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A comparative study of purple membranes partially rehydrated with water and deuterium oxide

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Abstract. The photoelectric signals of dried oriented purple membrane samples were studied at various hydration degrees (humidities between 0.036–0.13 gH_2O/gbR) in water and deuterium oxide. A modified photocycle was found both in water and deuterium oxide at very low humidities, as obtained previously in the case of water. The dependence of the lifetime on temperature and hydration degree, for the L↔M and M→bR transitions, was calculated by using an exponential decomposition of the electric signals. The Eyring parameters were calculated from the temperature dependence, in order to obtain comparative information concerning the isotope effect following deuteration. The activation enthalpies and entropies for the L decay showed an abrupt change at a water content of about 0.06 gH_2O/gbR, but the isotope effect was present only at humidities below this value. In the case of the M decay, an isotope effect was found at all humidities, the values of Eyring parameters being smaller in deuterium oxide. The activation entropies have negative values in the case of strongly dehydrated samples, both in water and deuterium oxide.

Key words: Bacteriorhodopsin – Photocycle – Electric signals – Thermodynamics – Humidity – Deuteration

Introduction

Bacteriorhodopsin (bR), a retinal protein in the plasma membrane of *Halobacterium halobium*, when illuminated in its light-adapted form, undergoes a photocycle resulting in the release of a proton at the external surface of the membrane and the uptake of another one on the cytoplasmic side (Lozier et al. 1975, 1976; Stoeckenius et al. 1979). Thus, bacteriorhodopsin acts as a light-driven proton pump, transporting protons across the plasma membrane and creating an electrochemical gradient used by the cell for ATP synthesis and other energy requiring processes (Stoeckenius et al. 1979; Lanyi 1984). Absorption of a photon by the all-trans retinal chromophore of the light-adapted bR causes its rapid 13-cis isomerization, leading through an excited state to the photoproduct *J*. This is followed by a linear sequence of thermal interconversions, spectroscopically characterized by the intermediates *K*, *L*, *M*, *N* and *O* (Lozier et al. 1975; Smith et al. 1985). Finally bR returns in tens of milliseconds to its initial state. Three of these reactions are associated with proton transfers, namely the $L \leftrightarrow M$, $M \leftrightarrow N$ and $N \leftrightarrow (O) \rightarrow$bR transitions.

Advances in vibrational spectroscopy, recent studies on site-directed (Braiman et al. 1988; Marinetti et al. 1989) and natural mutagenesis (Soppa and Oesterhelt 1989) as well as a high-resolution structural model (Henderson et al. 1990a), suggested the following sequence for the three steps of proton transfer. First, in the $L \leftrightarrow M$ step, the proton of the Schiff base is transferred to the Asp 85 (Braiman et al. 1988; Gerwert et al. 1989; Butt et al. 1989; Stern et al. 1989), which lies in a hydrophilic region of the protein open to the extracellular medium (Henderson et al. 1990a), and is then released to the external side of the cell membrane. Next, in the $M \leftrightarrow N$ step, the Asp 96, located in a hydrophobic region near the cytoplasmic side (Henderson et al. 1990a) and originally in a protonated state, deprotonates, transferring its proton to the Schiff base (Gerwert et al. 1989; Butt et al. 1989; Stern et al. 1989; Otto et al. 1989; Tittor et al. 1989; Holz et al. 1989). The third step is the uptake of a proton from the aqueous medium of the cytoplasm by Asp 96, in the $N \leftrightarrow (O) \rightarrow$ bR transition. While the first half of the photocycle, including the $K \leftrightarrow L \leftrightarrow M$ steps, is well characterized by an unambiguous reaction sequence which contains reverse reactions involving transient equilibria, the second part, consisting of the $M \leftrightarrow O$ steps, raises a lot of controversy about the role and spectrum of *O* and the existence of a branching (Váró and Lanyi 1990).

The structure and function of bR in purple membranes depend strongly on the water content of the sample (Korenstein and Hess 1977a, b). Studies on dehydrated and partially hydrated samples have shown changes in light

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absorption (Váró and Keszthelyi 1983, 1985; Váró and Lanyi 1991 a) and the modification of the photoelectric signals. If the humidity of the bR films was decreased below 90% (Váró and Lanyi 1991 a) the *O* intermediate was no longer observable. On further decreasing the humidiy, the appearance of the M intermediate accelerated while its decay slowed down and fewer and fewer protons were translocated across the membrane. Most of the protons seemed to return to the Schiff base on the *M*-bR pathway and below a water content of 0.06 $g H_2O/g bR$ this process was dominating. Under the same conditions, a negative slow electric signal appears, suggesting again a return of the proton to its initial position (Váró and Keszthelyi 1983, 1985). At a water content of 0.06 $g H_2O/g bR$ the Arrhenius parameters of the photocycle show an abrupt change, suggesting the appearance of some structural modifications which have been confirmed in an infrared study (Váró and Eisenstein 1987). In vacuum dried purple membrane samples, the spectrum of the chromophore shifts to 530 nm (Hildebrandt and Stockburger 1984), the proton undergoes a backward and a forward motion with respect to the proton-pumping direction of bR, and the photocycle goes until the *L* state and back (Kovács and Váró 1988). Similar modifications of the photocycle were found in bR incorporated in PVA films (Bryl et al. 1991), where the *O* intermediate disappeared and the negative photoelectric signal appeared, suggesting a backflow of protons to their original site. All these findings showed that bulk water is necessary for long-range proton transport.

In order to obtain more information on the possible role of water in the structure and photochemical reactions of bR, we followed up the effects of progressive rehydration of dried oriented purple membrane samples, with deuterium oxide instead of water.

Materials and methods

The photoelectric signals from purple membranes at different hydration levels were studied in water and deuterium oxide. The purple membranes were obtained from *Halobacterium halobium* strain S9 by the standard method (Oesterhelt and Stoeckenius 1974) and the oriented dried samples were prepared as previously described (Váró 1981). The sample consisted of about 1000 layers of purple membrane sheets having a resistance of 10^{11} Ohm and a capacitance of 100 pF. The measuring system and all the methods used have already been reported (Váró and Keszthelyi 1983, 1985). The temperature of the sample could be varied by means of a sample holder controlled by Peltier elements. The purple membranes were light-adapted by illumination with a mercury lamp (HBO 200, C. Zeiss) through heat and green light filters.

The sample was totally dehydrated by keeping it in a desiccator over P_2O_5 for one week. In order to obtain the deuterated sample, the rehydration took place in a vacuum tight sample holder and the humidity of the sample was controlled using various saturated salt solutions (Pethig 1979) prepared with deuterium oxide (Merck 99.9%). Similarly, the controls were incubated with saturated salt solutions prepared in normal water. To ensure a complete equilibration, the sample was kept for more than a day at the given humidity. The H_2O/bR ratio at a given humidity was determined from the measurements described elsewhere (Váró and Keszthelyi 1983).

The interpretation of the signals was made on the basis of the paper Váró and Keszthelyi (1983). The photoelectric signals associated with the *L* decay in the microsecond range and with the M decay in the millisecond range were recorded for several different humidities in a temperature range of $-10\,^{\circ}C$ and $26\,^{\circ}C$ in non-deuterated and deuterated samples. They were fitted with two exponentials and from the temperature dependence of the lifetimes, the Eyring parameters were calculated as given by the expression:

$$\tau = (h/k_B\,T)\exp(-\Delta S/R)\exp(\Delta H/R\,T)$$

where k_B is the Boltzmann constant and R is the gas constant.

As the lifetimes of the electric signals are macroscopic parameters, the calculated energy barriers correspond to a simplified model used earlier (Váró and Keszthelyi 1985) and give only general information about comparative changes between samples rehydrated with water and deuterium oxide.

Results

The measured photoelectric signals are shown in Fig. 1. The lifetimes of the two components given by the exponential fit to the *L* decay increased with increasing humidity of the sample, both in water and in deuterium oxide, as can be seen from Table 1. An isotope effect is present, the lifetimes being larger in the deuterated samples, and this effect seems to be more pronounced at higher humidities. In dehydrated purple membranes, the photoelectric signal corresponding to the *M*-bR transition has inverse polarity compared to suspensions (Váró and Keszthelyi 1983). At low humidities (below 0.06 $g H_2O/g bR$), the signal has only a negative part (Fig. 1 B). On increasing the hydration degree, the form of the signal begins to change and a positive component appears, indicating that more and more bR molecules are undergoing the normal photocycle.

In order to study the part characteristic of dehydration, we took for data analysis only the negative part of the signal, which is slowed down as compared to the *M* decay in suspensions. The values calculated for the *M* decay (Table 2) show an isotope effect too, but while the lifetimes for the fast component of the *M* decay are longer in deuterium oxide, those corresponding to the slower component are unexpectedly smaller, with a mean ratio of the liftetimes in heavy water and in water of about 0.5. Another peculiarity of the lifetimes for both components of the *M* decay is their apparent independence of the humidity in the deuterated samples, unlike in the case of the non-deuterated ones, at least in the range of humidities between 0.046 and 0.083 $g H_2O/g bR$.

The temperature dependence of the lifetimes in the case of the *L* decay show a different slope in water and

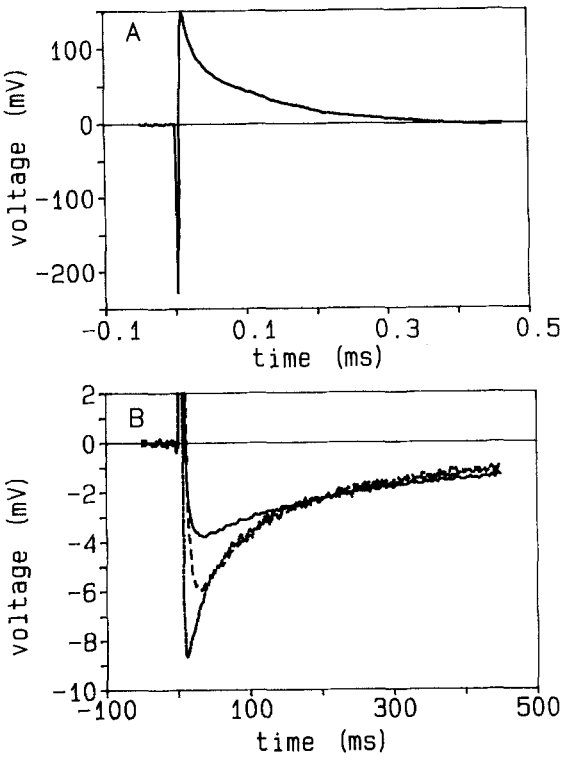

Fig. 1. Electric response signals of dried purple membrane sample at room temperature: **A** Fast and medium signals, water content 0.13 gH_2O/gbR, R = 10 kOhm; **B** slow signals, water content –·–· 0.046 gH_2O/gbR, ---- 0.06 gH_2O/gbR, — 0.083 gH_2O/gbR, R = 10 MOhm

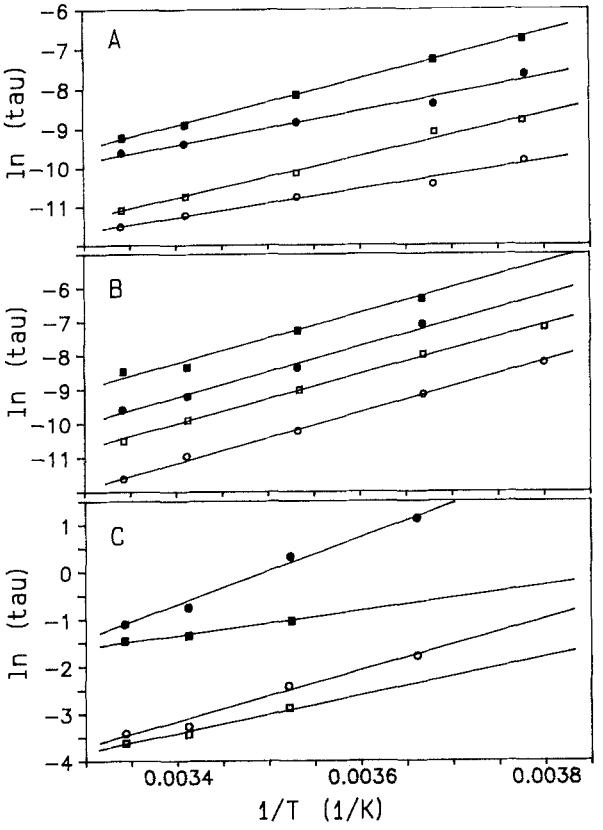

Fig. 2. Temperature dependence of the photoelectric signal time constants for the *L* decay at humidity 0.046 gH_2O/gbR **A**, at humidity 0.06 gH_2O/gbR **B** and for the *M* decay at humidity 0.036 gH_2O/gbR **C**, in water and deuterium oxide. ○ fast component and ● slow component in water; □ fast component and ■ slow component in deuterium oxide respectively

Table 1. The liftimes of the two components of the *L* decay at various humidities, at 20 °C, in water and deuterium oxide. The humidities corresponding to deuterated salt solutions have been considered as not significantly different from those in non-deuterated ones (no precise data are available in the literature)

Humidity (gH_2O/gbR)	τ_1 (s) H_2O	D_2O	Isotope effect	τ_2 (s) H_2O	D_2O	Isotope effect
0.036	12	15	1.25	80	95	1.19
0.046	14	20	1.42	87	130	1.49
0.061	16	31	1.93	100	200	2.00
0.083	26	42	1.61	130	285	2.19
0.130	31	52	1.67	220	375	1.70

Table 2. The lifetimes of the two components of the *M* decay at various humidities, at 20 °C, in water and deuterium oxide

Humidity (gH_2O/gbR)	τ_1 (ms) H_2O	D_2O	Isotope effect	τ_2 (ms) H_2O	D_2O	Isotope effect
0.036	39	32	0.82	500	260	0.52
0.046	48	120	2.50	450	310	0.69
0.061	60	122	2.05	330	280	0.85
0.083	95	120	1.26	580	240	0.42
0.130	80	120	1.50	900	350	0.39

deuterium oxide, for humidities below 0.06 gH_2O/gbR, but approximately the same slope for humidities above this value (Fig. 2a, b). The activation enthalpies and entropies were calculated from this temperature dependence. The abrupt change already reported for dehydrated samples (Váró and Keszthelyi 1985) at a water content of 0.06 gH_2O/bR, is also present in deuterium oxide (Fig. 3a). Below this humidity the energy barriers show an isotope effect. There is not a significant difference between the values obtained for deuterated and non-deuterated samples at a higher water content. For lower hydration degrees, the activation enthalpy of the first component of the *L* decay was about 45.7 KJ/mol in the case of the deuterated sample, as compared to 27.3 KJ/mol in the non-deuterated one, showing an isotope effect of 1.66. For the second component, the corresponding values were 49.9 KJ/mol in deuterium oxide and 33.6 KJ/mol in water, which means an isotope effect of 1.4. For higher hydration degrees the values of the activation enthalpies were 57.6 KJ/mol in heavy water as compared to 57.3 KJ/mol in water for the slower one, showing the lack of an isotope effect. The same difference between the behaviour at lower and higher humidities is true for the activation entropies (Fig. 3b). Thus, the mean valve of the activation entropy in the case of the *L* decay was −10.3 J/mol K in deuterium oxide and −48.7 J/mol K in water with an isotope effect of 0.21 for the lower hydration

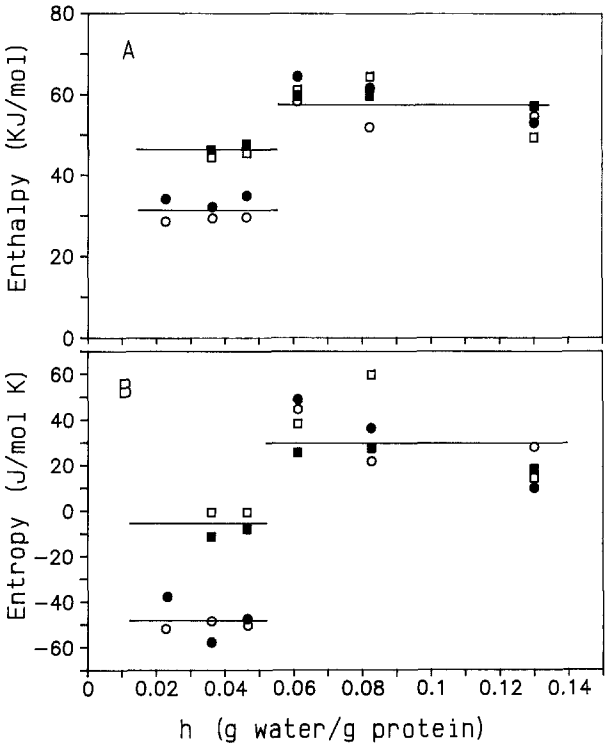

Fig. 3. Dependence of the activation enthalpy **A** and activation entropy **B** of the *L* decay on the water content at 20 °C; ○ fast component in water and ● deuterium oxide; □ slow component in water and ■ deuterium oxide. The fitting error was less than 10%. The lines are drawn just to guide the eye

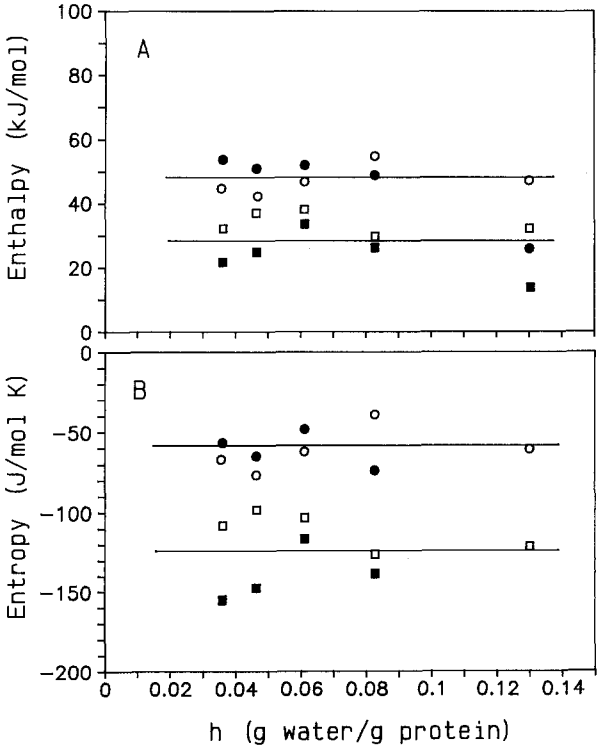

Fig. 4. Dependence of the activation enthalpy **A** and activation entropy **B** of the M_1 decay on the water content at 20 °C: ○ fast component in water and ● deuterium oxide; □ slow component in water and ■ deuterium oxide. The fitting error was less than 15%. The lines are drawn just to guide the eye

degree. At the higher water content the activation entropies were about 30.3 J/mol K in the deuterated sample and 31.8 J/mol K in the non-deuterated one.

In the case of the *M* decay the Eyring plots of the lifetimes show different slopes in water and deuterium oxide at all humidities (Fig. 2c). The calculation of the activation enthalpies surprisingly revealed lower values in the case of deuterated samples, for both components (Fig. 4a). Thus, the mean value of the activation enthalpy was about 33.6 KJ/mol in deuterium oxide as compared to 47 KJ/mol in water, for the fast component of the *M* decay, with a ratio of 0.7 and about 27 KJ/mol in deuterium oxide as compared to 46 KJ/mol in water with a ratio of 0.59 for the slower component. The mean values of the activation entropies were −125.6 J/mol K in the deuterated sample and −61 J/mol in the non-deuterated one, with an isotope effect of about 2.

Discussion

The studies on dried oriented purple membrane samples revealed a modified photocycle, characterized by the lack of proton pumping activity, with the intramolecular return of protons to their initial site (Váró and Keszthelyi 1985; Váró and Lanyi 1991 a). This means that some of the protons can only move from the Schiff base to the Asp 85 and back again to the Schiff base, without having the necessary energy to jump over the barrier and reach the extracellular side. Below a water content of 0.06 gH_2O/gbR a reduced reaction scheme (*K-L-M*-bR) is valid. Previous experiments showed that in dehydrated samples the *L* decay, associated with the proton transfer from the Schiff base to the Asp 85, was accelerated (Váró and Lanyi 1991 a). In our deuterated samples the isotope effect of about 1.57–1.7 obtained for the liftetimes of the *L* decay (Table 1) is much smaller than that of about 5.2 found by Keszthelyi (1982) in suspensions of purple membranes, but it is closer to the values given by Fahr et al. (1981). The difference is probably due to different measuring conditions and analysing methods. On the other hand, the values of the activation enthalpies show an isotope effect of about 1.6 only for strongly dehydrated purple membranes (below 0.06 gH_2O/gbR water content) whereas for a higher hydration this effect disappears (see Fig. 3a). This could be explained by taking into account the presence of water molecules in the vicinity of the Schiff base when the purple membranes are in the normal state of hydration. These water molecules might shield the counterions which contribute to the proton transfer from the Schiff base to Asp 85 (the non-protonated Asp 212 and Asp 85).

On water removal the transient states will become less stable owing to decreased shielding, as stated in previous studies. The energetic barrier will be lower, but in the new more rigid structure the hydrogen/deuterium bonds dominate and so the isotope effect can be observed. At the same time the activation entropies even better reflect the conformational change induced by the strong dehydration. From positive values at a higher water content they pass to negative ones both in water and deuterium oxide, indicating a more packed and rigid conformation. With

an increased water content the protein passes into a more flexible conformation, allowing fluctuations which make the access easier to its different parts. Together with the increased shielding of separate charges this leads to practically the same values for the energetic barriers in water and deuterium oxide as the fluctuations are now the dominating effect. The results obtained for the *M*-bR step with deuterated samples are quite surprising, deviating from the behavior generally expected in a deuteration experiment. It is important to remember that the processed signals reflect only the negative part of the charge displacement connected with the *M*-bR transition at all humidities. This represents the direct return of the proton from the Asp 85 to the Schiff base. A general feature of the *M* decay in dehydrated samples, deuterated or not, is that it slows down, but while the lifetimes for the non-deuterated samples depend on humidity, those in the deuterated ones seem to be independent of it (Table 2). For the slower part of the *M* decay they are smaller than in water. The activation enthalpies are smaller in deuterated samples, showing a more pronounced tendency of deuterons to go back the Schiff base (Fig. 4a). These data are hard to interpret. We consider them as a consequence of the particular way they were obtained, namely by processing only the last negative part of the slow signal for the samples having a humidity above 0.06 g H_2O/g bR and ignoring the positive one. But for very low humidities there was no positive part of the signal and the results were the same. Possible causes for these unexpected changes in deuterated samples could be associated with a modified pK around the Schiff base, lowering the energetic barrier and/or modifications in the lattic which proved to be an important factor for the mechanism of proton transfer, as demonstrated by experiments with bR in monomeric state (Váró and Lanyi 1991b). On the other hand, the negative activation entropies suggest that the *M* intermediates has, in the case of dehydrated bR molecules, another conformation to the normally hydrated one which is characterized by positive values of the entropy.

All the changes reported here for the *L* and *M* decays in dehydrated bacteriorhodopsin show not only a decreased capability of pumping protons/deuterons, but even its total disappearance. They support the idea that each particular structure of a membrane-bound retinal protein has to be able to give an optimal response to the ionic species which is supposed to be transported through the membrane by that protein (Henderson and Schertler 1990b). This involves not only the amino acid chains and the conformation of the chromophore, but also the environment in which they are embedded. Thus, in the case of bacteriorhodopsin, the presence of water molecules is necessary for the proton pumping activity. Recent neutron diffraction studies localize about four tightly bound water molecules in the vicinity of the Schiff base (Papadopoulos et al. 1990). On the other hand, "the proton channel" (Henderson et al. 1990a), allows the placing of water molecules in both the hydrophilic open part, which forms the channel between the Schiff base and the extracellular surface, and the hydrophobic one, which leads from the Schiff base to the cytoplasmic surface. Studies concerning the influence of osmotically active solutes on the photocycle reactions show that of all these reactions, the most strongly influenced by removal of internal water is the proton exchange between the Schiff base and Asp 96 (Cao et al. 1991). This indicates that for maximal proton transfer at least 15 water molecules are required.

The results in this study confirm once more the importance of water in the optimization of the proton pumping in bR.

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References

Braiman MS, Mogi T, Marti T, Stern, Khorana HG, Rothschild KJ (1988) Vibrational spectroscopy of bacteriorhodopsin mutants: Light-driven proton transport involves protonation changes of aspartate residues 85, 96, and 212. Biochemistry 27: 8516–8520

Bryl K, Váró G, Drabent R (1991) The photocycle of bacteriorhodopsin immobilizd in poly (vinyl alcohol) film. FEBS Lett 285:66–70

Butt H-J, Fendler K, Dér A, Bamberg E (1989) Temperature jump study of charge translocation during the bacteriorhodopsin photocycle. Biophys J 56:851–859

Cao Y, Váró G, Chang M, Ni B, Needleman R, Lanyi JK (1991) Water is required for proton transfer from aspartate 96 to the bacteriorhodopsin Schiff base. Biochemistry 30:10972–10979

Fahr A, Lauger P, Bamberg E (1981) Photocurrent kinetics of purple-membrane sheets bound to planar bilayer membranes. J Membrane Biol 60:51–62

Gerwert K, Hess B, Soppa J, Oesterhelt D (1989) Role of aspartate-96 in proton translocation by bacteriorhodopsin. Proc Natl Acad Sci USA 86:4943–4947

Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH (1990a) Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J Mol Biol 213:899–929

Henderson R, Schertler G (1990b) The structure of bacteriorhodopsin and its relevance to the visual opsin and other seven-helix G-protein coupled receptor. Phil Trans R Soc Lond B 326:379–389

Hildebrandt P, Stockburger M (1984) Role of water in bacteriorhodopsin's chromosphere: resonance Raman study. Biochemistry 23:5539–5548

Holz M, Drachev LA, Mogi T, Otto H, Kaulen AD, Heyn MP, Skulachev, Khorana HG (1988) Replacement of aspartic acid-96 by asparagine in bacteriorhodopsin slows both the decay of the *M* intermediate and the associated proton movement. Proc Natl Acad Sci USA 86:2167–2171

Keszthelyi L (1982) Orientation of purple membranes by electric field. Methods Enzymol 88:287–297

Korenstein R, Hess B (1977a) Hydration effects on cis-trans isomerization of bacteriorhodopsin. FEBS Lett 82:7–11

Korenstein R, Hess B (1977b) Hydration effects on the photocycle of bacteriorhodopsin in thin layers of purple membrane. Nature 270:184–186

Kovács I, Váró G (1988) Charge motion in vacuum-dried bacteriorhodopsin. J. Photochem Photobiol B: 1:469–474

Lanyi JK (1984) Bacteriorhodopsin and related light-energy converters. In: Comparative biochemistry: bioenergetics, ed by Ernster, L. Amsterdam: Elsevier, p 315–350

Lozier RH, Bogomolni RA, Stoeckenius W (1975) Bacteriorhodopsin: a lightdriven proton pump in Halobacterium halobium. Biophys J 15:955–963

Lozier RH, Niederberger W, Bogomolni RA, Hwang S, Stoeckenius W (1976) Kinetics and stoichiometry of light-induced proton release and uptake from purple membrane-fragments,

Halobacterium halobium cell envelopes, and phospholipid vesicles containing oriented purple membrane. Biochim Biophys Acta 440:545–556

Marinetti T, Subramaniam S, Mogi T, Marti T, Khorana HG (1989) Replacement of aspartic residues 85, 96, 115, or 212 affects the quantum yield and kinetics of proton release and uptake by bacteriorhodopsin. Proc Natl Acad Sci USA 86:529–533

Oesterhelt D, Stoeckenius W (1974) Isolation of the cell membrane of Halobacterium halobium and its fractionation into red and purple membrane. Methods Enzymol 31:667–678

Otto H, Marti T, Holz M, Mogi T, Lindau M, Khorana HG, Heyn MP (1989) Aspartic acid-96 is the internal proton donor in the reprotonation of the Schiff base of bacteriorhodopsin. Proc Natl Acad Sci USA 86:9228–9232

Papadopoulos G, Dencher NA, Zaccai G, Büldt G (1990) Water molecules and exchangeable hydrogen ions at the active centre of bacteriorhodopsin localized by neutron diffraction. Elements of the proton pathway. J Mol Biol 214:15–19

Pethig R (1979) Dielectric and electric properties of biological materials, John Wiley and Sons, New York, pp. 100–149

Smith SO, Lugtenburg J, Mathies RA (1985) Determination of retinal chromophore structure in bacteriorhodopsin with resonance Raman spectroscopy. J Membr Biol 85:95–109

Soppa J, Oesterhelt D (1989) Bacteriorhodopsin mutants of halobacterium sp. GRB 1. The 5-bromouridine-selection as a method to isolate point mutants in halobacteria. J Biol Chem 264:13043–13048

Stern LJ, Ahl PL, Marti T, Mogi T, Duñach M, Berkovitz S, Rothschild KJ, Khorana HG (1989) Substitution of membrane-embedded aspartic acids in bacteriorhodopsin causes specific changes in different steps of the photochemical cycle. Biochemistry 28:10035–10042

Stoeckenius W, Lozier RH, Bogomolni RA (1979) Bacteriorhodopsin and the purple membrane of halobacteria. Biochim Biophys Acta 505:215–278

Tittor J, Soell C, Oesterhelt D, Butt H-J, Bamberg E (1989) A defective proton pump, point-mutated bacteriorhodopsin Asp 96 → Asn is fully reactivated by azide. EMBO J 8:3477–3482

Váró G (1981) Dried oriented purple membrane samples. Acta Biol Sci Hung 32:301–310

Váró G, Eisenstein L (1987) Infrared studies of water induced conformational changes in bacteriorhodopsin. Eur Biophys J 14:163–168

Váró G, Keszthelyi L (1983) Photoelectric signals from dried oriented purple membranes of Halobacterium halobium. Biophys J 43:47–51

Váró G, Keszthelyi (1985) Arrhenius parameters of the bacteriorhodopsin photocycle in dried oriented samples. Biophys J 47 (2 Pt 1):243–246

Váró G, Lanyi JK (1990) Pathways of the rise and decay of the *M* photointermediate of bacteriorhodopsin. Biochemistry 29:2241–2250

Váró G, Lanyi JK (1991 a) Distortions in the photocycle of bacteriorhodopsin at moderate dehydration. Biophys J 59:313–322

Váró G, Lanyi JK (1991 b) Effects of the crystalline structure of purple membrane on the kinetics and energetics of the bacteriorhodopsin photocycle. Biochemistry 30:7165–7171