal absorption maximum at 570 nm):

$$bR_{570} \xrightarrow[0.4\,ps]{h\nu} J_{625} \xrightarrow[2\,ps]{} K_{610} \xrightarrow[2\,\mu s]{} L_{550} \xrightarrow[60\,\mu s]{-H^+}$$

$$M_{412} \xrightarrow[ms]{+H^+} N_{550} \rightarrow O_{640} \rightarrow bR_{570}$$

The photocycle causes protons to be pumped across the cell membrane to the outside surface of the membrane, establishing a pH gradient used by the organism for metabolic processes such as ATP synthesis.[5–8] The protons are ejected from the cell at a rate comparable to that for the formation of the M_{412} intermediate.[9–11] This intermediate is the only one in which the Schiff

base is unprotonated.[12–16] Consequently, many studies have inferred that the PSB deprotonation is closely associated with the proton pump mechanism.[1] Preceding M_{412} formation (or PSB deprotonation) is the formation of the early intermediates, J_{625}, K_{610}, and L_{550}. The retinal in bR_{570} is in the all-trans form, while in K it has a distorted 13-cis conformation.[17–19] In the L_{550} form, the isomerization is complete.[17–19] The pK_a value of the PSB is 13.3 in bR_{570},[20,21] yet it deprotonates during the $L_{550} \rightarrow M_{412}$ step of the cycle even if the pH of the medium is lowered to below 3, suggesting a large change in its pK_a value during the photocycle.[22]

There are several important reviews that the interested reader should consult for detailed studies carried out by many active researchers in different aspects of this field (i.e., the photochemistry,[23,24] the biochemistry,[1,25,26] the protein structure,[27–29] and the vibration spectroscopy[30–32]). A number of models for the proton pumping process have been proposed.[1,33–39] By combining the results of extensive site specific mutant studies[40] with refined electron diffraction studies,[29] it was possible to reach detailed conclusions regarding the important active amino acids present in the retinal pocket and the proton channel. A better qualitative "sketch" of the mechanism of the proton pump process that accompanies the photocycle is beginning to emerge, but much more extensive results are needed before conclusions regarding the correct and exact model can be drawn.

This Account summarizes part of the work done by

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several members of our research group during the past decade in an attempt to answer several questions regarding this very important photosynthetic system. First, does this system have an antenna (as in the case of chlorophyll) which transfers the absorbed solar energy to a specific region of the membrane (a reaction center) where the retinal isomerization takes place and the proton pump is initiated? The second question: how fast is the first step in the energy conversion process, the retinal isomerization? The third question concerns the molecular mechanism of the deprotonation process (which is the "switch" of the proton pump): What are the factors that determine its rate? Different laser spectroscopic techniques are developed and used in order to answer these different questions.

Does bR Have an Antenna?[41]

The electron diffraction pattern demonstrates that the bR sample shows a hexagonal structure containing trimers of bR molecules.[27] In addition, the CD spectrum of the retinal in the visible region shows a biphasic band shape. This was fitted[42] to an exciton model from which the coupling energy between the excited and the unexcited retinal molecules within the trimer structure was found[42] to be ~200 wavenumbers. Using the uncertainty between energy and time, one would calculate[43] an energy transfer time between excited and unexcited retinals on the order of tens of femtoseconds.

We decided to perform a photoselection experiment to determine whether or not the excitation energy randomizes (as a result of energy-transfer processes between retinals) and whether or not it occurs before the first daughter is formed. If bR indeed has an antenna (i.e., rapid energy transfer), initiating the photocycle with polarized light, which excites those retinal molecules with their long axes (the axes with maximum absorption strength) most parallel to the light electric field direction, would give daughters whose retinal long axes have an equal probability of being in a direction parallel or perpendicular to the excitation polarization direction. This is because the retinal molecules in bR are not parallel to one another, and energy transfer will indeed randomize the direction of the initial excitation.

A photoselection method was used to confirm this prediction. In this method two lasers were employed,[41] an Nd-YAG pulsed laser to initiate the photocycle and a cw He–Ne laser to monitor the absorption of retinal in the K_{610} intermediate. The change in the sample transmission at the He–Ne cw laser wavelength (633 nm) was monitored upon initiating the photocycle in parallel and perpendicular polarization directions with respect to the polarization direction of the photolysis YAG laser (i.e., the retinal absorption direction of the parent (bR_{570})). If energy transfer occurs prior to the formation of K_{610}, no correlation is expected between the absorption direction of the daughter and that of the parent molecule, as energy transfer destroys (depolarizes) the initial excitation memory. Thus the ratio of the retinal absorption intensity of K_{610} (measured from the transmission to the He–Ne laser) in parallel and in perpendicular directions with respect to the YAG laser

polarization direction (anisotropy ratio) should be 1.0. On the other hand, if the absorbing bR_{570} molecule is photochemically transformed into a daughter molecule (i.e., there is no antenna system), its retinal absorption axes will be near parallel to that of the parent. In this case the theoretical value of the anisotropy ratio should be[41] 3.

The value of the linear dichroism extrapolated to low intensity of the photolysis laser is found[41] to be 2.7 ± 0.3. This is so near to 3 that one can conclude that energy transport does not take place during the time between excitation and photochemistry, i.e., *unlike chlorophyll, bR photosynthesis does not use an antenna system.*

The above results suggest that the excitation energy is localized on the molecule whose long axis is most aligned with the laser electric field (excitation) direction. The observed localization of the excitation energy can be due either to rapid isomerization or to other rapid energy trapping (dephasing) processes, or else it suggests that the CD spectrum is not a reflection of exciton coupling but rather results from heterogeneity.[43,44] Femtosecond studies[45–48] conclude that the isomerization process occurs in 400 fs. More recent studies[49] report a rapid dephasing process on the 10 fs time scale. The latter process (whose origin is not yet known) could indeed localize the excitation and explain the photoselection results, but leaves the proposed exciton origin for the bilobal band shape of the CD unsupported. However, there are a number of other observations that argue for and others that argue against such an explanation. Thus the origin of the visible CD of bR remains unresolved.

Does Retinal Isomerization Occur on the Femtosecond Time Scale?[50,51]

Using Raman spectroscopy, it has already been concluded[50] that isomerization occurs on a time scale equal to or shorter than 40 ps. Using optical spectroscopy,[45–48] changes in the broad visible retinal absorption, attributed to isomerization, were observed in 0.4 ps. Below, we discuss a resonance Raman experiment by which we examine whether or not isomerization indeed occurs on the subpicosecond time scale.

In our efforts to develop new time-resolved resonance Raman methods,[32,52] we extended the technique that recorded the Raman spectra of transients on the tens of picosecond to the subpicosecond time scale. In this technique,[32,50,52] we used pulses[51] from a Spectra-Physics YAG-DYE laser of 700 fs duration which had a me-

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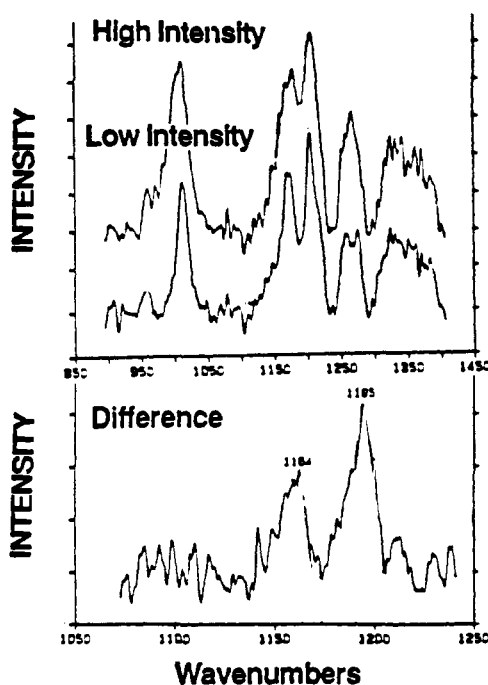
Figure 1. Resonance Raman spectrum[51] of transients formed at time ≤700 fs (the laser pulse width) in the spectral region most sensitive to retinal conformation changes (bottom). The spectrum was determined with the flow technique[32,52] and then subtracting the spectrum taken at low laser intensity from that at high laser intensity (top). The band at 1195 cm^{-1} strongly indicates that isomerization from *all-trans*- to distorted 13-*cis*-retinal occurs on this time scale in bacteriorhodopsin.

gahertz repetition rate. The laser excitation beam used was of a few nanojoules per pulse and was focused to a few micrometers. By flowing the bR suspension at a speed of ~80 m/s across the focused laser beam, the sample residence time in the laser beam becomes shorter than the time between pulses (1 μs). This insures that the collected scattered Raman radiation is that from the unphotolyzed bR as well as from its photoproducts that are formed *during the pulse width of each laser pulse*. The Raman spectrum recorded at low laser powers (which is mostly due to the unphotolyzed bR) is subtracted from that recorded at high powers (which is due to the parent and any daughter formed within 700 fs) in order to obtain the spectrum of the femtosecond transients.

The Raman spectrum[51] of the subpicosecond intermediate in the C=C stretching region has two bands at 1518 and 1510 cm^{-1}. Using the expected linear correlation between the C=C stretching frequency and the wavelength of the absorption maximum of polyenes (e.g., the other intermediates in the bR photocycle[51]), one concludes that the absorption maxima of these intermediates are at 625 and 640 nm, respectively. The 625-nm absorption corresponds to the J_{625} intermediate, which is observed optically[45] with a rise time of 400 fs.
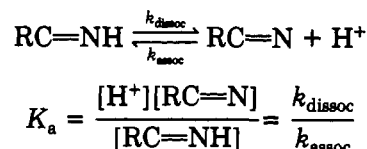
Figure 1 shows the Raman spectra of these intermediates in the vibration region that is sensitive to retinal conformation changes (1000–1400 cm^{-1}). The fact that we see a new spectrum upon subtraction in this region (bottom spectrum) suggests that the intermediates observed in the optical region have retinal conformations different from that in bR. In particular, the band at 1193 cm^{-1} is characteristic of a distorted 13-cis configuration and has been observed for the intermediates

appearing at longer time scale.[32,50] Furthermore, the correspondence between these transients and those observed optically, whose spectra are not observed if the C_{13}–C_{14} bond is clamped[45] with CH_2 groups (to prevent isomerization), is strong evidence that isomerization indeed occurs on such a short time scale.

This kind of isomerization leads to the separation of the positively charged PSB from its counterion(s) and changes in H-bonding strength and in the nature of the retinal distortions.[53] All of these lead to the storage of solar into electrochemical energy.

The Deprotonation Process: The Switch of the Proton Pump

Kinetic Considerations of the Protonation–Deprotonation Processes. *The Protonated Schiff Base.* What are the factors that determine the rates and efficiency of the deprotonation of the PSB? This is the process by which some of the initially absorbed solar energy is converted into the long-lived form of electric energy: the proton gradients. Is the observed rate of deprotonation determined by the rate of proton dissociation (proton transfer) or is it determined by the rate of protein (solvent) conformation reorganization? It is not immediately obvious that the rate of proton dissociation should be rapid. Due to the much longer decay of M, one may assume an L $\rightleftharpoons$ M (deprotonation $\rightleftharpoons$ protonation) equilibration prior to the M decay, i.e., one may write

$$RC=NH \underset{k_{assoc}}{\overset{k_{dissoc}}{\rightleftharpoons}} RC=N + H^+$$

$$K_a = \frac{[H^+][RC=N]}{[RC=NH]} = \frac{k_{dissoc}}{k_{assoc}}$$

in which the rate of deprotonation equals the rate of proton association. From this, $k_{dissoc} = K_a k_{assoc}$, where K_a is the acid constant of the PSB in the environment in which it dissociates. Assuming a pK_a value of 3 and a proton association process that is diffusion controlled with $k_{assoc} = 10^8–10^{11}$ M^{-1} s^{-1} (where 10^{11} M^{-1} s^{-1} is in aqueous medium and the slow value is in nonaqueous systems), one calculates $k_{dissoc} = 10^5–10^8$ s^{-1}. Since the observed rate constant of deprotonation is $10^4–10^5$ s^{-1} (i.e., near this range), an answer to the above question cannot be found from simple theoretical arguments.

Amino Acids; Acid–Base Equilibrium and bR Heterogeneity. bR has a number of carboxylic groups (e.g., in aspartic and glutamic acids) with pK_a values ranging from 2 to 9.5.[54] At any pH, some of these groups would be in the protonated form while others would be in the negatively charged deprotonated form. This leads to (inhomogeneous) broadening of the spectral bands of nearby molecules and causes the bR sample to be heterogeneous. The observed shift in the bR absorption[55] at pH 8.5 is a manifestation of this type of heterogeneity.

If the acid–base equilibration of the amino acids occurs faster than the formation of all of the photocycle intermediates, only one cycle should be observed and the inhomogeneity will be averaged out in the kinetics

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of the photocycle. However, more than one parallel photocycle and, thus, nonexponential rise and decay of certain intermediates are expected to be observed, if some of the aspartic or glutamic acids that affect the rates of formation of some of the photocycle intermediates have protonation–reprotonation rates on a comparable or slower time scale. Using the equation, k_{dissoc} = $K_a k_{assoc}$, the limits on the pK_a values of 2–9, and k_{assoc} = 10^6–10^{11}, one can calculate deprotonation times that range from a nanosecond to a quarter of an hour. Thus, it is possible that some of carboxylic acids would equilibrate on a longer time scale than the rise time of some of the intermediates. The observed changes[56] in the tryptophan (Trp) fluorescence quenching by retinal during the cycle at pH values near some of the pK_a values of the different aspartic acids in bR have been attributed to this kind of heterogeneity. The deprotonation and reprotonation processes of the PSB of bR are observed to have two components. These are proposed to be due to this kind of protein heterogeneity.[57] Back reactions[58,59] in the PSB acid–base equilibrium were recently introduced to explain the observed multicomponent kinetics in the bR photocycle.

Deprotonation of the PSB and the Rate of Protein Conformational Changes. While global protein conformational change is not expected to occur nor is it observed during the bR photocycle, local conformational changes near and around the retinal are expected to take place. There are a number of observations discussed below that strongly suggest that, while the rate of the initial process of converting solar to electric energy is completely determined by the rate of retinal absorption and isomerization (the action), the rate of the deprotonation, and thus that of the formation of the proton gradients, is determined solely by the rate at which the protein responds to the electrostatic strains induced by the retinal isomerization. These are observations on some amino acids, presumably not very far from the retinal (i.e., within the active site), that undergo changes with rates comparable to that of the deprotonation process. Furthermore, the rates of all of these changes have comparable activation energies, which are similar to that for the deprotonation of the PSB. This, together with the fact that the observed value of the activation energy is comparable to hydrogen-bond energies and much larger than that known for diffusion-controlled processes in aqueous medium, led us to propose that the rate of deprotonation of the PSB is determined by the rate of protein conformational changes.[22,57] This brings proton acceptor near the PSB and *further* changes its environment sufficiently[22] to make it acidic enough to induce proton transfer and proton pumping process.

Tryptophan Fluorescence as a Monitor for Protein Conformational Changes. Trp Fluorescence Quenching in bR.[60] There are seven Trp residues in a molecule[1] of bR. The Trp fluorescence decay can be resolved into four different components[60] with lifetimes
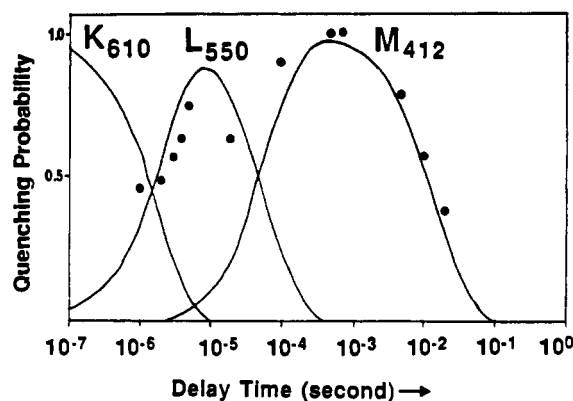

Figure 2. Time dependence of the observed quenching probability of the Trp fluorescence (●) and the calculated formation probability of the K_{610}, L_{550}, and M_{412} daughters (—). It is clear that the maximum quenching (due to energy transfer to the retinal) occurs at the maximum formation probability of M_{412} (i.e., of the deprotonation process). This suggests that the rate of deprotonation of the protonated Schiff base is determined by the rate of the protein conformational changes (as monitored by the changes in the Trp fluorescence intensity).

of 100, 200, 1000, and 2800 ps. The short-lived components are found to have shorter wavelengths, suggesting emission from Trp residues in a more hydrophobic medium (i.e., within the protein). Furthermore, the short-lived emission disappears and the total fluorescence intensity greatly increases, if retinal is removed. These results suggest that the observed difference in the lifetimes reflects differences in the quenching probability of the Trp emission by retinal as a result of differences in the relative orientation (distance and angles) of these emitting Trp molecules with respect to the retinal transition moment direction (the long axis). The Trp with short-lived emission must then be located closer to retinal and/or oriented with its absorption transition moment more parallel to that of the retinal than the ones with the long lifetimes. It is thus expected that the fluorescence intensity changes of the Trp during the photocycle can be an excellent monitor of the protein conformational changes.

Changes of Trp Fluorescence Intensity during the Photocycle.[61] The changes in the quenching probability of the Trp fluorescence are indeed observed on the L_{550} and M_{412} formation times[61] (see Figure 2). The rapid retinal isomerization apparently does not lead to large changes in the relative orientation of the retinal and the Trp residues whose emission is observed. Only when the amino acids within the protein begin to change their relative orientation during the K → L or the L → M process would the probability of both the energy transfer and the quenching processes begin to change. This leads to changes in the observed Trp fluorescence intensity, as shown in Figure 2.

pH Dependence of Trp Fluorescence Quenching during the Photocycle.[56] We have recently studied[56] the pH dependence of the Trp fluorescence quenching probability during the photocycle. Only the quenching on the deprotonation time scale is found to be very sensitive to the pH changes of the medium (see Figure 3). In fact, sudden observed changes in the probability occur at pH values that correspond to pK_a values of different acidic groups in the purple membrane (PM),

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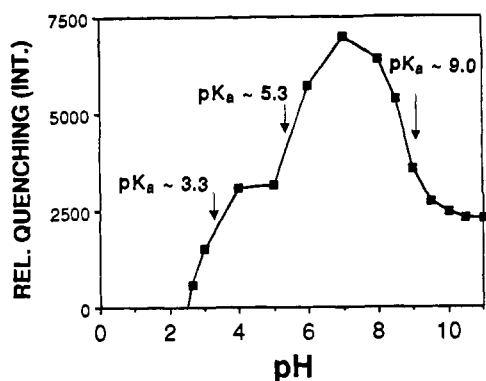
Figure 3. pH dependence of the Trp fluorescence quenching probability during the deprotonation process. Changes in the probability of quenching occur at pH's near pK_a values of amino acids in the protein (e.g., aspartic acid in different environments). This suggests that changing the acid–base equilibrium within the protein changes its conformation and thus the coupling between its amino acids (e.g., Trp) and the retinal in bacteriorhodopsin during its photocycle.

for example, pK_a = 2.6, 3.3, 5.3, and 9.0. Interestingly enough, the total amount of M_{412} formed,[62] and thus the efficiency of the cycle, also changes at a pH near some of these values, confirming the proposal that some of the emitting Trp residues and the retinal system are coupled through the protein conformational changes.

Certainly, when the different acidic groups in PMbR (e.g., the phosphates in the lipids and the glutamates, aspartates, and tyrosinates in the protein) change from the acidic to the conjugate basic form, the electrostatic forces within the protein and on the surface change, possibly leading to changes in the protein conformations. This could lead to changes in the relative orientation of, and/or distances between, the retinal and some of the amino acids during the photocycle. The coupling between some of the excited Trp residues and the retinal chromophore would then change and thus leads to changes in the probability of the dipolar energy transfer between them. This, in turn, results in changing the quenching probability of the Trp fluorescence.

Interestingly enough, the maximum quenching of the Trp fluorescence occurs at physiological pH ($\sim$6.8). It has been shown that UV photons absorbed by Trp and Tyr can initiate the photocycle,[63] although with lower efficiency. Thus in nature, some of the UV solar photons absorbed by Trp (and by Tyr, which are quenched by Trp) can be used via an effective quenching of the Trp excitation by retinal to initiate the photocycle.

The Transient Absorption at 298 nm. It has been observed[64-66] that an increase in the absorption intensity at 298 and 244 nm takes place on the deprotonation time scale. Tyrosinate is found to have similar maxima. Because of this and the fact that this transient absorption decreases with pH with an apparent pK_a value near 9.6, the transient absorption was attributed to the dissociation of tyrosine to form a tyrosinate[64,65] (Tyr⁻) during the photocycle. Spectral FTIR studies[66,67] as

well as Raman studies[68] have suggested the presence of a tyrosinate species in bR during the M formation. However, NMR[69,70] and more recent UV Raman studies[71] suggest that no tyrosinate is present in bR and that none is formed during the cycle. Using Raman techniques,[72] it was suggested earlier that the transient absorption at 296 nm was a result of charge perturbation of the lowest absorption band of a Trp residue.

We recently carried out studies on the intensity of this transient absorption[73] in different Trp mutants in which Trp residues were individually replaced by an amino acid which had an absorption at much higher energy and whose perturbation would not give rise to a transient absorption at 296 nm. We have observed this absorption for all of the Trp mutants *but Trp 182*. Since the latter mutant is found to have a normal photocycle with an observed strong M_{412} type absorption, we conclude that the 296-nm transient absorption is a result of charge perturbation of Trp 182.

Previously, we have studied[55] the relative amplitudes of the fast and slow rise components of the M_{412} transient as a function of pH. A titration type curve was obtained with a pK_a value of $\sim$9.6. Since at that time tyrosine was implicated[64,65] in the cycle, we have proposed that the observed heterogeneity resulting from the tyrosine–tyrosinate equilibrium could be responsible for the two cycles with two different rise times for their M formation. Since recent studies give evidence against the presence of tyrosinate whereas recent FTIR results suggest[54] that some aspartic acids could have pK_a values near 9.5, the heterogeneity might thus arise from these aspartic acids. Asp 115 is very much within the retinal pocket, and thus its ionized and nonionized forms would give quite different environments around the PSB, leading to different kinetic parameters for its deprotonation (i.e., for the M_{412} formation).

From the above discussion, it can be concluded that changes in the absorption and fluorescence changes from amino acid residues occur on the same time scale as those for the deprotonation process. Equally important, the observed activation energies for both the Trp fluorescence quenching and the 296-nm transient absorption of these protein molecules occurring on the deprotonation time scale are found to be comparable[56,57] to each other and to the activation energy of the deprotonation process. The observed values ($\sim$50 kJ/ mol) are much larger than those for diffusion-controlled processes ($\sim$13 kJ/mol), suggesting that the deprotonation process is controlled by protein conformational changes that probably involve H-bond or metal–ligand bond-rearranging processes.

Other Observations Supporting Protein Conformational Changes upon Deprotonation. The above kinetic results suggest that the rate of protein conformational changes controls the rate of the depro-

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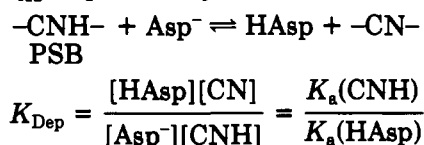(65) Hess, B.; Kuschmitz, D. *FEBS Lett.* **1979**, *100*, 334–340.

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tonation process (i.e., of the M formation). This thus suggests that protein conformational changes occur upon M formation. There are indeed structural and thermodynamic results that support this notion. As early as 1977, Ort and Parson[74] proposed such changes to account for the large enthalpy decrease associated with M formation. More recent calorimetric results[75-77] are all accounted for by a protein conformational change when bR is transformed into M. Spectrally, the FTIR spectra of the amide I and II modes,[78] of the Pro 186 and Pro 50 mutants,[79,80] and of a number of aspartic acids[81-84] all show changes that suggest protein conformational changes indeed occur upon the formation of M.

The Deprotonation Process: Thermodynamic Considerations. Why would conformational changes lead to the deprotonation process? It could bring an appropriate proton acceptor near the PSB to allow for the proton transfer to take place and/or it could change the environment around the PSB to make it acidic enough for the proton dissociation to occur. From the thermodynamic arguments given below, one can show that the first possibility alone can be eliminated. Thus it is essential that conformational changes lead to drastic changes in the pK_a value of the PSB (by more than 10 units.)

It is now believed that an aspartate 85 and/or 212 act(s) as a proton acceptor[29,41] for the PSB during the $L_{550} \rightarrow M_{412}$ step of the cycle:

$$-CNH- + Asp^- \rightleftharpoons HAsp + -CN-$$
$$PSB$$

$$K_{Dep} = \frac{[HAsp][CN]}{[Asp^-][CNH]} = \frac{K_a(CNH)}{K_a(HAsp)}$$

Prior to the cycle, the values[85,86] of $K_{CNH} = 10^{-13.3}$ and of $K_a(Asp) = 10^{-2}$. This gives the deprotonation equilibrium constant K_{Dep} a value of 10^{-11}. In order to observe the deprotonated form ($-CN-$) during the M_{412} formation, at least a 50% conversion of $-CNH-$ into $-CN-$ and Asp^- into HAsp must take place, (i.e., K_{Dep} must be ≥ 1). For this to be true, $K_a(CNH)$ must be $\geq K_a(HAsp)$ for L_{550} as it is transformed into M_{412} (i.e., for the deprotonation process to take place). Since at pH below 2.6 the deprotonation process ceases[22,86] to occur (presumably due to the protonation of the aspartate proton acceptor), one can use $\sim 10^{-2.6}$ for K_a-(HAsp). This suggests that $K_a(CNH)$ must have in-

creased from $10^{-13.3}$ in bR to $>10^{-2.6}$. Thus the K_a value of the PSB has increased by a factor of $>10^{10.7}$!

The next question concerns how this large change in the pK_a of the PSB can be accomplished as the bR changes into L_{550} just before its deprotonation. A number of proposals are made. The distortion[37,53] of the retinal at C_{14}–C_{15} presumably could decrease the Schiff base pK_a. Change in pK_a as a result of changes in the strength and the geometry of the hydrogen bond to the PSB could also lead to reduction in its pK_a.[87] An increase in the electrostatic repulsion between the PSB and the positive end of a dipole or a positively charged cation (e.g., Arg 82 or metal cation) during the L → M step was proposed by us several years ago.[22,57,88] From the recently published structure,[29] one concludes the presence of a very highly inhomogeneous electric field within the retinal pocket. There is no specific counterion associated with the PSB. In the retinal pocket, there are negatively charged Asp 85 and Asp 212 residues, positively charged Arg 82 and the PSB, strongly hydrogen-bonded Tyr 185, unprotonated Asp 115, and available space for a few water molecules. More recently, a specific metal cation (Ca^{2+} or Mg^{2+}) is proposed to be bound within the pocket.[89] The presence of all these charged species in not too large a volume is expected to give large field gradients that are very sensitive to small protein conformational changes that take place during the photocycle.

Summary

Bacteriorhodopsin is not only one of the most efficient solar energy converters[90] we presently have but it is also becoming an important optical material of potential[91] use as the future eyes of robots. The conversion of solar energy into chemical energy (photosynthesis) by bR is found to occur via conversion first into electric energy, as in chlorophyll. Unlike chlorophyll, bR is found not to possess an antenna system. Like chlorophyll, the conversion into electric energy is observed on two different time scales: rapid charge separation and slow creation of proton gradients. While the rapid charge separation in chlorophyll involves electron transfer, it results from femtosecond isomerization of the retinal chromophore in bR. This separates the —C=NH— (protonated Schiff base, PSB) group from its counterion(s). The electrostatic strain thus induced initiates protein conformational relaxation. The rate of these changes controls the rate of the deprotonation process (which is responsible for the creation of the proton gradients). As a result of protein conformational changes, the PSB and its proton acceptor (an aspartate) find themselves in an environment in which the PSB changes its acidity constant by a factor larger than 10 billion. One of the important questions yet to be answered is in regard to the molecular mechanism of the reduction of the acidity con-

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stant of the PSB during the photocycle. Among many other facets of the bR structure and function that need to be understood are the details of the proton movement that leads to the creation of the proton gradients.

Titan: A Laboratory for Prebiological Organic Chemistry

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Introduction

While the presence of complex kerogen-like organic solids in some meteorites has been recognized since the time of Berzelius, a general understanding that organic molecules are pervasive in the solar system has been slow in achieving consensus in the astronomical community. In part this has been due to the fact that the inner solar system in which the Earth is embedded is not in a reducing oxidation state. But when we examine the atmospheres of the Jovian planets (Jupiter, Saturn, Uranus, and Neptune), the satellites in the outer solar system, comets, and even—through microwave and infrared spectroscopy—the cold dilute gas and grains between the stars, we find a rich organic chemistry, presumably abiological, not only in most of the solar system but throughout the Milky Way galaxy. In part because the composition and surface pressure of the Earth's atmosphere 4×10^9 years ago are unknown, laboratory experiments on prebiological organic chemistry are at best suggestive; but we can test our understanding by looking more closely at the observed extraterrestrial organic chemistry. The present Account is restricted to atmospheric organic chemistry, primarily on the large moon of Saturn.

Titan is a test of our understanding of the organic chemistry of planetary atmospheres. Its atmospheric bulk composition (N_2/CH_4) is intermediate between the highly reducing ($H_2/He/CH_4/NH_3/H_2O$) atmospheres of the Jovian planets and the more oxidized ($N_2/CO_2/H_2O$) atmospheres of the terrestrial planets Mars and Venus. It has long been recognized[1] that Titan's organic chemistry may have some relevance to the events that led to the origin of life on Earth. But with Titan surface temperatures $\simeq 94$ K and pressures $\simeq 1.6$ bar, the oceans of the early Earth have no ready analogue on Titan. Nevertheless, tectonic events in the water ice-rich interior[2] or impact melting and slow refreezing[3] may lead to an episodic availability of liquid water. Indeed, the latter process is the equivalent of a $\sim 10^3$-year-duration shallow aqueous sea over the entire surface of Titan.

When the Voyager 1 and Voyager 2 spacecraft flew by Titan in 1980 and 1981, the cameras uncovered an enveloping pinkish-orange haze layer unbroken even at kilometer resolution. Through differential refraction, a radio-occultation experiment determined the atmospheric structure from high above the visible haze down to the surface. The atmospheric chemistry was examined[4] by an infrared interferometric spectrometer (IRIS). Six simple gas-phase hydrocarbons ($HC{\equiv}CH$, $H_2C{=}CH_2$, H_3CCH_3, $H_3CCH_2CH_3$, $H_3CC{\equiv}CH$, $HC{\equiv}CC{\equiv}CH$) and three nitriles ($HC{\equiv}N$, $N{\equiv}CC{\equiv}N$, $HC{\equiv}CC{\equiv}N$) were found. Stratospheric volume mixing ratios for these compounds varied from the 10 ppm range for ethane down to the 1–10 ppb range for C_3H_4, C_4H_2, HC_3N, and C_2N_2. The pole-to-equator abundance ratios range from ~ 1 for the C_2-hydrocarbons to ~ 10–100. The observations are summarized (E for equatorial abundances, P for polar) in Figure 1. One important question, posed by Voyager, is why these minor organic constituents and not others are prominently produced from the major constituents (N_2 and CH_4) in the atmosphere of Titan.

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