

THE PINK MEMBRANE: THE STABLE PHOTOPRODUCT OF DEIONIZED PURPLE MEMBRANE

CHUNG-HO CHANG, SU-YI LIU, ROY JONAS, AND RAJNI GOVINDJEE

Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

ABSTRACT When cations are removed from the purple membrane of *Halobacterium halobium* it turns blue ($\lambda_{\max} = 603$ nm); continuous irradiation with intense red light ($\lambda \geq 630$ nm) converts this deionized blue membrane into a pink membrane ($\lambda_{\max} \approx 491$ nm). The rate and extent of the transformation from the blue to the pink membrane is facilitated by the removal of the last twenty COOH-terminal amino acids of bacteriorhodopsin. While the chromophore of the blue membrane is a 32:68 mixture of the 13-*cis* and all-*trans* isomers of retinal, the chromophore of the pink membrane is 9-*cis* retinal. The quantum efficiency of the pink to blue membrane photoconversion is relatively high compared with that of the blue to pink membrane photoconversion. Proton release is observed when the pink membrane is converted to the blue form, and proton uptake occurs during the reverse transition. Unlike the blue membrane, the absorbance maximum of the pink membrane is only slightly affected by cation addition at low pH and ionic strength.

INTRODUCTION

Light absorbed by bacteriorhodopsin leads to proton translocation across the purple membrane of *Halobacterium halobium*. The chromophore of bacteriorhodopsin is retinal, attached to the protein via a protonated Schiff base to the ϵ -amino group of a lysine (see reviews by Ebrey, 1982; Stoekenius and Bogomolni, 1982). The purple membrane binds ~ 4 mol of Ca^{2+} and Mg^{2+} per mole of bacteriorhodopsin at pH 6.0 (Chen et al., 1984; Chang et al., 1985). After these divalent cations are removed, bacteriorhodopsin loses its purple color and turns blue ($\lambda_{\max} = 603$ nm, the deionized blue membrane) (Kimura et al., 1984; Chang et al., 1985). Thus divalent cations play a role in regulating the color of the pigment.

The purple membrane also turns blue when the pH of the medium is lowered to 2.0 (the acid blue membrane) (Oesterhelt and Stoekenius, 1971). Extended exposure of the acid blue membrane to red light ($\lambda \geq 670$ nm) converts it into a pink species ($\lambda_{\max} \approx 500$ nm, the acid pink membrane) (Maeda et al., 1980; Fischer et al., 1981). In a previous paper (Chang et al., 1985), we reported that the formation of the blue membrane at acid pH's is due to the loss of Ca^{2+} and Mg^{2+} from their binding sites, and that the photochemistry of the acid blue membrane is the same as that of the deionized blue membrane. The only distinguishable difference between the two membranes is that the acid blue membrane aggregates but the deionized blue membrane does not.

In this paper, we show that intense red light can convert the deionized blue membrane into a stable pink-colored

membrane ($\lambda_{\max} \approx 491$ nm), the deionized pink membrane. The deionized pink membrane studied here is quite similar to the acid pink membrane (Fischer et al., 1981; Maeda et al., 1980). Using different types of the blue and pink membrane samples, we have performed a series of experiments to investigate both the nature of the pink membrane and its relationship to the blue and purple membranes.

MATERIALS AND METHODS

Preparation of the Purple Membrane and Various Types of the Blue Membrane

The purple membrane was prepared from *Halobacterium halobium* strain S-9 cells according to the method of Becher and Cassim (1975), except that the DNase treatment was omitted. The EDTA-treated, papain/EDTA-treated, and 1-aminonaphthalene-3,6,8-trisulfonic acid (ANTS)-treated blue membranes were prepared as described previously (Chang et al., 1985).

Treatment of the purple membrane with 5 mM MgCl_2 leads to all of the divalent cation binding sites being occupied by Mg^{2+} (Chang et al., 1985); this was used therefore to insure a standard preparation. Some of the EDTA-treated and papain/EDTA-treated blue membranes were also incubated with 5 mM MgCl_2 , which caused them to turn purple. After treatment, the membrane suspensions were washed with distilled water by centrifugation seven times (20 K for 35 min [model J2-21; Beckman Instruments, Inc., Fullerton, CA]) and then resuspended in distilled water.

Preparation of Various Types of the Pink Membranes

To prepare the pink membrane, a sample of the blue membrane contained in a quartz cuvette was irradiated with red light ($\lambda \geq 630$ nm; 150-W slide projector plus a CS 2-58 glass cutoff filter; [Corning Glass Co., Corning, NY] the distance between the sample and projector was ~ 6.5 cm). The sample cuvette was cooled by ice water during illumination. The final pH of the samples was ~ 4.5 .

The pink membrane, prepared from the EDTA-treated or papain/

Dr. Chang's present address is Department of Cell Biology, Stanford University, Stanford, California 94305.

EDTA-treated blue membrane, was also incubated with 5 mM MgCl_2 in distilled water, $\sim\text{pH}$ 5.5. The membrane suspensions were washed seven times with distilled water (20 K for 35 min [model J2-21; Beckman Instruments, Inc.]) and then resuspended in distilled water. The concentration of the pink membrane was calculated by photoconverting the Mg^{2+} -treated pink membrane to the purple membrane and assuming the extinction coefficient of the light-adapted purple membrane is $62,700 \text{ cm}^{-1} \text{ M}^{-1}$ (Rehorek and Heyn, 1979). (The above method gives only an approximate estimate of the concentration of the pink membrane because the photoconversion of the pink membrane back to purple membrane is not 100% [see below]; therefore, the estimated concentration of the pink membrane may be slightly low.)

Dry films of the pink membrane were prepared by evaporating a drop of the pink membrane suspended in water, under vacuum, on a quartz slide.

Atomic Absorption Spectrophotometry

An atomic absorption spectrophotometer (model 305; Perkin-Elmer Corp., Instrument Div., Norwalk, CT) equipped with Mg^{2+} cathode tube was used to determine the concentration of Mg^{2+} in the membrane samples.

Calcium Electrode Measurement

A Ca^{2+} microelectrode made from a silicon-treated glass capillary containing the ionophore ETH1001 was calibrated according to the method of Tsien and Rink (1980). The tip diameter of the electrode was 0.7 mm. The electrode was made 4 h before the measurement.

Chromophore Extraction

The chromophore was extracted from the membranes using the retinal oxime extraction method (Tsuda, 1982). The isomeric composition was determined by high performance liquid chromatography (HPLC) using an HPLC System (model 60000A; Waters Associates, Millipore Corp., Milford, MA) equipped with a μ -Porasil column and a Schoeffel Model SF 7870 detector (Schoeffel Instruments Div., Westwood, NY) set at 365 nm. The retinal oxime peaks were identified by comparison of their retention times with those of the authentic retinal isomers. Since both syn and anti retinal oxime conformations exist, but only the anti-isomers of the various retinal oximes can be sufficiently separated by HPLC, the areas of the anti-oxime peaks were measured to calculate the relative

concentration of each isomer. The following extinction coefficient ratios at 365 nm were used: all-*trans* anti/13-*cis* anti = 1.00, all-*trans* anti/11-*cis* anti = 1.71, and all-*trans* anti/9-*cis* anti = 1.11. The syn/anti ratios were determined by converting pure samples of 13-*cis*, 11-*cis*, 9-*cis*, and all-*trans* retinals into retinal oximes. Under our conditions the syn/anti isomer ratios were 2.5 ± 0.4 , 2.0 ± 0.4 , 2.5 ± 0.4 , and 2.5 ± 0.4 for 13-*cis*, 11-*cis*, 9-*cis*, and all-*trans* retinal oximes, respectively (20–30 trials). The total percentage of each retinal isomer was then obtained by calculating the relative amount of the syn-oxime and adding it to the anti-oxime.

RESULTS

Formation of Various Types of Pink Membrane

The purple membrane turns blue ($\lambda_{\text{max}} \approx 603 \text{ nm}$) upon removal of the bound Ca^{2+} and Mg^{2+} by any of the various treatments discussed in Chang et al. (1985, 1986). The λ_{max} for the EDTA-treated, papain/EDTA-treated, and ANTSA-treated blue membranes are at 603, 603, and 593 nm, respectively. When a sample of the blue membrane is illuminated for up to 3 h by red light ($\lambda' \geq 630 \text{ nm}$; 150-W projector plus CS 2-58 glass cutoff filter [Corning Glass Co.]) it is slowly converted into a 491-nm species (the pink membrane) (Fig. 1) with a minor band at 340 nm (Fig. 2). The fairly good isosbestic point at 530 nm (Fig. 1) indicates that only two principle species are involved in this photochemical conversion. The pigment undergoes a significant conformational change during the transition between the blue and pink membranes as reflected by changes in the extinction coefficient of the aromatic amino acids at 280 nm (Fig. 2).

A blue membrane sample cannot be completely converted into the pink membrane because the quantum efficiency for the pink to the blue membrane transition is much greater than the blue to the pink membrane transition (see next section). The residual absorption at $\sim 600 \text{ nm}$

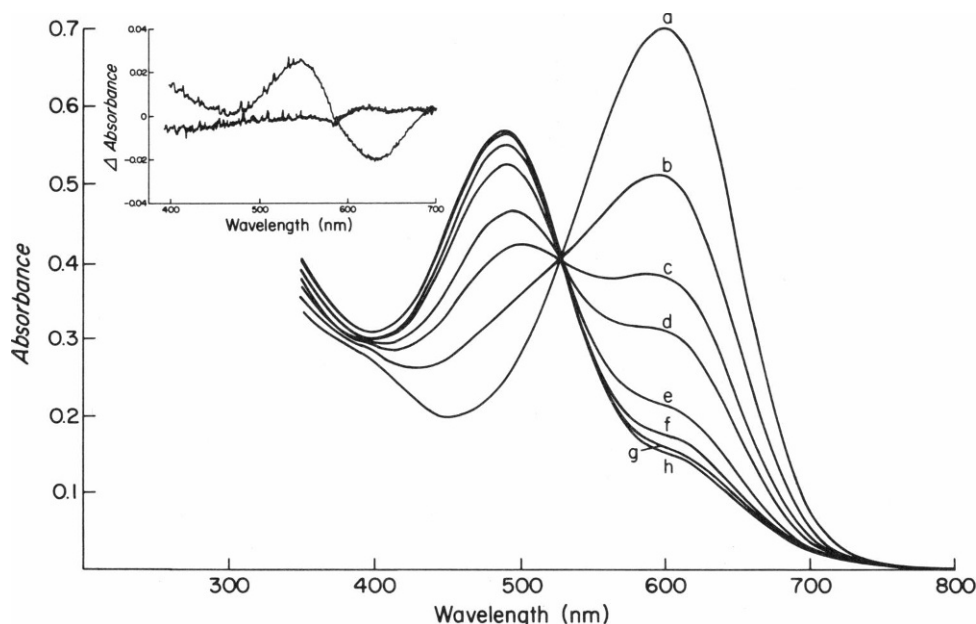


FIGURE 1 Photoconversion of the EDTA-treated blue membrane to the pink membrane. The irradiation times were: (a) 0, (b) 10, (c) 20, (d) 35, (e) 60, (f) 90, (g) 150, and (h) 300 min (150-W projector plus CS 2-58 glass cutoff filter [Corning Glass Co.], $\lambda' \geq 630 \text{ nm}$). Inset: The difference spectrum observed by adding Ca^{2+} to the pink membrane showing the transition of the residual blue membrane to the purple membrane. Sample h was used for this measurement.

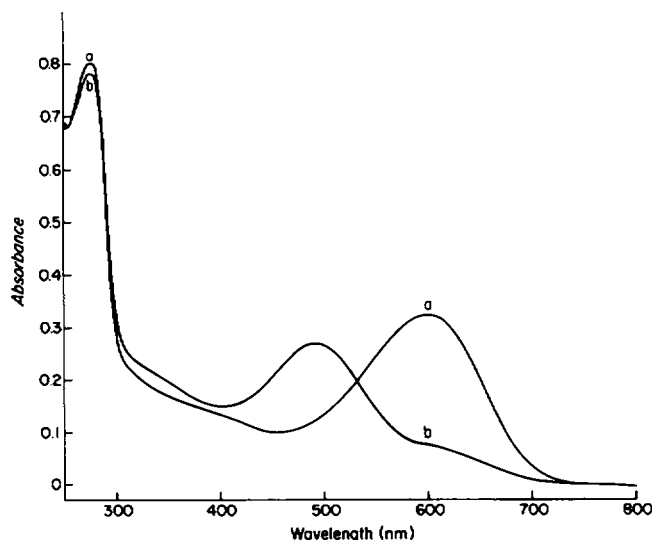


FIGURE 2 Photoconversion of the pink (curve *b*) to the blue (curve *a*) membrane. The increase in absorbance at 280 nm indicates a change in the environment of the aromatic amino acid residues.

is due to the blue membrane. This is demonstrated by the typical difference spectrum of the blue to the purple membrane conversion which is seen when cations are added to the pink membrane (Fig. 1, *inset*). Interestingly, the absorption band of the pink membrane at 491 nm is only slightly affected by cation addition at low pH and ionic strength. However, at high ionic strength, a slight decrease in absorbance at 500 nm and an increase at 420 nm is observed. We do not know if this is actually a cation-specific effect or an effect due to changes in the local pH (see below) caused by differences in the ionic

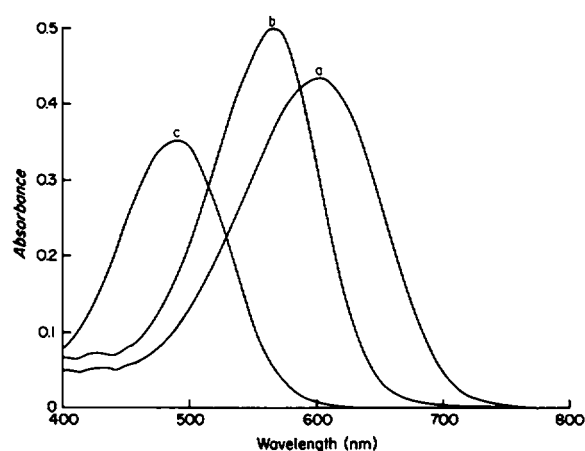


FIGURE 3 The relationship of the pink, blue, and purple membranes. Spectra: (a) the blue membrane; (b) the purple membrane; (c) the pink membrane. The purple membrane was prepared from the blue membrane by the addition of 10 mM Tris(hydroxymethyl)aminomethane (TRIS) buffer, pH 8.0, and 0.1 mM CaCl_2 . The sample was then light adapted. The spectrum of the pink membrane was obtained by a method similar to that of Maeda et al. (1980). The scattering contribution to the spectra was corrected by subtracting a bleached membrane spectrum normalized to the purple membrane spectrum at 280 nm.

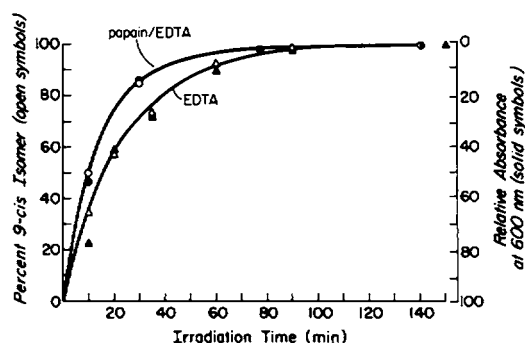


FIGURE 4 Concomitant isomeric and spectral changes associated with the formation of the pink membrane from the EDTA and papain/EDTA-treated blue membranes as a function of time of irradiation. Note that the papain/EDTA-treated sample converts faster.

strength of the suspension. Based on the absorption at 600 nm and the isomeric composition (see below), the concentration of the blue membrane in the samples was ~10–15% of the total pigment for the EDTA-treated membrane and 5–10% for the papain/EDTA-treated pink membrane (data not shown). The calculated “pure” pink membrane spectrum (with the contribution of the blue membrane removed) is shown in Fig. 3 along with both the blue and purple membrane spectra. The maximum absorbance of the pink membrane is ~491 nm. Longer irradiation times could convert slightly more of the blue membrane into the pink membrane, but destroyed some of the pigment, especially in the case of the papain/EDTA-treated and ANTSA-treated blue membranes. The photoconversion of the blue membrane to the pink membrane was improved by cooling the samples to 4°C.

The rate of the pink membrane formation is different for the papain/EDTA-, EDTA-, and ANTSA-treated blue membrane samples. Using the decrease in the absorbance at 600 nm as an index of the formation of the pink membrane, Fig. 4 shows that the rate of pink membrane formation from the papain/EDTA-treated blue membrane is ~1.5 times that of EDTA-treated blue membrane.

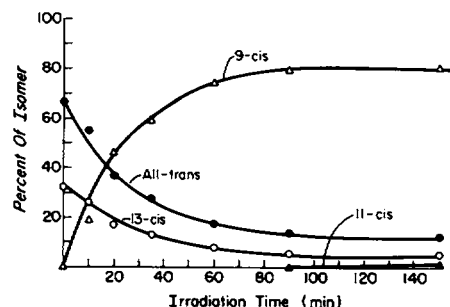


FIGURE 5 Changes in the retinal isomer composition during the transition from the blue to the pink membrane by irradiation with red light (λ 's ≥ 630 nm, 150-W slide projector plus CS 2-58 glass cutoff filter [Corning Glass Co.]). The final retinal percentages were ~80% 9-*cis* retinal, 13% all-*trans* retinal, 4-5% 13-*cis* retinal, and 3% 11-*cis* retinal for the trial shown in this figure.

Incorporating ANTSA into bacteriorhodopsin slows down the rate of the blue to the pink membrane transition (data not shown). The rate of the pink membrane formation from the ANTSA-treated blue membrane is increased when its COOH-terminal tail is removed by further treatment with papain (data not shown). Thus removing the COOH-terminal tail from bacteriorhodopsin enhances the rate of the blue to the pink membrane transition.

The absorption spectrum of the pink membrane is pH dependent. As the pH increases, the pink membrane peak at 491 nm decreases, while a new peak appears at ~420 nm (data not shown). The ~420-nm species is probably similar to the short wavelength species observed by Maeda et al. (1980) and Fischer et al. (1981) when the pH of the acid pink membrane was increased.

Isomeric composition of the chromophore of the pink membrane was determined by HPLC. Fig. 5 shows the changes in retinal isomer composition during irradiation of the EDTA-treated blue membrane. The blue membrane contains all-*trans* and 13-*cis* retinal in the ratio of 68:32. The amount of 9-*cis* retinal increased with time at the expense of the all-*trans* and 13-*cis* retinal isomers, and reached ~80% of the total isomer composition within 3 h of irradiation (other trials resulted in 80–85% 9-*cis* retinal). The ratio of 13-*cis* to all-*trans* retinal remains unchanged during conversion to pink membrane. This suggests that either the two isomers convert to 9-*cis* at the same rate or only one of them converts to 9-*cis* while subsequent equilibration of the isomer converted keeps the ratio of the 13 *cis* and *trans* isomer constant. By comparing the disappearance of the 600-nm peak, due to the blue membrane, in Fig. 1 with the time course of the appearance of the 9-*cis* isomer (Fig. 4), it is apparent that the chromophore of the pink membrane is almost exclusively 9-*cis* retinal. Fig. 5 also shows that there is a small amount of 11-*cis* retinal ($\leq 3\%$) present during irradiation, whose significance is not clear. Maeda et al. (1980) obtained similar, yet slightly different results for the acid blue and the acid pink membranes. They reported that the acid blue membrane had an all-*trans*:13-*cis* retinal ratio of 60:40 and that the acid pink membrane had 75% 9-*cis* retinal, 15% all-*trans* retinal, 10% 13-*cis* retinal, and <1% 11-*cis* retinal.

The Pink to Blue and the Ca^{2+} -Pink to Purple Membrane Transitions

When the pink membrane is irradiated with a broad band of blue light (150-W projector plus CS 5-60 glass filter [Corning Glass Co.]) it is rapidly converted back to the blue membrane (Fig. 6). The isosbestic point is at ~530 nm, which is the same as in the blue to pink membrane transition. In contrast to the formation of the pink membrane, which takes hours, the pink to blue membrane transition is complete within a few minutes. However, the

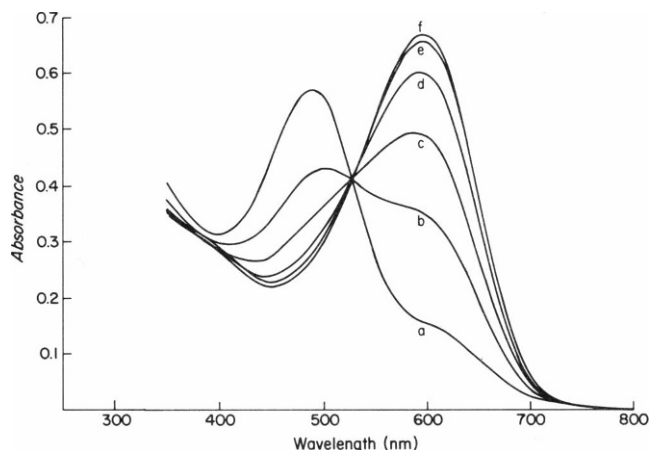


FIGURE 6 Photoconversion of the EDTA pink membrane to the blue membrane as a function of irradiation time (150-W projector plus CS glass 5-60 filter [Corning Glass Co.]). The irradiation times were: (a) 0, (b) 10, (c) 25, (d) 50, (e) 120, and (f) 1,920 s.

absorbance of the blue membrane at 600 nm does not quite return to what it was before the photoconversion to the pink membrane. The recovery of the EDTA-treated blue membrane (~97%) is higher than that of the papain/EDTA-treated blue membrane (~92%). Again, the cleavage of the COOH-terminal tail of bR affects the pink to blue membrane transition. The rate of the pink to blue membrane transition is faster for the papain/EDTA pink membrane than for the EDTA pink membrane (Fig. 7). It seems that removal of the COOH-terminal tail somehow facilitates the phototransitions.

When a suspension of the blue membrane is photoconverted to the pink membrane the pH of the suspension increases (data not shown). This suggests that something in the membrane suspension is protonated during the blue to the pink membrane transition. Since the transition from the blue to the pink membrane requires several hours of irradiation, too long for the stable monitoring of the pH, we measured changes in the pH of the medium during the pink to blue membrane transition. Fig. 8 (left side) shows

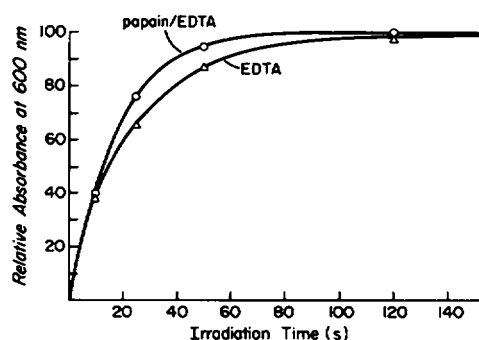


FIGURE 7 The formation of the blue membrane from the EDTA and the papain/EDTA pink membranes as a function of irradiation time. Same irradiation conditions as in Fig. 6.

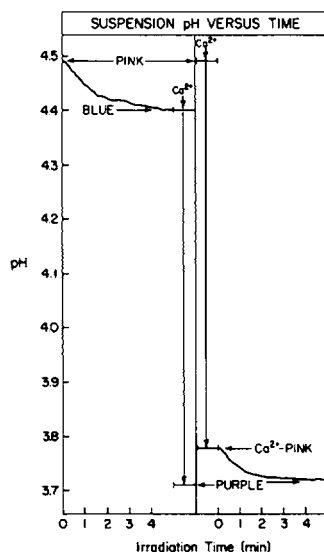


FIGURE 8 pH changes during the pink to blue membrane photoconversion (*left side*) and the Ca^{2+} -pink to purple membrane photoconversion (*right side*). pH changes observed by cation addition ($15 \mu\text{l}$ of 0.1 M CaCl_2) to the blue membrane (*left side*) and to the pink membrane (*right side*) are also included. Approximately 4.8×10^{-8} mol of bR was suspended in 4 ml of deionized water for the experiments. The samples were constantly stirred by a small magnetic stirrer and the pH electrode was completely covered to avoid light artifacts. Samples were titrated with HCl to determine the number of protons.

that the pH decreases during the transition from the pink to the blue membrane, indicating that a proton(s) is/are released and some functional group(s) in the membrane is/are deprotonated during this transition. Under the condition described in the figure legend, an average of 0.7 mol H^+ /mol bR was released. 13.1 protons were released when Ca^{2+} was added to the blue membrane (Fig. 8, *left side*).

The absorption spectrum of a dry film of the pink membrane is identical to that of a suspension of the pink membrane in water. However, the light-induced transition between the pink and the blue membranes is inhibited in dry films. This suggests that water is required in the transition and that the deprotonation of the functional group(s) during the transition between the pink and blue membranes is mediated by water.

As noted above, unlike the blue membrane, the color of the pink membrane is only slightly affected by divalent cations at low pH. We and others have reported that when the blue membrane is converted into the purple membrane by adding Ca^{2+} , the pH of the medium decreases (Kimura et al., 1984; Chang et al., 1985, 1986). We also see this decrease of pH with the formation of Ca^{2+} -pink membrane (Fig. 8, *right side*). Approximately 11.2 H^+ per bR were released during the pink to Ca^{2+} -pink membrane transition. Irradiation of the Ca^{2+} -pink membrane leads to the formation of the purple membrane. Like the pink to the blue membrane transition, the Ca^{2+} -pink to purple membrane transition is very rapid, taking ~ 2 min of irradiation under our experimental conditions. The pH changes during the Ca^{2+} -pink to purple membrane transition is shown in Fig. 8 (*right side*) and was determined to be equivalent to 1.9 H^+ per bR. The above results can be interpreted as follows: when the pink membrane is converted into the Ca^{2+} -pink membrane by Ca^{2+} addition, protons are released; additional protons are released when the Ca^{2+} -pink membrane is irradiated and becomes purple.

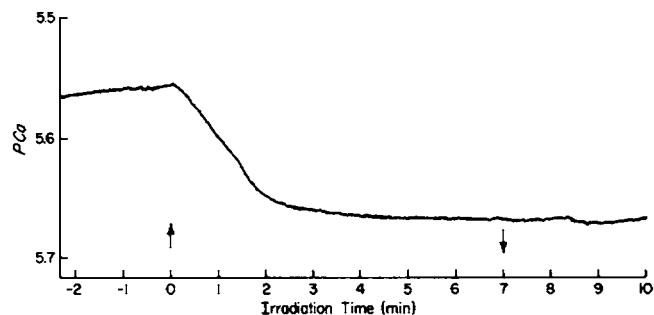


FIGURE 9 Changes in calcium binding during the pink to purple membrane photoconversion. The measurement of pCa was made with a Ca^{2+} -sensitive microelectrode. The upward arrow indicates the onset of irradiation and the downward arrow indicates the end of irradiation. The concentration of bacteriorhodopsin was $12 \mu\text{M}$. The concentration of free Ca^{2+} was $2.9 \mu\text{M}$ before the irradiation and $2.1 \mu\text{M}$ after the photoconversion to the purple membrane. The sample was in 2 mM HEPES buffer, pH 6.8.

Cation Changes During the Pink to the Purple Membrane Transition

Atomic absorption spectrophotometry measurements of our samples show that the MgCl_2 -treated pink membrane binds approximately the same number of moles of Mg^{2+} per mole of pigment as the MgCl_2 -treated purple membrane, 3.0–4.5, depending upon the pH of the deionized water (but see below).

We also used a calcium-sensitive electrode to monitor the changes in free calcium concentration when the Ca^{2+} -loaded pink membrane is photoconverted to the purple membrane. As shown in Fig. 9, upon irradiation with 500 nm light, the free Ca^{2+} in the medium decreases at about the same rate as the Ca^{2+} -pink membrane photoconverts back to the purple membrane, indicating that the purple

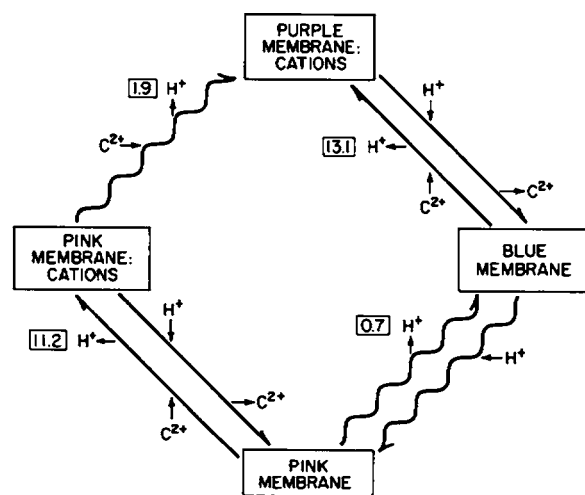


FIGURE 10 Schematic representation of the proton and cation changes accompanying the interconversions between the purple, blue, pink, and Ca^{2+} -pink membranes.

membrane binds slightly more calcium than the Ca^{2+} -pink membrane at pH 6.8. We could not precisely measure this Ca^{2+} uptake because of problems with quantitatively reproducing the small microelectrode changes (in Fig. 9 $\sim 0.07 \text{ Ca}^{2+}$ per bacteriorhodopsin are taken up). We assume that the change involves a small number of Ca^{2+} ions, which could not be detected by atomic absorption measurements.

Additionally, the effect of cleaving the COOH-terminal tail on the binding of Mg^{2+} was studied in deionized water with the MgCl_2 /papain-treated pink membrane and the MgCl_2 /papain-treated purple membrane by atomic absorption spectrophotometry. The results indicate that cleaving the COOH-terminal tail from bR slightly reduces the Mg^{2+} binding to both the pink and the purple membranes (data not shown). The total scheme of the proton and cation changes during the color transitions is summarized in Fig. 10.

DISCUSSION

The Pink Membrane

The purple membrane turns blue at acidic pHs (the acid blue membrane) (Oesterhelt and Stoerkenius, 1971). In a previous paper we reported that the purple membrane, which normally has several Ca^{2+} and Mg^{2+} ions bound per bacteriorhodopsin, turns blue when the bound cations are removed, and lowering the pH results in the loss of the bound Ca^{2+} and Mg^{2+} (Chang et al., 1985). Maeda et al. (1980) and Fischer et al. (1981) reported that irradiation of the acid blue membrane also leads to the formation of a pink species ($\lambda_{\text{max}} \approx 500 \text{ nm}$) (the acid pink membrane). The chromophore of the acid pink membrane was shown to be primarily 9-*cis* retinal. Here we have developed a method for preparing the pink membrane at less acidic pH's. Such a preparation has several advantages over the acid pink membrane since at very low pH: (a) the membrane samples aggregate, giving samples of poor optical quality; (b) with the high background concentration of protons, small pH changes that occur during the transitions between the pink and the blue membranes and between the Ca^{2+} -treated pink and the purple membranes are difficult to detect; and (c) the pigment may have an abnormal conformation at very low pH's.

The experiments in this study have established several interesting points: (a) the papain/EDTA-, EDTA-, and ANTSA-treated blue membrane samples can all be slowly photoconverted into the pink membrane; however, their rates of conversion are different. The papain/EDTA-treated samples have the highest quantum efficiency of conversion while the ANTSA-treated samples have the lowest. It is possible that the proteolysis and ANTSA-treatment lead to changes in the protein conformation that favor or disfavor, respectively, the photoconversions. Alternatively, since papain cleaves four carboxyl groups from the COOH-terminal tail and ANTSA introduces two extra

negative charges per mole of ANTSA incorporated per pigment (generally two to four ANTSA are incorporated per bacteriorhodopsin [Chang et al., 1985, 1986]), it may be that the surface potential or the local electrostatic environment can affect the rate of the blue to pink membrane transition; if so, an increase in the negative surface potential would slow down the blue to pink color transition. (b) The reverse photochemical reaction (i.e., pink to blue or pink to purple color transition) has a much higher quantum efficiency than the blue to pink transition (about fifty-five times [Liu, S., and T. Ebrey, manuscript in preparation]), and the quantum efficiency of the papain-treated membrane is slightly higher than the unproteolysed samples. (c) The chromophore of the pink membrane is 9-*cis* retinal. This result is also confirmed by resonance Raman (Pande et al., 1986) and Fourier Transform Infrared Spectroscopy (Chang et al., 1987). (d) The protein undergoes a conformational change during the transitions between the blue and pink membranes. The absorbance at 280 nm due to the aromatic amino acids is less in the pink membrane. (e) Proton release is observed during the pink to blue membrane transition. Moreover, Fourier Transform Infrared Spectroscopy shows that carboxyl groups of bacteriorhodopsin undergo changes during the transition from the pink to the blue membrane (Chang et al., 1987). Therefore, the above experiments suggest that aspartic or glutamic residues in bacteriorhodopsin are involved in the pink to blue membrane transitions. (f) The pink to blue membrane transition is inhibited in dry films. In dried films, most carboxyl groups are already protonated. Thus, since proton release is observed when the pink membrane is converted to the blue membrane, it may be that the blocking of this proton release, due to the acceptor group(s) (the surface carboxyls) being protonated in the dry film, causes the inhibition of the pink to blue membrane photoconversion.

COOH-Terminal Tail of Bacteriorhodopsin

There has been a controversy about both the conformation and the functional role of the COOH-terminal tail of bacteriorhodopsin (Wallace and Henderson, 1982; Govindjee et al., 1982; Renthall et al., 1983; Govindjee et al., 1984; Liao and Khorana, 1984; Ovchinnikov et al., 1986; Marque et al., 1986). In this paper we examined the effect of removing the COOH-terminal tail with papain on the blue to pink, the pink to blue, and the Ca^{2+} -pink to purple membrane transitions, and on divalent cation binding to the purple membrane. The rate and yield of the blue to pink membrane transition is higher in the papain-treated sample. The rates of the pink to blue and Ca^{2+} -pink to purple transitions are also higher in papain-treated compared with unproteolysed samples; however, the yield is less in the papain-treated sample. It seems that the removal of COOH-terminal residues with papain enhances the different membrane phototransformations. We also found that cleaving the COOH-terminal tail slightly decreases

the number of cations bound to the purple and pink membranes.

We thank Dr. Ebrey for his support and help in writing this manuscript and to Burr Nelson for determining the retinal compositions.

This work was supported by National Science Foundation grant PCM 83 40569 and Department of Energy grant DEA CO2-82-ER12087 to Thomas G. Ebrey.

Received for publication 27 January 1987 and in final form 1 June 1987.

REFERENCES

- Becher, B., and J. Y. Cassim. 1975. Improved isolation procedures for the purple membrane of *Halobacterium halobium*. *Prep. Biochem.* 5:161-178.
- Chang, C.-H., J.-G. Chen, R. Govindjee, and T. Ebrey. 1985. Cation binding by bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA.* 82:396-400.
- Chang, C.-H., R. Jonas, S. Melchiorre, R. Govindjee, and T. Ebrey. 1986. Mechanism and role of divalent cation binding of bacteriorhodopsin. *Biophys. J.* 49:731-739.
- Chang, C.-H., R. Jonas, T. Ebrey, M. Hong, and L. Eisenstein. 1987. Biophysical studies of retinal proteins. T. Ebrey, H. Frauenfelder, B. Honig, and K. Nakanishi, editors. University of Illinois Press, Urbana, Illinois.
- Chen, J.-G., C.-H. Chang, R. Govindjee, and T. Ebrey. 1984. *Biophys. J.* Bacteriorhodopsin's color is partially due to divalent cations. 45:214a. (Abstr.)
- Ebrey, T. 1982. Light-energy transduction in *Halobacterium halobium*. *Membr. Transport.* 2:323-328.
- Engleman, D. M., R. Henderson, A. D. McLachlan, and B. A. Wallace. 1980. Path of the polypeptide in bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA.* 77:2023-2027.
- Fischer, U. C., P. Townner, and D. Oesterhelt. 1981. Light induced isomerization, at acidic pH, initiates hydrolysis of bacteriorhodopsin to bacterio-opsin and 9-*cis*-retinal. *Photochem. Photobiol.* 33:529-537.
- Govindjee, R., K. Ohno, and T. G. Ebrey. 1982. Effect of the removal of the COOH-terminal region of bacteriorhodopsin on its light-induced H⁺ changes. *Biophys. J.* 38:85-87.
- Govindjee, R., K. Ohno, C.-H. Chang, and T. G. Ebrey. 1984. The C-terminal tail of bacteriorhodopsin: its conformation and role in proton pumping. In *Information and Energy Transduction in Biological Membranes*. C. L. Bolis, E. J. Helmreich, and H. Passow, editors. Alan R. Liss, Inc., New York. 13-25.
- Kimura, Y., A. Ikegami, and W. Stoeckenius. 1984. Salt and pH-dependent changes of the purple membrane absorption spectrum. *Photochem. Photobiol.* 40:641-646.
- Liao, M.-J., and H. G. Khorana. 1984. Removal of the C-terminal peptide does not affect refolding or function of bacteriorhodopsin as a light-dependent proton pump. *J. Biol. Chem.* 259:4194-4199.
- Maeda, A., T. Iwasa, and T. Yoshizawa. 1980. Formation of 9-*cis* and 11-*cis*-retinal pigments from bacteriorhodopsin by irradiating purple membrane in acid. *Biochemistry.* 19:3825-3831.
- Marque, J., K. Kinoshita, Jr., R. Govindjee, A. Ikegami, T. G. Ebrey, and J. Otomo. 1986. Environmental modulation of C-terminus dynamic structure in bacteriorhodopsin. *Biochemistry.* 25:5555-5559.
- Oesterhelt, D., and W. Stoeckenius. 1971. Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. *Nature (Lond.)*. 233:149-152.
- Ovchinnikov, Yu. A., N. G. Abdulaev, A. V. Kiselev, L. A. Drachev, A. D. Kavlen, and V. P. Skulachev. 1986. The water-exposed C-terminal sequence of bacteriorhodopsin does not affect H⁺ pumping. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 194:16-20.
- Pande, C., R. H. Callender, C.-H. Chang, and T. Ebrey. 1986. Resonance Raman study of the pink membrane photochemically prepared from the de-ionized blue membrane of *H. halobium*. *Biophys. J.* 50:545-549.
- Renthal, R., N. Dawson, J. Tuley, and P. Horowitz. 1983. Constraints on the flexibility of bacteriorhodopsin's C-terminal tail at the purple membrane surface. *Biochemistry.* 22:5-12.
- Rehorek, M., and M. P. Heyn. 1979. Binding of all-*trans*-retinal to the purple membrane. Evidence for cooperativity and determination of the extinction coefficient. *Biochemistry.* 22:4977-4983.
- Stoeckenius, W., and R. A. Bogomolni. 1982. Bacteriorhodopsin and related pigments of *Halobacteria*. *Annu. Rev. Biochem.* 52:587-616.
- Tsien, R. Y., and T. J. Rink. 1980. Neutral carrier ion-selective micro-electrodes for measurement of intracellular free calcium. *Biochim. Biophys. Acta.* 599:623-638.
- Tsuda, M. 1982. Methods for extraction of pigment chromophore. *Methods Enzymol.* 88:552-561.
- Wallace, B. A., and R. Henderson. 1982. The location of the C-terminal of bacteriorhodopsin in purple membrane. *Biophys. J.* 39:223-239.