PHOTOCHEMISTRY OF TWO RHODOPSINLIKE PIGMENTS IN BACTERIORHODOPSIN-FREE MUTANT OF *HALOBACTERIUM HALOBIUM*

NORIO HAZEMOTO, NAOKI KAMO, YOSHIO TERAYAMA,
YONOSUKE KOBATAKE, AND MOTOYUKI TSUDA
Department of Physics, Sapporo Medical College, Sapporo 060, Japan

ABSTRACT Two photocycles due to two different pigments were found in membrane vesicles of a bacteriorhodopsin-free mutant of *Halobacterium halobium*. A pigment absorbing ~590 nm halorhodopsin (HR) underwent a faster photocycle with a phototransient at ~490 nm (half-time of decay, $\tau_{1/2}$ = 10 ms). Another third rhodopsinlike pigment (TR) absorbing ~580 nm underwent a slower photocycle accompanying a phototransient absorbing below 410 nm ($\tau_{1/2}$ = 0.8 s). The photocycles were measured under various conditions of temperature, NaCl concentration, pH, and in the presence of cholate. All results obtained support the notion that the two photocycles are independent of each other, and the fast or the slow cycle can be abolished after these treatments. At alkaline pH, the wavelength of maximum absorbance of both pigments shifted to blue, but the magnitude of the shift of the pigment undergoing the slow photocycle was much greater than the other. The ratio of the content of the two pigments varies among bacteriorhodopsin-free mutants.

INTRODUCTION

It is well known that under appropriate conditions the cytoplasmic membrane of *Halobacterium halobium (H. halobium)* contains bacteriorhodospin (BR), which transports protons actively from inside to outside upon illumination (1). This transport is detected by an acidification in the medium. In 1977 Matsuno-Yagi and Mukohata (2, 3) isolated an apparently BR-free (BR^-) mutant of *H. halobium* that produced only light-dependent alkalinization (proton uptake). A hypothesis that the unexpected observation by Matsuno-Yagi and Mukohata is attributable to a second pigment that works as a light-driven Na^+-pump has been presented (4, 5, 6). Extensive illumination on envelope vesicles from a BR^- mutant in the presence of hydroxylamine caused decreases of both the absorption band at 588 nm and the response to light (7, 8). The band could be restored by the addition of retinal. The absorption band of 588 nm agreed with the maximum wavelength of the action spectra of the proton uptake and the membrane potential. These observations indicated that the second pigment has retinal as a chromophore, named halorhodopsin (HR). The characterization of HR was started quite recently (3, 7, 8).

Weber and Bogomolni (9) showed that HR has a photocycle. They observed that an actinic laser pulse caused the depletion of absorbance at ~600 nm (i.e., disappearance of the original pigment), which was followed by a net increase in the absorbance at 500 nm. This intermediate was called P_{500}. The decay of P_{500} matched the formation of P_{380} whose absorption maximum is located at 380 nm. The decay of P_{380} regenerated the original pigment.

In a previous paper (10), we reported that in a BR^- mutant of *H. halobium* there exists two phototransients whose absorption maxima are similar to P_{380} and P_{500}. The results obtained, however, suggested that these two intermediates belong to different photocycles. Here we report our detailed observations. Our results suggest that there exist two light-reactive pigments in the membrane of the BR^- mutant of *H. halobium* used, and that these two pigments are not interconvertible to each other. The flash-photolysis experiments were performed under various conditions of temperature, NaCl concentration, pH, and cholate to characterize and separate these two pigments.

MATERIALS AND METHODS

The strains used were JW-1 (formerly ET-15, the generous gift of Dr. H. J. Weber, University of California, San Francisco, CA), T-2, and KH-10. These strains are BR^- mutants. Strain T-2 and KH-10 were isolated from S9. T-2 and KH-10 (11) are free from carotenoids. The growth of cells was carried out as described previously (12). Cells in 10 liter medium were harvested by centrifugation and washed three times with 4 M NaCl, which was adjusted to pH 6.9–7.0 with 10 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N'*-2-ethanesulfonic acid)/NaOH, and suspended in 200 ml of the same solution. Vesicles were prepared by sonication with a sonicator (Sonifier model 200; Branson Sonic Power

N. Hazemoto, N. Kamo, and Y. Kobatake's permanent address is Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan.

Co., Danbury, CT) at a power setting of 8 for 3 min at 20% duty cycle. After removal of the undisrupted cells and cell debris by centrifugation, (15,000 *g* for 10 min) (RP 50-2 rotor; Hitachi Co., Tokyo, Japan), the vesicles were collected at 87,000 *g* for 50 min (RP 50-2 rotor; Hitachi Co.). The vesicles were resuspended in the buffered 4 M NaCl and centrifuged again at 87,000 *g* for 50 min. The washing by this procedure was repeated until the supernatant became clear. The washed vesicles in the buffered 4 M NaCl were subjected to resonication under the same conditions as above to reduce turbidity for optical measurement. The sample suspension of 30–60 mg protein/ml was stored at −15°C. Proteins were determined by the Lowry method using bovine serum albumin as the reference standard.

Flash spectroscopy was performed in an apparatus essentially identical to that described by Tsuda (13, 14). The flash lamp had a half duration of ~200 μs. A fluorescence cuvette (10 × 10 mm) containing the sample was placed in a thermostated jacket and the temperature in the sample solution was monitored with a thermocouple. The flash was delivered to the sample through an interference filter (600 ± 15 nm) (KL-60; Toshiba Co., Tokyo, Japan) and a cut-off filter (λ > 550 nm) (VO-55; Toshiba Co). Though the phototransient could be observed with a single flash, several kinetic traces were accumulated in a kinetic processor (RA450; Union Giken Co., Hirakata, Japan) to improve the signal-to-noise ratio. When the NaCl concentration in the sample was changed, its exact concentration was determined by conductivity measurements.

RESULTS

Fig. 1 shows the flash-induced transient difference spectra of the vesicles of JW-1 at pH 6.9 (4 M NaCl). Within the duration of the flash (Fig. 1 *a*), the band below 410 nm and the band at 490 nm rose with a concomitant decrease of the band at ~590 nm, which corresponded to the depletion of the original pigment. At 50 ms, the band at 490 nm fell and the band at 590 nm recovered partially with an isosbestic point at 530 nm, while the absorption below 410 nm remained unchanged (Fig. 1 *a*). During the next 2 s, the band below 410 nm fell and the band at 590 nm rose with an isosbestic point of 430 nm (Fig. 1 *b*). These results suggest that there are two phototransients whose absorption maxima are at ~490 nm and at somewhat below 410 nm. The phototransient absorbing at ~490 nm (half-time of the decay, $\tau_{1/2}$ = 10 ms at 20°C) will be referred to as HR_{490}, hereafter. On the other hand, the phototransient absorbing below 410 nm ($\tau_{1/2}$ = 0.8 s at 20°C) will be referred to as $TR_{<410}$. Note that the maximum wavelength of the negative band in Fig. 1 *b* is ~580 nm, which is blue shifted by 10 nm from that of the spectrum shown in Fig. 1 *a*. This suggests that there are two light-reactive pigments whose maximum wavelengths are slightly different: one (referred to as halorhodopsin; HR) undergoes a faster photocycle and the other (referred to as a third rhodopsin-like pigment; TR) undergoes a slower photocycle. The assignment of these pigment will be published elsewhere. The existence of isosbestic points as described above suggests that HR_{490} and $TR_{<410}$ transform to HR and TR, respectively.

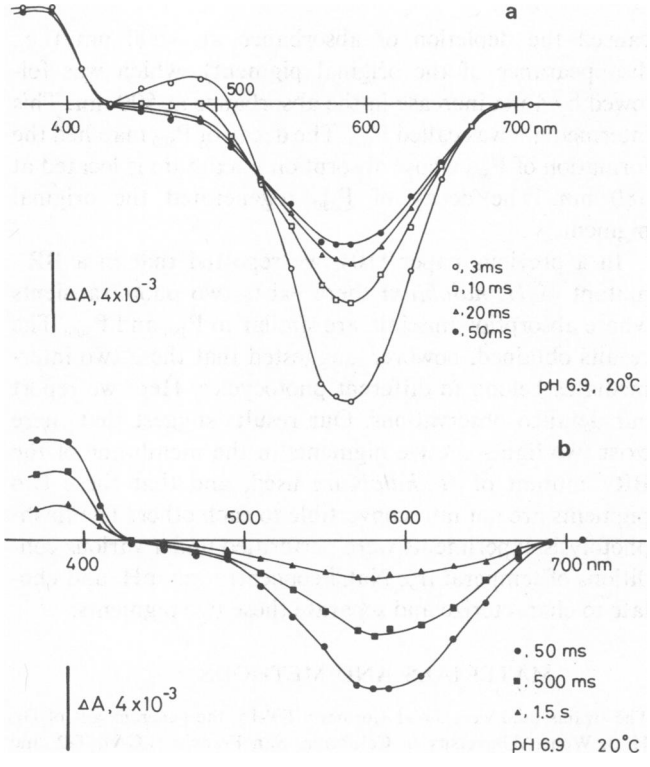

FIGURE 1 Flash-induced absorbance difference spectra of membrane vesicles from JW-1. Vesicles (3 mg protein/ml) were suspended in 4 M NaCl adjusted at pH 6.9 with 10 mM HEPES. Temperature was 20°C. (*a*) Difference spectra at 3 to 50 ms after the flash. ○, 3 ms; □, 10 ms; △, 20 ms; ●, 50 ms after the flash. (*b*) Difference spectra at 50 ms to 1.5 s. ●, 50 ms; ■, 500 ms; ▲, 1.5 s after the flash. The vertical bar represents an absorbance change of 4 × 10^{-3}.

To confirm the above scheme and to clarify whether HR and TR belong to a single or distinct pigments, the photochemistry of the vesicles of JW-1 was studied under various conditions of temperature, NaCl concentration, pH, and cholate. Kinetic data were analyzed by the following equations

$$\Delta A_{590}(t) = -\Delta A^{f}_{590} \exp(-k^{f}_{590}t) - \Delta A^{s}_{590} \exp(-k^{s}_{590}t)$$

$$\Delta A_{490}(t) = \Delta A^{f}_{490} \exp(-k^{f}_{490}t) - \Delta A^{s}_{490} \exp(-k^{s}_{590}t)$$

$$\Delta A_{390}(t) = \Delta A^{s}_{390} \exp(-k^{s}_{390}t),$$

where $\Delta A_{590}(t)$, $\Delta A_{490}(t)$, and $\Delta A_{390}(t)$ stand for the absorbance change at 590 nm, 490 nm, and 390 nm at the time t after the flash, respectively. Superscripts f and s represent the fast and slow components of each wavelength. The first term of the right-hand side of $\Delta A_{590}(t)$ represents the change in HR, and the other, TR. The main contribution to $\Delta A_{490}(t)$ is from HR_{490}, but the change is affected by a change of TR since TR has a broad absorption band (see Fig. 1 *b*). Thus, the second term of the right-hand side in the equation of $\Delta A_{490}(t)$ is required. The absorption change at 390 nm stands for the change in $TR_{<410}$.

Fig. 2 shows the effect of temperature on the rate constant, k^{f}_{λ} or k^{s}_{λ} (Fig. 2 *a*) and the amplitudes, ΔA^{f}_{λ} or ΔA^{s}_{λ} (Fig. 2 *b*). The results clearly show that $k^{f}_{490} = k^{f}_{590}$ and that $k^{s}_{390} = k^{s}_{590}$ for all temperature examined. The Arrhenius plot for the fast photocycle shows a break at

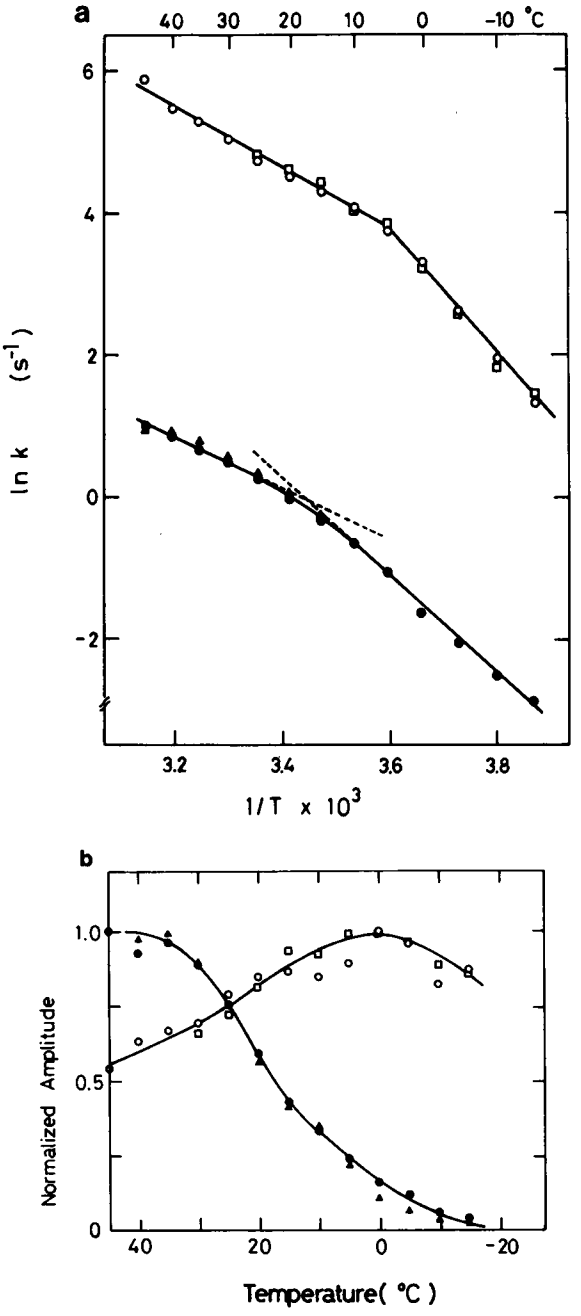

FIGURE 2 (*a*) Temperature dependence of the rate constants and (*b*) the amplitudes. The medium was 4 M NaCl buffered with 10 mM HEPES at pH 6.9. O, ΔA^f_{590}; ●, ΔA^s_{590}; □, ΔA^f_{490}; ▲, $\Delta A^s_{<410}$.

5°C. The activation energy calculated was 9.5 Kcal/mol above 5°C and 18.0 Kcal/mol below 5°C. The Arrhenius plot for the slow photocycle also showed the break at around 15°C, which is distinctly higher than that of the fast photocycle. The activation energy for the higher temperature region was 7.8 Kcal/mol and that for the lower temperature region was 12.5 Kcal/mol. These values are smaller than those of the fast photocycle. The temperature dependence of ΔA^s_{390} and ΔA^s_{590} was the same and they decreased with a decrease in temperature. On the

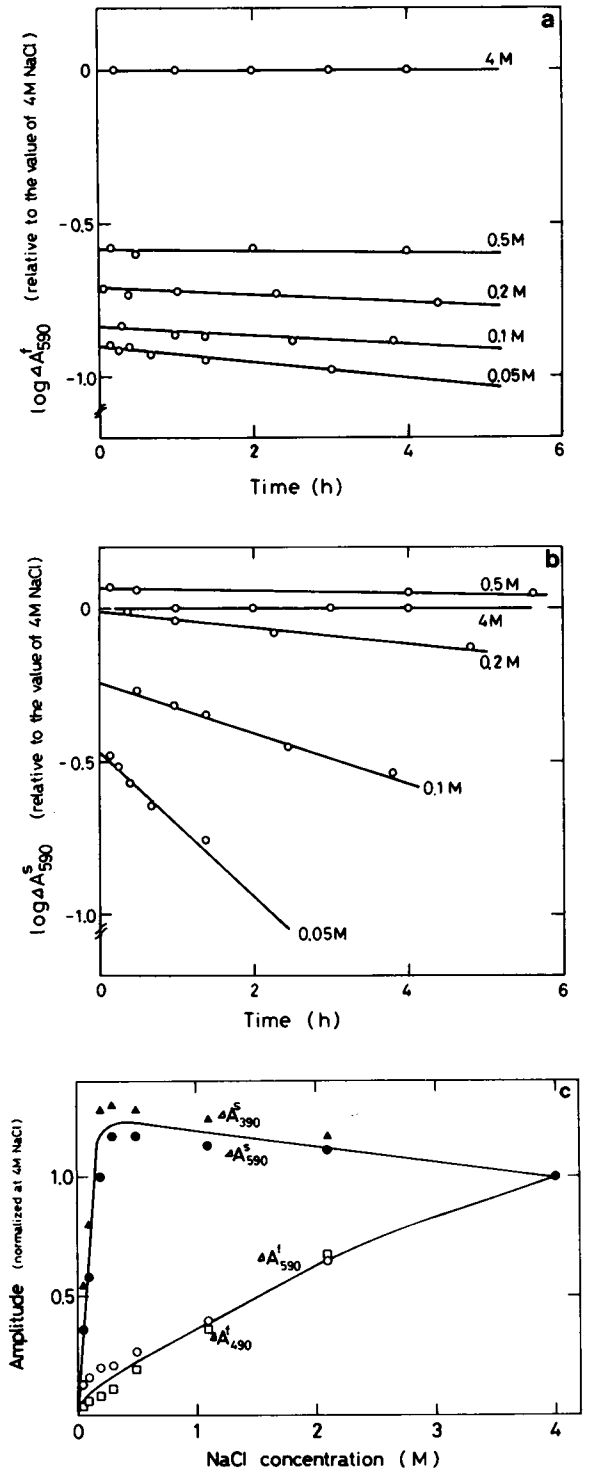

FIGURE 3 NaCl concentration dependence of the fast and the slow photocycle. (*a*) The values of ΔA^f_{590} in logarithmic scale were plotted against the incubation time after the reduction of NaCl concentration to the respective concentrations indicated. The value of ΔA^f_{590} was represented by the value relative to that obtained in 4 M NaCl, where no decrease of amplitude was observed within the period of the experiment. Vesicles from JW-1 were suspended in the solution of varying NaCl at pH 7.0 and 20°C. (*b*) The plot of ΔA^s_{590} against time, as is similar with *a*. (*c*) NaCl concentration dependence of the amplitude, which were taken from ordinate intercept of plots *a* and *b*. O, ΔA^f_{590}; □, ΔA^f_{490}; ●, ΔA^s_{590}; ▲, ΔA^s_{390}.

other hand, ΔA^{f}_{490} and ΔA^{f}_{590} increased with decrease of temperature until ~0°C, and then decreased with a further decrease in temperature.

The effect of the concentration of NaCl on photolysis of the vesicles was examined. Once the concentration of NaCl of the solution was reduced, the amplitude of the phototransients decreased with time. Figs. 3 *a, b* show the plot of ΔA^{s}_{590} and ΔA^{f}_{590} on a logarithmic scale against the incubation time after the reduction of NaCl concentration from 4 M NaCl to the value indicated. The slope in these figures became steeper with a larger decrease in NaCl concentration, indicating that TR and HR are not stable in the low-salt environments. Note that under these conditions the slope of ΔA^{s}_{590} is much steeper than that of ΔA^{f}_{590}. The amplitude of the phototransient recovered, though not fully, when the NaCl concentration of the solution returned to 4 M. Table I shows the recovery of the amplitude of phototransients when the NaCl concentrations are returned to 4 M after incubation of the vesicles in 0.1 M NaCl for a given period. The recovery was smaller as the incubation period became longer. The recovery of ΔA^{f}_{590} is always larger than that of ΔA^{s}_{590}.

The values obtained when extrapolated to the incubation time of zero in Figs. 3 *a* and *b* were plotted against the NaCl concentration in Fig. 3 *c*. The figure shows that ΔA^{f}_{590} and ΔA^{f}_{490} decreased with decreasing NaCl concentration. In addition, ΔA^{s}_{590} is proportional to ΔA^{s}_{390} for each NaCl concentration examined. The amplitude increases slightly until 250 mM and then, decreases steeply as the concentration decreases. The results in Fig. 3 *c* support the scheme that the phototransients HR_{490} and $TR_{<410}$ transform to the original pigments HR and TR, respectively. The amplitude of all phototransients went to zero as the concentration of NaCl decreased to zero.

The amplitude of phototransients in the vesicles was studied as a function of the pH of the medium (4 M NaCl). Fig. 4 shows that the amplitude is relatively constant within the pH range 5.5–8, and became smaller at both more acidic and more alkaline conditions. Note that the curve of the amplitude vs. pH for ΔA^{s}_{390} and ΔA^{s}_{590} is different from that for ΔA^{f}_{490} and ΔA^{f}_{590}. The reversibility of the amplitude of the phototransients was examined after bathing in alkaline solution (Fig. 4 *b*). ΔA^{f}_{490} and ΔA^{s}_{390} decreased with the increase of pH and became almost zero at pH 11.7. A decrease in pH restored ΔA^{f}_{490} to approximately the same level as before the alkaline treatment. On the other hand, ΔA^{s}_{390} was irreversible: ΔA^{s}_{390} at pH 7.8 after the alkaline treatment was reduced to 16% of the original level. The reversibility of the acid treatment was similar to the alkaline treatment (data not shown), indicating that TR (and $TR_{<410}$) was labile at extreme pH's.

Fig. 4 *c* shows the flash-induced transient difference spectra of the vesicles in alkaline solution (4 M NaCl, pH = 10). These spectral changes were similar to those at neutral pH (Fig. 1) in that the band below 410 nm and at 490 nm rose with a concomitant decrease of the band at 590 nm within the duration of the flash. During 50 ms the band at 490 nm fell and the band at 590 nm partially recovered, but after that time, the maximum wavelength of the negative band shifted to ~550 nm (580 nm at neutral pH). Finally, this band recovered to the original level with concomitant decrease of the band below 410 nm at 5 s. An outstanding difference in the spectral changes at pH 6.9 and pH 10.0 was the large blue shift of the maximum wavelength of the negative band due to the depletion of the original pigments at 50 and 500 ms. The maximum wavelength of the negative bands at 3 and 50 ms was determined for a wide range of pH's and was plotted against pH in Fig. 4 *d*. The maximum wavelengths of pigments' absorption bands shift to the blue in alkaline solution, but the shift of TR is much greater than that of HR. This result definitely reveals that there exist two pigments in this membrane, i.e., HR and TR.

As shown in Fig. 3 *c*, only HR was diminished at low concentrations of NaCl (250–300 mM) while TR was still active. On the other hand, solubilization of the vesicles with cholate removed the slower photocycle completely and we observed only the fast photocycle of HR. Fig. 5 is the flash-induced transient spectral changes of the preparation solubilized with 1% cholate (pH 8). The rise of the band at 490 nm (HR_{490}) and the fall of the band at 590 nm (HR) were observed, although the time constant became larger. However, no band characteristic to $TR_{<410}$ was ever observed. After cholate was removed by dialysis, no recovery of the absorbance change at 390 nm was found, indicating that the treatment caused the irreversible denaturation of TR. This reveals clearly that there exists a photocycle without the rise of the band below 410 nm, i.e., the formation of $TR_{<410}$. Flash-photolysis experiments of vesicles derived from other BR^{-} mutants were performed. As shown in Fig. 1, the ratio of ΔA^{f}_{590} to ΔA^{s}_{590}, which is proportional to the ratio of the content of HR and TR, was approximately 1.2, but that of T-2 and KH-10 was 1.8 and 3.9, respectively. These values show that the ratio of the contents of the two pigments varies among strains.

TABLE I
RECOVERY OF PHOTOCYCLE BY INCREASE OF NaCl CONCENTRATION

Amplitude	Incubation time in minutes		
	2	100	3,300
ΔA^{f}_{590}	0.93	0.83	0.31
ΔA^{s}_{390}	0.83	0.39	0.10

The vesicles of JW-1 (5 mg protein/ml) were incubated in 0.1 M NaCl for the given period and the amplitude of flash-induced absorbance change ΔA at 4 M was measured. As a control experiment, the photocycle was measured for the sample that was incubated in 4 M NaCl for the given period and the amplitude obtained was denoted as ΔA (4 M). The values of ΔA (0.1 M)/ΔA (4 M) are listed. Note that ΔA (4 M) was not changed within the period in this experiment.

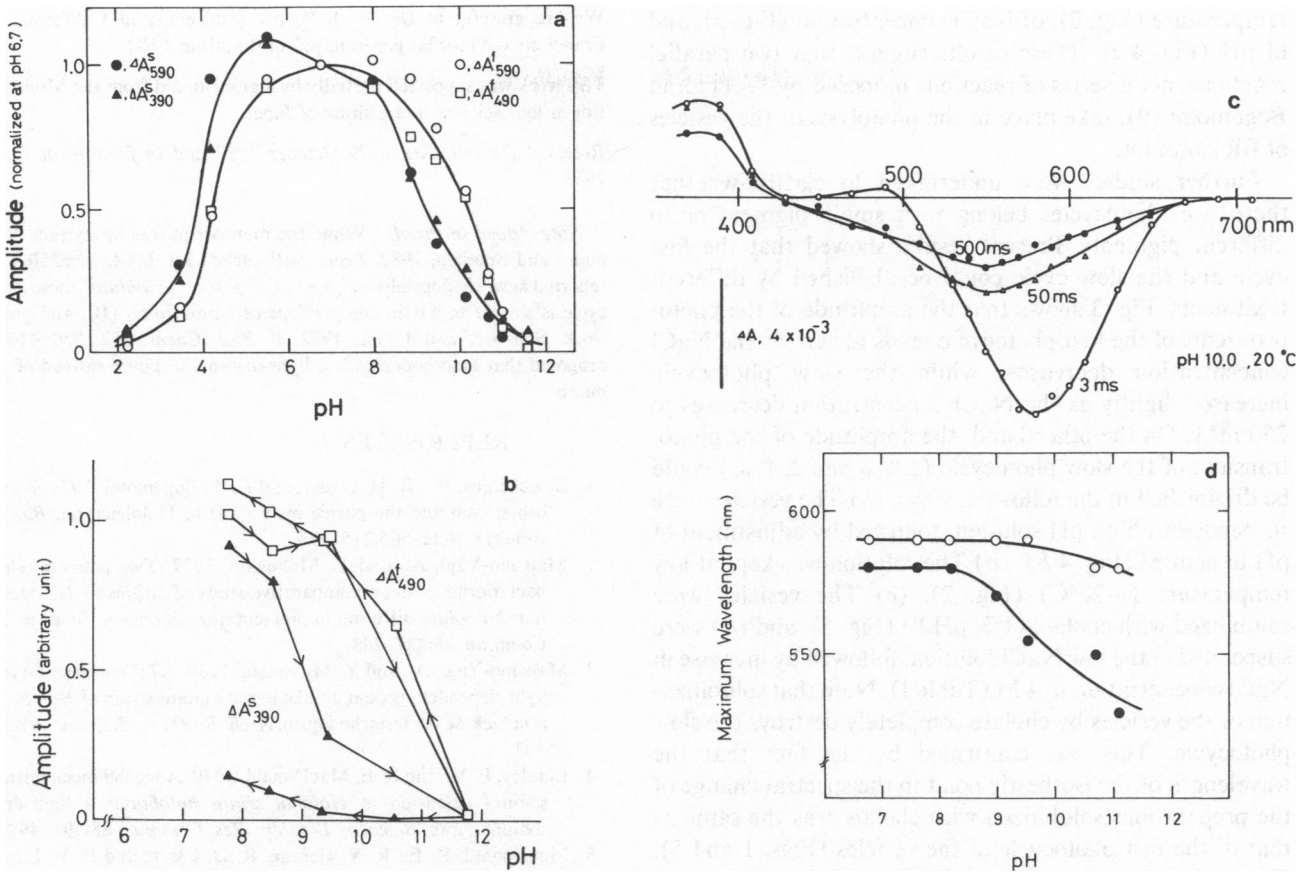

FIGURE 4 Effect of pH on the fast and slow photocycles. (*a*) pH dependence of ΔA^f_{590}, ΔA^f_{490}, ΔA^s_{590}, and ΔA^s_{390}. Symbols are the same as in Fig. 3. The values were normalized by the value of pH 6.7. (*b*) Selective inhibition of the slow photocycle by alkaline treatment. The experiment was started at pH 7.75, and the pH in the sample solution was changed to increase successively to 11.8, where no signals were observed. Then, the pH was decreased to the initial pH. □, ΔA^f_{490}; ▲, ΔA^s_{390}. (*c*) Flash-induced absorbance difference spectra of membrane vesicles at alkaline solution (pH 10.0). ○, 3 ms. △, 50 ms; ●, 500 ms. (*d*) Blue shift to maximum wavelength of HR (○) and TR (●). All these experiments were done with use of vesicles from JW-1. Vesicles (3 mg protein/ml) were suspended in 4 M NaCl of varying pH. Temperature was 20°C.

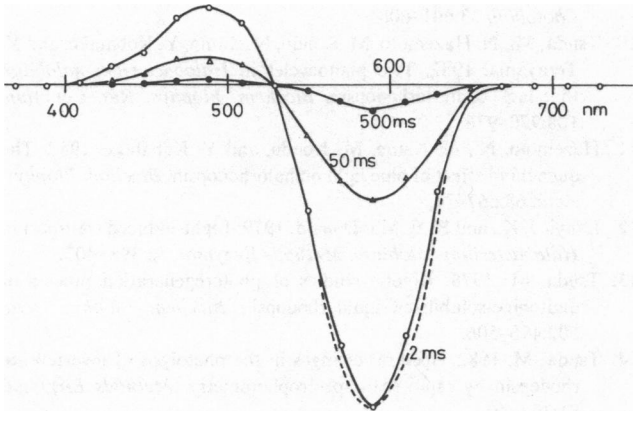

FIGURE 5 Flash-induced difference spectra of the membrane solubilized with cholate. Vesicles (10 mg protein/ml) from JW-1 were solubilized with 1% cholate in 4 M NaCl at pH 8.1. Temperature was 20°C. Broken line (---) represents the difference of the absorbance between 3 and 50 ms in Fig. 1.

DISCUSSION

Weber and Bogomolni (9) have presented a scheme of a single photocycle of HR that is completed in several tens of milliseconds. They found the phototransients have absorbance maxima at 500 nm (P_{500}) and 380 nm (P_{380}). P_{380} is formed with concomitant disappearance of P_{500}, followed by conversion to that original pigment, P_{588}. We also observed the phototransients, HR_{490} and $TR_{<410}$ whose absorbance maximum are close to P_{500} and P_{380}, respectively. However, no relation in the photochemical sequence could be found between HR_{490} and $TR_{<410}$, both of which appeared within the duration of the flash. The reformation of the original pigment (absorbance increase at 590 nm) is clearly biphasic, the fast component matches the decay of the band at 490 nm (HR_{490}) and the slow component matches the decay at 390 nm ($TR_{<410}$). This matching of the paired absorbance changes was observed under various conditions. These two photocycles showed the different dependencies with the experimental conditions. The evidence of the matching has been provided by the effect of

temperature (Fig. 2), of NaCl concentration (Fig. 3), and of pH (Fig. 4 *a*). These results suggest that two parallel reactions, not a series of reactions proposed by Weber and Bogomolni (9), take place in the photolysis of the vesicles of BR^- mutant.

Further studies were undertaken to clarify whether these two photocycles belong to a single pigment or to different pigments. Present results showed that the fast cycle and the slow cycle could be abolished by different treatments. Fig. 3 shows that the amplitude of the photo-transients of the fast photocycle tends to zero as the NaCl concentration decreases, while the slow photocycle increases slightly as the NaCl concentration decreases to 250 mM. On the other hand, the amplitude of the photo-transient of the slow photocycle (ΔA^s_{590} and $\Delta A^s_{<410}$) could be diminished in the following ways. (*a*) The vesicles were suspended in high pH solution, followed by adjustment of pH to neutral (Fig. 4 *b*). (*b*) The solution was kept at low temperature (−20°C) (Fig. 2). (*c*) The vesicles were solubilized with cholate (1%, pH 8) (Fig. 5), and (*d*) were suspended in the low NaCl solution, followed by increase in NaCl concentration to 4 M (Table I). Note that solubilization of the vesicles by cholate completely destroys the slow photocycle. This was confirmed by the fact that the wavelength of the isosbestic point in the spectral change of the preparations solubilized with cholate was the same as that of the fast photocycle of the vesicles (Figs. 1 and 5). The broken line shows the difference of the absorbance between 3 and 50 ms in Fig. 1 and agrees with the spectra of solubilized preparation, indicating that only HR is observable in the preparation solubilized with cholate. The ratio of the content of HR and TR varied among various BR^- mutants. The results presented here strongly support the conclusion that these two photocycles belong to the different pigments and that these pigments are not interconvertible.

The wavelength of absorption maximum of these two pigments (~590 nm for HR and ~580 nm for TR) is very close, but TR shifted to blue at alkaline solution (Fig. 4 *d*). Lanyi and Weber (8) also reported the blue shift of the maximum wavelength of a pigment in a BR^- mutant by means of the difference spectra between bleached and reconstituted preparations. Therefore the present results are consistent with their results. It seems strange that ΔA^s_{590} and ΔA^s_{390} decrease with decrease in temperature (Fig. 2 *b*). The reason is not clear at present. Experiments at shorter times may clarify this point. Another point to be mentioned is the instability of the pigments in the low-salt environment. It is known that some proteins from halophilic bacteria are labile in low salt (15). The present data show that both HR and TR are inactivated irreversibly when they are exposed to a low-salt medium in sharp contrast to BR.

We are grateful to Dr. H. J. Weber (University of California, San Francisco, CA) for his generous gift of the strain JW-1.

This work was supported partially by a grant-in-aid from the Ministry of Education, Science, and Culture of Japan.

Received for publication 29 October 1982 and in final form 7 June 1983.

Note Added in Proof: While the manuscript was in revision, Bogomolni and Spudich, 1982, *Proc. Natl. Acad. Sci. USA.* 79:6250–6254, reported slow rhodopsinlike pigment (SR) in *H. halobium* whose photocycle is similar to TR in our previous communication (10) and present work. Schobert and Lanyi, 1982, *J. Biol. Chem.* 257:10306–10313, proposed that halorhodopsin is a light-driven Cl^- pump instead of Na^+ pump.

REFERENCES

1. Stoeckenius, W., R. H. Lozier, and R. A. Bogomolni. 1979. Bacteriorhodopsin and the purple membrane of Halobacteria. *Biochim. Biophys. Acta.* 505:215–278.
2. Matsuno-Yagi, A., and Y. Mukohata. 1977. Two possible roles of bacteriorhodopsin; a comparative study of strains of *Halobacterium halobium* differing in pigmentation. *Biochem. Biophys. Res. Commun.* 78:237–243.
3. Matsuno-Yagi, A., and Y. Mukohata. 1980. ATP synthesis linked to light-dependent proton uptake in red mutant strain of *Halobacterium* lacking bacteriorhodopsin. *Arch. Biochem. Biophys.* 199:297–303.
4. Lindley, E. V., and R. E. MacDonald. 1979. A second mechanism for sodium extrusion in *Halobacterium halobium:* a light-driven sodium pump. *Biochem. Biophys. Res. Commun.* 88:491–499.
5. MacDonald, R. E., R. V. Greene, R. D. Clark, and E. V. Lindley. 1979. Characterization of the light-driven sodium pump of *Halobacterium halobium. J. Biol. Chem.* 254:11831–11838.
6. Greene, R. V., and J. K. Lanyi. 1979. Proton movement in response to a light-driven electrogenic pump for sodium ions in *Halobacterium halobium* membranes. *J. Biol. Chem.* 254:10986–10994.
7. Lanyi, J. K. 1981. Halorhodopsin—a second retinal pigment in *Halobacterium halobium. Trends Biochem. Sci.* 6:60–62.
8. Lanyi, J. K., and H. J. Weber. 1980. Spectrophotometric identification of the pigments associated with light-driven primary sodium translocation in *Halobacterium halobium. J. Biol. Chem.* 255:243–250.
9. Weber, H. J., and R. A. Bogomolni. 1981. P_{588}, a second retinal-containing pigment in *Halobacterium halobium. Photochem. Photobiol.* 33:601–608.
10. Tsuda, M., N. Hazemoto, M. Kondo, N. Kamo, Y. Kobatake, and Y. Terayama. 1982. Two photocycles in *Halobacterium halobium* that lack bacteriorhodopsin. *Biochem. Biophys. Res. Commun.* 108:970–976.
11. Hazemoto, N., N. Kamo, M. Kondo, and Y. Kobatake. 1982. The quenching effect of blue light on halorhodopsin. *Biochim. Biophys. Acta.* 682:67–74.
12. Lanyi, J. K., and R. E. MacDonald. 1979. Light-induced transport in *Halobacterium halobium. Methods Enzymol.* 56:398–407.
13. Tsuda, M. 1978. Kinetic studies of photoregeneration process of digitonine-solubilized squid rhodopsin. *Biochem. Biophys. Acta.* 502:495–506.
14. Tsuda, M. 1982. Spectral changes in the photolysis of invertebrate rhodopsin by rapid scan spectrophotometry. *Methods Enzymol.* 81:392–402.
15. Lanyi, J. K. 1974. Salt-dependent properties of protein from extremely halophilic bacteria. *Bacteriol Rev.* 38:272–290.