Bacteriorhodopsin에 의한 태양에너지 저장

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ON THE SOLAR ENERGY STORAGE BY THE OTHER PHOTOSYNTHETIC SYSTEM BESIDES CHLOROPHYLL: BACTERIORHODOPSIN

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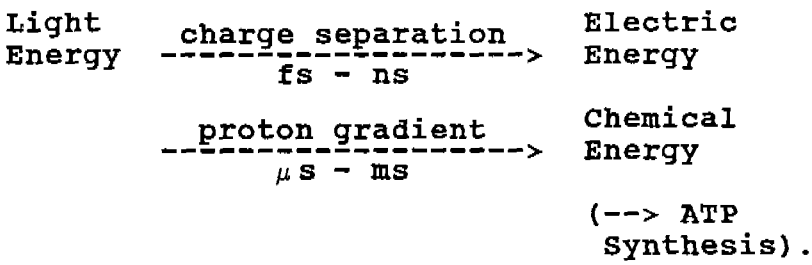
Photosynthesis is the process in which light energy is converted to chemical energy by a photosynthetic biological system:



Light energy is first converted to electric energy by rapid charge separation in the time scale of femtoseconds to nanoseconds. Using this electric energy as a driving force, the photosynthetic biological systems pump protons across a membrane in the time scale of microseconds to milliseconds. The created proton gradient across the membrane is then used in a slower time scale to synthesize ATP and to drive other transport processes. More complicated processes such as carbon trapping occur in a much slower time scale to convert light energy finally to more stable chemical energy. Since light energy is already converted to (electro)-

chemical energy by creating proton gradient, the understanding of proton pumping mechanism across a membrane becomes essential to our understanding of the molecular mechanism of solar energy storage in nature.

1. INTRODUCTION

Since the discovery of photosynthesis, all organisms investigated have been found to use chlorophylls as the primary light energy absorbing protein pigments. The chromopore in chlorophylls is porphyrin. According to the generally accepted chemiosmotic theory, a series of oxidation-reduction reactions converts the absorbed energy to an electrochemical proton gradient across a membrane, which contains the pigments and components of the redox chain (Mitchell, 1979). Recently (Oesterhelt and Stoeckenius, 1971), however, a new pigment has been discovered which, when illuminated, can directly generate an electrochemical proton gradient across a membrane. This pigment, bacteriorhodopsin, is found in the cell membrane of Halobacterium halobium. This prokaryotic organism can only live in environments with high NaCl concentrations and is naturally found in salt lakes, where the salt concentration is near saturation. Both in bacteriorhodopsin and in chlorophyll-based photosynthesis, the electrochemical energy is used by cell to synthesize ATP and to drive other transport processes, such as the uptake of nutrients or the ejection of Na^+. In prokaryotes, locomotion is also directly driven by the proton gradient (Manson et al., 1977).

Bacteriorhodopsin (bR) is a protein with a molecular weight of 26000 which is amazingly similar to the visual pigments of animals. Its chromophore responsible for the purple color is retinal (vitamin A aldehyde) and is bound via a protonated Schiff base linkage to the ε-amino group of the lysine-216 residue in the

apoprotein, known as bacterioopsin (Oesterhelt and Stoeckenius, 1971; Byley et al., 1981). In the bacterial membrane bR is concentrated in patches, where it forms a two-dimensional hexagonal lattice (plane group p3) excluding all other proteins (Henderson et al., 1975). Purple membrane contains a variety of diether lipids, amounting to about 25% by weight, that fill the spaces between bR molecules and are all in close contact with the protein (Henderson and Unwin, 1975).

A protonated Schiff base of retinal in solution absorbs at ~370 nm. However, light adapted bR has a strong absorption band around 568 nm. If bR is kept in the dark, its absorbance maximum shifts to 558 nm and the absorbance decreases by ~15%. This form is known as dark-adapted bR. Illumination with moderate light intensity restores the light-adapted form. Light adapted bR has all-trans retinal while dark adapted bR has the 1:1 mixture of 13-cis-retinal and all-trans-retinal (Pettei et al., 1977). bR uses the light energy absorbed by its chromophore to pump protons from the inside to the outside of the cell (Dencher and Wilms, 1975). Upon absorption of visible light, light adapted bR at neutral pH undergoes a photochemical cycle of at least five intermediates on time scales varying from femto- to milliseconds (Lozier et al., 1975; Mathies et al., 1988):

$$bR_{568} \xrightarrow[0.5\ ps]{light} J_{625} \xrightarrow[5\ ps]{} K_{610} \xrightarrow[2\ \mu s]{} L_{550}$$

$$\xrightarrow[60\ \mu s]{-\ H^+} M_{412} \xrightarrow[ms]{+\ H^+} O_{640} \longrightarrow bR_{568},$$

where the subscript for each intermediate indicates the absorption peak wavelength of the retinal for the intermediate. As a result, the protonated Schiff base is deprotonated during the L_{550} -> M_{412} step, leading to the proton-pumping. Thus, the understanding of the

deprotonation mechanism of the protonated Schiff base becomes important to our understanding of the molecular mechanism of solar energy storage in nature.

bR normally contains bound Ca^{2+} and Mg^{2+} as natural metal cations. Acidification or removal of metal cations from bR produces the purple-to-blue color transition (Kimura et al., 1984). The blue form is incapable of forming the M_{412} intermediate but allows isomerization of the retinal and formation of the K_{610} and L_{550} intermediates (Chronister et al., 1986). bR that is 75% delipidated bR does not change the color from purple to blue by deionization but forms M_{412} with half the efficiency of bR (Jang and El-sayed, 1988). "Acid purple bR" (bR at pH ~0) is found to form K_{610} and L_{550} analogues but does not form M_{412} or pump protons (Chronister et al., 1986).

In this paper the author summarizes very briefly what he has studied on the proton pumping mechanisms of bR (Bitting et al., 1990; Jang and El-Sayed, 1988, 1989a, b; Jang et al., 1988, 1990a, b, c; van den Berg et al., 1990a, b) while he was in the Professor El-Sayed's research group at the Department of Chemistry and Biochemistry, University of California, Los Angeles as a postdoctoral student in 1987 - 1989. The research was mainly focused on understanding the roles of protein in the proton translocation mechanism. The protein conformation and its change in different perturbations and during the photocycle were studied using various methods. Single substituted bR mutant samples prepared with site-directed mutagenesis were also used to understand the roles of individual amino acids in the proton pumping process. A picosecond Nd:YAG laser-streak camera system was used for the measurements of tryptophan emission decay kinetics. A N_2 laser-transient digitizer system was used for the measurement of transient absorption and emission during the photocycle in the time scale of microseconds to seconds. Raman spectra were measured using a subpicosecond

Nd:YAG dye laser-reticon system. Static spectrometers of circular and magnetic circular dichroim, emission and absorption and other static measurement systems such as difference scanning calorimetry and pH meter were also used as experimental methods.

2. DELIPIDATION STUDIES

Delipidation effect on the deprotonation (Jang and El-Sayed, 1988)

Szundi and Stoeckenius (1987) reported that partially delipidated bR (absorption peak at 561 nm) could be reversibly converted to the blue form by acid titration but not by metal cation deionization. In this work we study the effect of lipids on the deprotonation process of the protonated Schiff base in bR by comparing M_{412} absorption kinetics and the emission kinetics from bound Eu^{3+} sites in regular bR and delipidated bR. The absorption and emission kinetics were measured using a N_2-pumped dye laser-transient digitizer system.

The removal of 75% of the lipid from bR caused the following: (i) decreased efficiency and rate of deprotonation of the protonated Schiff base (as monitored by absorption of the M_{412} intermediate); (ii) increased efficiency of the deprotonation of deionized samples; (iii) a decrease by 1 unit in the pH at which deprotonation ceases; (iv) increase of intensity of Eu^{3+} emission in Eu^{3+}-regenerated deionized delipidated samples; (v) increased exposure of the Eu^{3+} sites to water; and (vi) ellimiation of dependence of the deprotonation efficiency on the metal cation concentration. These results are discussed in terms of changes in the protein conformation upon delipidation, which in turn control the deprotonation mechanism. The protein conformation is sensitive to lipid charges, metal cation charges and the charges and dipoles of the protein itself.

3. TRYPTOPHAN FLUORESCENCE STUDIES

It contains 8 tryptophan residues and 11 tyrosine residues out of total 248 amino acid residues in the bR polypeptide chain (Ovchinnikov, 1982). Optical probes of these amino acid residues have been used to understand the protein structure and the interactions of the protein with the retinal in bR and its photocycle intermediates (Jang and El-Sayed, 1989a, b; Jang et al., 1988, 1990c; van den Berg et al., 1990b). Protein fluorescence of bR and its retinal-free form, bacterioopsin, is characteristic of tryptophan fluorescence only (Sherman, 1982). Most of the tryptophans in bR interacts with the retinal (Jang and El-Sayed, 1989b).

3(A). Effects of metal cation, retinal, and the photocycle on the tryptophan emission (Jang et al., 1988; Jang and El-sayed, 1989a)

The picosecond fluorescence kinetics of tryptophan residues in bR and some perturbed analogs are measured, using a system of picosecond laser-streak camera with UV optics, to study the different tryptophan environments and their changes upon metal cation removal, retinal removal, and M_{412} trapping. In bR, the emission shows four decay components designated $C1_R$, $C2_R$, $C3_R$, and $C4_R$ in order of increasing lifetimes. The emission wavelength of $C3_R$ and $C4_R$ is near that found in aqueous solution, while that of $C1_R$ is the shortest. The removal of retinal triples the total emission intensity and reduces the number of components to two, suggesting that the observed variation of the lifetimes in bR results from the variation of the energy transfer efficiency between different tryptophans and retinal. We conclude that the $C1_R$ and $C2_R$ emission is from the closest tryptophans to the retinal. The quenching of the $C3_R$ emission by all metal cation, including those that cannot act as energy acceptors, e.g. Ca^{2+}, is attributed to protein conformation

changes caused by metal cation binding which leads to a stronger energy transfer coupling between tryptophans and retinal. The additional quenching of the $C2_R$ emission in Eu^{3+} bound bacterioopsin is proposed to result from direct energy transfer between tryptophans and Eu^{3+}.

3(B). Absence of tryptophan fluorescence quenching by metal cations in delipidated bR (Jang et al., 1990c)

In this work we have carried out both steady-state study as well as picosecond time-resolved study using a laser-streak camera system on the influence of delipidation of bR and the addition of Eu^{3+} and Ca^{2+} to deionized bR and deionized delipidated bR on the tryptophan emission. The addition of metal cations to deionized bR quenches the steady-state tryptophan fluorescence intensity and reduces the decay times of some of its picosecond components. Similar quenching processes of the tryptophan emission are not observed in deionized 75% delipidated bR. The results are discussed in terms of conformational changes taking place in the protein upon delipidation.

3(C). Tryptophan fluorescence quenching occurring during the photocycle under different perturbations (Jang and El-Sayed, 1989b)

The rates of the quenching and recovery of tryptophan fluorescence are determined in the microsecond-millisecond time scale during the photocycle of bR under different perturbations. The photocycle of bR was initiated by a N_2-pumped dye laser pulse and the variation of tryptophan fluorescence excited by Hg lamp was monitored using a transient digitizer. The kinetics suggest the presence of two quenching processes, a rapid one (on the time scale of photocycle intermediate L_{550} formation or faster) and a slow one (slightly slower than the slow component of intermediate M_{412} formation). The slow quenching process is found to respond

to different perturbations in the same manner as the slow component of M_{412} formation. It has the same activation energy, it is inhibited if metal cations are removed, it is negligible at pH values > the pK_a of tyrosine, and its rate is slowed down when 75% of the lipids are removed. These results, together with the observed value of the quenching activation energy, suggest that the rates of the tryptophan fluorescence quenching, like those of tyrosinate and M_{412} formations during the cycle, are all determined by the rates of the protein conformation changes. The pH studies of the slow quenching process show that the maximum quenching probability occurs at neutral pH. A rapid decrease in quenching occurs at lower pH (~3 and ~5.5) and higher pH (~9). Two quencing mechanisms involving energy transfer to either retinal or tyrosinate are considered. Protein conformation changes resulting from a change in the ionization state of amino acids of different pKa values could change the tryptophan-retinal (or tryptophan-tyrosinate) coupling and thus the quenching efficiency.

3(D). Decay of the tryptophan fluorescence anisotropy in bR and its modified forms (van den Berg et al., 1990b)

In this work we study the decay of the polarization of the tryptophan fluorescence in native bR, dionized bR, and the retinal-free form of bR, bactrioopsin, using picosecond laser-streak camera system. Two types of depolarization processes are observed. One around 250 ps, which is temperature independent around room temperature, and the other in the 1-3-ns range, which is sensitive to temperature and certain bR modifications. This suggests the presence of at least two different environment for the 8 tryptophan molecules in bR. Native bR and deionized bR gave the same depolarization decay times, suggesting that the removal of metal cations does not change the microenvironment of the emitting tryptophan molecules. The slow component is faster in

bacterioopsin than in bR, suggesting a change in the environment of the tryptophan molecules upon the removal of the retinal chromophore. All these results are discussed in terms of the different mechanisms of tryptophan fluorescence depolarization. A comparision between the depolarization decay in rhodopsin and bR is made.

4. THERMAL STABILITY STUDIES

Thermal stability of native, delipidated, deionized and regenerated bR (Kresheck et al., 1990)

The above results indicate that the protein plays a dominant role in the mechanism of the deprotonation processes. A thermodynamic analysis of the structural stabiliy of bR can be obtained from studies of the thermal unfolding by differential scanning calorimetry. In this work, the effect of several different perturbations on the overall macroscopic protein is studied by this technique and circular dichroism spectrometry. A comparision is then made between the response to these different perturbations of the protein binding and helical content on one hand and the structure and function of the active site (as measured by the changes in the deprotonation efficiency) on the other hand.

The differential scanning calorimeter curves and the circular dichroism spectra are determined for the protein of native bR, deionized bR, acid blue and acid purple bR, 75% delipidated bR, deionized bR and Mg^{2+} regenerated deionized bR. The effect of the different perturbation on the thermal stability (melting temperature) and the apparent helix content of the protein is examined. It is found that the response of the deprotonation efficiency or the retinal color change is much more sensitive to these perturbations than that

of the thermal stability or the apparent helix content of the protein. Although the addition of Mg^{2+} to deionized bR restores the photocycle, it does not completely restore the thermal stability to that of the native protein.

5. MUTAGENETIC SUBSTITUTION STUDIES

In an approach to understanding the mechanism, a variety of site-directed mutagenetic methods now permit alteration of any amino acid residue in bacterioopsin (Khorana, 1988). Thus, bacterioopsin mutants that contain single-amino acid substitutions have been prepared and studied by a number of biochemical and biophysical techniques to test current hypotheses regarding bR structure and function (Ahl et al., 1988; Mogi et al., 1988; Holz et al., 1989). Previously, the proton pumping across the membrane has been postulated to occur as protons are transfered through amino acid chains. Among the several models involving specific residues, a role of Tyr in light-driven proton pumping by bR was suggested (Merz and Zundel, 1981). A tyrosine deprotonates on the same time scales as M_{412} formation (Hanamoto et al., 1984).

5(A). Effect of genetic modification of Tyr-185 on the proton pump and the blue-to-purple transition (Jang et al., 1990b)

The retinylidence chromophore mutant of bR, in which Tyr-185 is substituted by phenylalanine, is examined and compared with wild-type bR expressed in Escherichia coli; both were reinstituted similarly in vesicles. The mutant shows (at least) two distinct spectra at neutral pH. Upon light absorption, the blue species (which absorbs in the red) behaves as if "dead"-i.e., neither its tyrosine nor its protonated Schiff base undergoes deprotonation nor does its tryptophan fluorescence undergo

quenching. This result is unlike either the purple species (which absorbs in the blue) or wile-type bR expressed in E. coli. As the pH encreases, both the color changes and the protonated Schiff base deprotonation efficiency suggest a blue-to-purple transition of the mutant near pH 9. If this blue-to-purple transition of the mutant corresponds to the blue-to-purple transition of purple-membrane (native) bR (occurring at pH 2.6) and of wild-type bR expressed in E. coli (occurring at pH 5), the protein-conformation changes of this transition as well as the protonated Schiff base deprotonation may be controlled not by surface pH alone, but rather by the coupling between surface potential and the general protein internal structure around the active site. The results also suggest that Tyr-185 does not deprotonate during the photocycle in purple-membrane bR.

5(B). Sensitivity of the retinal circular dichroism to the mutagenetic single substitution of amino acids: tyrosine (Jang et al., 1990a)

The circular dichroism (CD) spectrum of native bR in the visible spectrum region is composed of negative and positive (larger) components of unequal strength. This biphasic line shape was attributed to the presence of exciton coupling which splits the degeneracy of the 3 retinal excited states of the trimer into 'E and 'A states (Ebrey et al., 1977). Recently (El-Sayed et al., 1989), magnetic circular dichroism showed no signs for an 'E type state in bR. El-Sayed et al. (1989) suggested that the biphasic nature of CD spectra of bR results from the presence of the CD of more than one type of bR with the retinals having different protein environment giving them opposite signs.

The retinal CD spectrum of bR in the 400-700 nm range is compared with those of wild bR expressed in E. coli and reconstituted into lipid vesicles and its genetically modified bR in which one of the tyrosines, Tyr-26, Tyr-57,

Tyr-64, Tyr-79 or Tyr-185, is substituted by a phenylalanine (Khorana et al., 1988). In this work, only the substituted Tyr-185 mutant is found to show large effects on the CD spectrum of the photocycle of bR. bR in the native purple membrane, in wild type expressed in E. coli and reconstituted in lipid vesicles, and its constituted mutants with substitutions of Tyr-185 by a phenylalanine all are found to have different visible retinal CD spectra. The results strongly suggest that the environment of retinal in bR determines the sign and heterogeneity of its visible retinal CD spectrum. This supports the recent proposal that the observed biphasic CD spectrum of bR is due to the superposition of the CD spectra having opposite signs of more than one type of bR rather than due to exciton coupling.

6. PHOTOCYCLE STUDIES

On the multiple photocycles at high pH (Bitting et al., 1990)

The absorption of visible light initiates the photochemical cycle which drives the proton translocation (Lozier et al., 1975; Mathies et al., 1988). However, the number of intermediates and the pathways involved in the regeneration of the original pigment from M_{412} intermediate are still not understood. The discrepancy increases with increasing pH and ionic strength. In order to determine whether the slow bleach recovery of bR_{568} is due to a subsequent intermediate of M_{412} or a parallel intermediate, we investigated the precursor of slowly decaying M_{412} and the kinetics of the 350 nm-absorbing species, using a N_2-pumped dye laser-transient digitizer system. The long-lived bR photobleach is due to the formation of a parallel rather than subsequent intermediate of M_{412}.

bR shows at least two parallel photocycles at pH 10.5, suggesting that more than one form

of bR exist in alkaline bR sample. Upon the absorption of visible light, the different forms of bR at high pH yield different parallel intermediates: M_{412} with two rise and two decay components; and R, an extremely fast rising and extremely slow decaying intermediate with an absorption peak at 350 nm. The kinetics and spectra do not agree with the proposal of Kouyama et al. (1988) that the 350 nm-absorbing species is the N intermediate which follows M_{412} intermediate and that the slow decaying M_{412} is an M-like photoproduct of N. Our results basically agree with the proposal of Dacshazy et al (1988) that the fast and slow decaying M_{412} intermediates and R are in independent photocycles arising from different forms of bR. The different forms of bR are probably in dynamic equilibrium with their ratios controlled by pH and ionic strength.

7. SUBPICOSECOND RAMAN STUDIES

Suppicosecond resonance Raman spectra of the early intermediates in the photocycle (van den Berg et al., 1990a)

The primary photochemical event in the photocycle has been a subject of considerable interest. A number of experiments showed a redshifted precursor of K_{610} which forms in ~500 fs and relaxes to K_{610} on a 3-ps time scale (Sharkov et al., 1985; Nuss et al., 1985; Mathies et al., 1988). This precursor was labeled J_{625}, although there was some uncertainty about its absorption maximum. Although transient absorption spectra are obtainable in the short-time domain, they are generally very broad and structureless, and give little or no information on configurational or conformational changes. We present the first resonance Raman spectrum which can exclusively be attributed to J_{625} intermediate.

The resonace Raman spectra are presented for

the species formed during the photocycle of bR on a timescale of 800-900 fs. In the ethylenic stretch region two intermediates were found with frequencies of 1510 and 1518 cm^{-1}, corresponding to species with optical absorption maxima at 660 nm and 625 nm, respectively. This leads to the assignment of the 1518 cm^{-1} band to the J_{625} intermediate. In the fingerprint region, the appearance of a vibration at 1195 cm^{-1} strongly suggests that the isomerization indeed has taken place in a time less than the pulsewidth of our laser. This support the previous proposals made on the basis of the time-resolved transient absorption spectra. The spectra are compared with those observed in tens of picoseconds up to nanoseconds.

ACKNOWLEDGEMENTS: All the works presented here were conducted under the supervision of Professor M. A. El-Sayed at the Department of Chemistry and Biochemistry, University of California, Los Angeles. The author also wishes to thank the group members of Professor El-Sayed and, especially, the collaborators of the works.

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