Journal of Bioenergetics and Biomembranes, Vol. 20, No. 5, 1988

Light-Induced Conductivity Changes of Purple Membrane Suspensions in Strong Electrolytes

M. A. Slifkin,[1] I. Bakarudin,[1] S. Bradley,[1] and S. R. Caplan[2]

Received February 17, 1988

Abstract

Measurements have been made of the modulated light-induced changes in conductivity and the associated relaxation times of bacteriorhodopsin in a variety of strong electrolytes, both unbuffered and buffered. The effects of pH and temperature variation have been studied as well as the effect of adding valinomycin. Two relaxation times can be distinguished: a fast lifetime associated with protonation–deprotonation, and a slow lifetime associated with ion binding. The ion-binding effects appear to be cation specific.

Key Words: Bacteriorhodopsin; conductivity; *Halobacterium halobium*; ion binding; modulation excitation; photocycle; protonation–deprotonation; purple membrane; relaxation times; valinomycin.

Introduction

Light-induced conductivity changes have been measured by Hara (1963) in rhodopsin suspensions and by Falk and Fatt (1968) in rod outer segments using conventional techniques. In an earlier communication (Slifkin *et al.*, 1979), we described how light-induced conductivity changes in suspensions of bacteriorhodopsin (bR)[3] are measured using a modulation method combined with phase-sensitivity detection. It was suggested that the observed changes were related to changes in the surface charge of the purple membrane fragments. More recently, Marinetti and Mauzerall (1983), using flash methods, have also observed light-induced changes in the conductivity of bR suspensions. In this article, we present a more detailed study of light-induced effects

[1]Department of Electronic and Electrical Engineering, University of Salford, Salford M5 4WT, UK.
[2]Department of Membrane Research, Weizmann Institute of Science, Rehovot 76100, Israel.
[3]Abbreviations: bR, bacteriorhodopsin; PM, purple membrane.

0145-479X/88/1000-0585$06.00/0

on the conductivity of bR suspensions in different electrolytes, both unbuffered and buffered, and the influence on these effects of pH, temperature, and the addition of valinomycin. Our results in general agree with those of Marinetti and Mauzerall (1983) in the case of buffered solutions without added electrolyte, and extend them to the case of specific added electrolyte.

Materials and Methods

Halobacterium halobium was grown according to the method described by Danon and Stoeckenius (1974) and purple membrane (PM) prepared using the technique of Oesterhelt and Stoeckenius (1974). Except where otherwise indicated, light-adapted PM fragments were used from a freeze-dried stock. "Recentrifuged" samples were centrifuged for 5 min at 4000 rpm, decanted, washed, and filtered, three times in succession. This process appears to remove a small quantity of lipid in the first stage only, but no change occurs in the photocycle. The pH values of the unbuffered samples were adjusted by the addition of small quantities of dilute acid or base. The approximate concentration of bR in the suspension was 8 μM to 16 μM as determined by absorption spectroscopy.

The conductivity measurements were carried out by the modulation excitation method described previously by Slifkin *et al.* (1979) (irradiation at 577 nm at an intensity of 350 W/m^2), and lifetimes were determined according to the methods of Slifkin and Darby (1978, 1980). In brief, the method is as follows. The sample is illuminated with chopped light (in the range 20–200 Hz) in a thermostatted Radiometer CDC 324 conductivity cell. Modulated changes in conductivity are detected using a phase-sensitive detector. The relaxation time of the process giving rise to the change in conductivity, t, can be obtained either by measuring the phase shift, ϕ, and using the relationship $\sin \phi = \omega t$ (where ω is the angular frequency of the modulation), or by measuring the signal amplitude, S, as a function of ω and using the relationship $S = Kt/(1 + \omega^2 t^2)$ (if only one lifetime is involved—see below). In these studies, filters were used to remove all irradiation > 650 nm. In an earlier study, the effect of temperature modulation on bR was examined, and shown to induce a specific chemical relaxation process (Slifkin *et al.*, 1986a). Here the optical perturbation, which gives rise to an energy transduction process, outweighs the small thermal effect due to degradation of a fraction of the absorbed light to heat. [Note that the intrinsic relaxation time of the apparatus in these experiments is $< 10^{-2}$ ms (Slifkin *et al.*, 1983), as determined using the dye dinitrosalicylic acid.]

Valinomycin was obtained from Sigma Chemicals.

Results

The frequency responses of the conductivity change in 4 M sodium chloride solution containing freeze-dried PM fragments show a characteristic complex behaviour involving both loss (negative) and gain (positive) in conductivity (Fig. 1). Two separate events involving different relaxation processes are clearly indicated. Crossover points obtained along the frequency axis depend on the pH of the medium as well as sample preparation. In centrifuged PM fragments resuspended in 4 M NaCl solution at different pH's (Figs. 2 and 3), three types of frequency response are seen involving both loss and gain in conductivity. At low pH ($\leqslant 2.7$), where the absorption band of PM undergoes a red shift (Oesterhelt and Stoeckenius, 1971), there are only losses in conductivity involving first-order processes. At high pH ($\geqslant 6.7$), there are only gains in conductivity, again involving only first-order

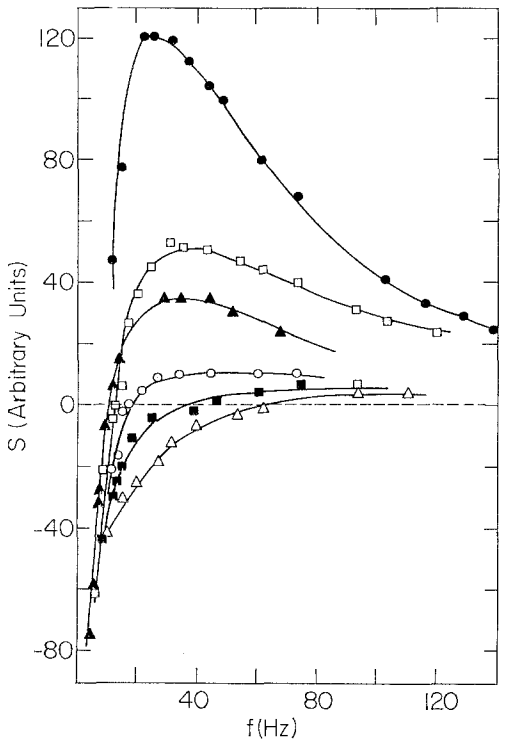

Fig. 1. Frequency spectra of photoconductivity change (S) in PM suspensions at various pH values (T = 18° C). PM fragments (freeze-dried) were suspended in 4 M NaCl solution. (■—■) pH 5.6, (▲—▲) pH 6.5, (□—□) pH 6.9, (●—●) pH 7.0, (○—○) pH 7.1, and (△—△) pH 9.3.

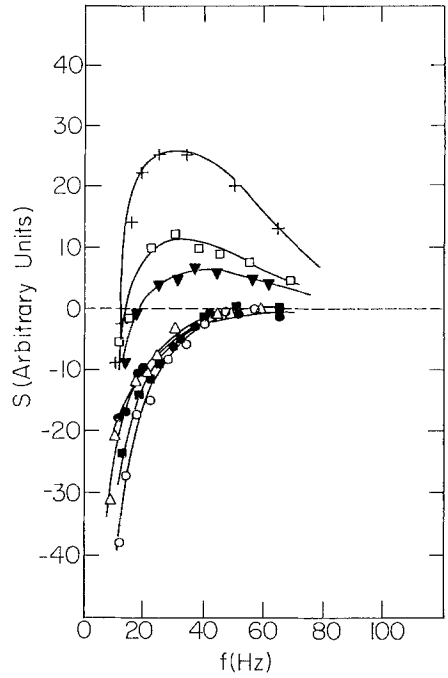

Fig. 2. Frequency spectra of photoconductivity change (S) in PM suspensions at various pH values between 0.8 and 5.2 (T = 18° C). Recentrifuged PM fragments (freeze-dried) were resuspended in 4 M NaCl solution. (●—●) pH 0.8, (△—△) pH 1.6, (■—■) pH 2.4 (○—○) pH 2.7, (□—□) pH 4.4, (▼—▼) pH 4.6, and (+—+) pH 5.2.

processes. For pH's between 4.4 and 6.3, a combination of both loss and gain in conductivity is observed with crossover points along the frequency axis. This complex behavior may well be due to a combination of first-order processes.

The conductivity changes seen here agree with previous results reported by Slifkin *et al.* (1979). In addition, the present results indicate a pH dependence of light-induced conductivity and hence the involvement of processes not previously considered. We have assumed that the conductivity changes shown in Fig. 1 consist of differences between two simple first-order processes characterized by two different apparent lifetimes, t_1 and t_2, respectively. Analyses of these conductivity changes (with estimated standard error $< 10\%$) are obtained by fitting the data to the following equation:

$$S = \frac{K_1 t_1}{1 + 4\pi^2 f^2 t_1^2} + \frac{K_2 t_2}{1 + 4\pi^2 f^2 t_2^2} \tag{1}$$

where S is the conductivity change, K_1 and K_2 are constants, f is the modulation frequency (Hz), and t_1 and t_2 are the relaxation times of the two different

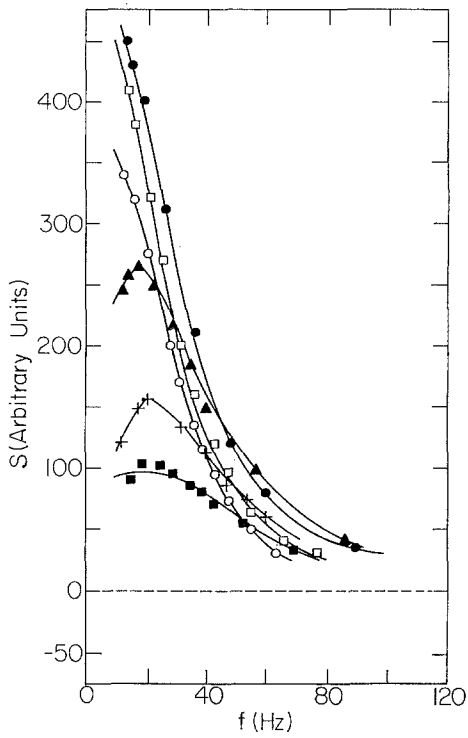

Fig. 3. Frequency spectra of photoconductivity change (S) in PM suspensions at various pH values between 5.5 and 7.0 (T = 18° C). Conditions are as in the legend to Fig. 2. (■—■) pH 5.5, (+—+) pH 5.9, (▲—▲) pH 6.3, (●—●) pH 6.7, (○—○) pH 6.8, and (□—□) pH 7.0.

processes involved. The algorithm used to obtain the best fit to equation (1) has been discussed in great detail by Slifkin and Ali (1985), and it was shown that, with care, accuracies of about 95% are realisable. The differences between the relaxation times measured here are such that we believe that we have accuracies of this order. The relative amplitudes K_1 and K_2 are also obtainable by the algorithm, but are not presented. Plots of the apparent lifetimes against the sample pH (from, *inter alia*, the data of Fig. 1) are shown in Fig. 4. These plots indicate a dramatic variation of the lifetimes around neutral pH. This may suggest that pK shifts associated with the photocyle give rise to more extensive association or dissociation at neutral pH. The effect of adding D_2O to these suspensions is to cause a fourfold increase in the lifetime of the fast process (not shown).

In the case of recentrifuged fragments resuspended in 4 M NaCl solution, at pH values between 4.4 and 6.3, frequency responses were obtained similar to those shown in Fig. 1, involving a combination of two different

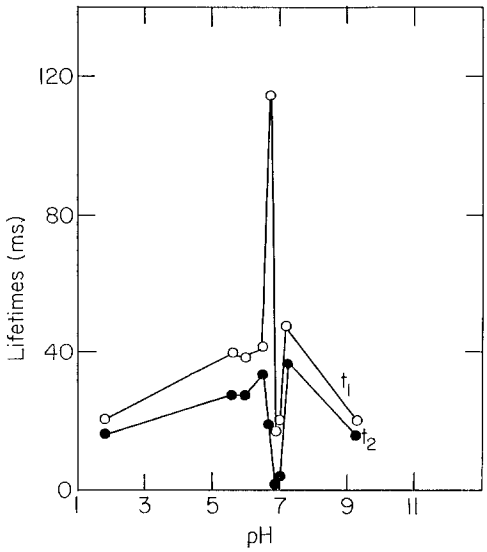

Fig. 4. The pH dependence of the lifetimes, t_1 and t_2, for the photoconductivity change (S) in PM suspensions in 4 M NaCl. Conditions are as in the legend to Fig. 1. The lifetimes are obtained by computer fitting to equation (1), and their standard error is ~ 10%.

processes. However, outside this range, simple first-order losses and gains in conductivity were observed as discussed above. In both the loss and the gain in conductivity, two events characterized by two lifetimes can be distinguished. Figure 5 shows kinetic plots of the inverse gain in conductivity vs. modulation frequency for recentrifuged PM fragments resuspended in 4 M NaCl. At pH 7.8, the gain in conductivity is characterized by a single relaxation time t_1 of ~ 5 ms. Plots of the lifetimes against pH (Fig. 6) show two characteristics. The apparent lifetimes for the loss in conductivity at low pH are much longer as compared with the lifetimes obtained at higher pH values. For neutral and alkaline conditions, the lifetimes obtained for the gain in conductivity correspond to the lifetimes of the longer-lived photointermediates in the bR photocycle. It is therefore quite likely that the photocycle is involved in the gain in photoconductivity at physiological pH.

Measurements of conductivity changes in PM fragments suspended in 2 M $CaCl_2$ solution (Fig. 7) do not show the complex behavior seen earlier for fragments suspended in 4 M NaCl. Here simple first-order processes involving loss of conductivity (at low pH's) and gain in conductivity (at pH > 5.5) were obtained. Analysis of these conductivity changes again revealed two lifetimes corresponding to two events for both the loss and gain in conductivity. Plots of the lifetimes against pH are shown in Fig. 8. These appear to show scaling between the lifetimes (unlike similar plots in different

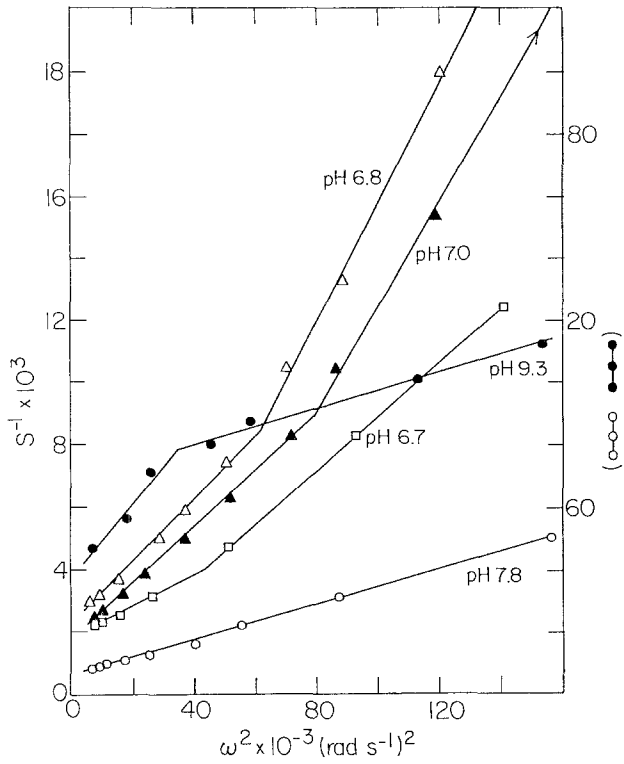

Fig. 5. Examples of kinetic plots of the photoconductivity change (S). Conditions are as in the legend to Fig. 2. Note the scale change at pH's 7.8 and 9.3. $\omega = 2\pi f$.

electrolytes) that may well be fortuitous. The frequency response of conductivity changes in PM fragments suspended in 2 M KCl (in the absence of valinomycin) is similar to that found in 4 M NaCl. Plots of conductivity change against modulation frequency for pH values around neutral pH are shown in Fig. 9. These curves again suggest that more than one first-order process is involved. Measurements of conductivity changes in freeze-dried PM suspended in 4 M $MgCl_2$ solution showed simple first-order processes with characteristic single lifetimes of ~ 5 ms (Fig. 10). However, experiments on freshly prepared purple membrane fragments indicated two lifetimes of about the same duration (Fig. 10). Evidently freeze-drying can bring about an irreversible change in the ligand-binding behavior.

The presence of valinomycin ($\sim 2\,\mu$M) in 4 M NaCl solution containing PM fragments has little effect on the photoconductivity (Fig. 11), but the lifetimes for the effect are somewhat increased (by ~ 1 ms for pH 6.4). In PM fragments suspended in the presence of valinomycin in 2 M KCl, however,

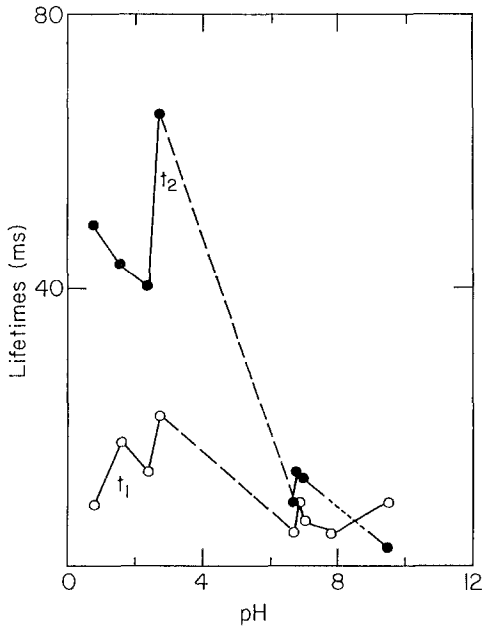

Fig. 6. The pH dependence of the parallel lifetimes, t_1 and t_2, for PM fragments suspended in 4 M NaCl. Conditions are as in the legend to Fig. 2.

the conductivity change reverses direction (i.e., instead of the gain in conductivity seen in the absence of valinomycin, there is a loss in conductivity in the presence of valinomycin as shown in Fig. 9). This effect is evidently mediated through the well-known potassium complex of valinomycin.

The dependence of photoconductivity change on pH of PM suspensions is shown in Fig. 12. The maximum conductivity change occurs at approximately pH 7. The magnitude and direction of the conductivity changes depend, of course, on the modulation frequency. Nevertheless, a simlar response was shown by all the PM suspensions. The effect of temperature on photoconductivity suggests that the process involved is sensitive to the physical state of the PM. Thus, in Fig. 13, "Arrhenius" plots of the signal are displayed for samples of pH 6.6 and 4.9 in 4 M NaCl at different modulation frequencies. These plots show at least two straight lines intersecting at $\sim 31 \pm 4°$ C. However, at low modulation frequency, three straight lines intersecting at 31° C and 41° C are observed (Fig. 13, plot A). At low pH (< 2), temperature has no apparent effect on the photoconductivity (not shown). However, at high pH (8.9), a transition point at 26° C is observed and at this pH value no apparent temperature effect is seen above 37° C. Similar results were obtained for PM suspended in the other electrolyte

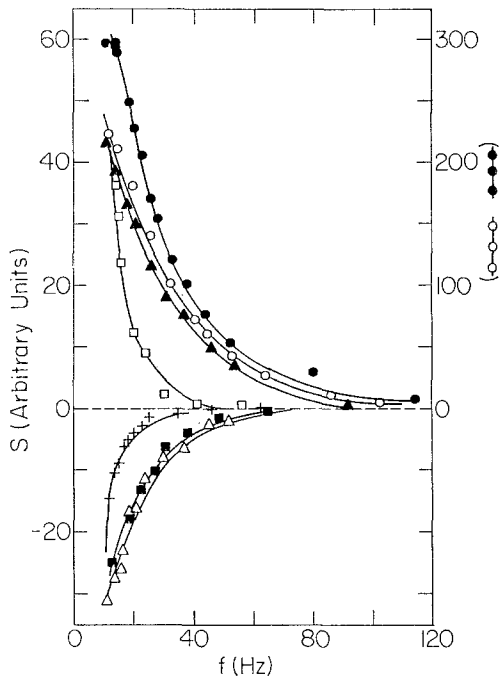

Fig. 7. Frequency spectra of the photoconductivity change (S) for freeze-dried PM fragments in 2 M $CaCl_2$ at various pH values (T = 18° C). (△—△) pH 2.0, (■—■) pH 3.9, (+—+) pH 4.5, (○—○) pH 5.5, (●—●) pH 6.1, (▲—▲) pH 6.5, and (□—□) pH 7.9. Note the scale change at pH's 5.5 and 6.1.

solutions; the transition point from the Arrhenius plots is ~ 31° C (to within ± 5°C).

In phosphate buffer, somewhat different effects were observed. Table I shows that, over a fairly wide range of pH, only one lifetime is observed, corresponding to the fast lifetime seen in unbuffered solutions. However, as in the unbuffered case, this lifetime is maximal at pH 7, where additionally a second even faster lifetime is seen. Measurements made on buffer alone at the same instrumental settings yielded negligibly low signals (not shown). Table II shows lifetimes for various concentrations of sodium chloride in phosphate buffer at different pH values. In general, these are longer than in buffer alone. Again, the lifetime reaches a maximum at pH 7. Figure 14 illustrates the same effect for potassium chloride, which is seen to be qualitatively similar to the case of sodium chloride over the pH range 5.6–8. Measurements down to pH 2.5 (not shown) give only the slow relaxation time varying between 12 and 20 ms, depending on ionic strength and pH.

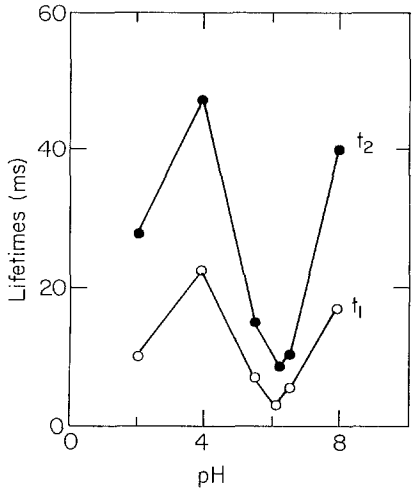

Fig. 8. The pH dependence of the lifetimes, t_1 and t_2, for PM fragments suspended in 2 M $CaCl_2$ (T = 18°C).

Table I. Lifetimes of the Photoconductivity Effect for PM Fragments Suspended in Phosphate Buffer Solution at Various pH's (for Signs and Amplitudes, See Figs)

(A) 100-mM Buffer Solution

	Lifetimes (ms)	
pH	t_1	t_2
2.5	3.98 ± 0.34	—
2.9	4.67 ± 0.21	—
4.0	4.79 ± 0.13	—
5.6	5.29 ± 0.17	—
6.3	5.50 ± 0.12	—
7.0	7.17 ± 0.45	1.52 ± 0.05
8.0	4.04 ± 0.10	—

(B) 10-mM Buffer Solution

	Lifetimes (ms)	
pH	t_1	t_2
3.5	5.07 ± 0.25	—
5.0	5.31 ± 0.31	—
6.1	6.06 ± 0.31	—
7.0	9.35 ± 0.66	3.30 ± 0.32
8.0	4.68 ± 0.37	—

The standard deviations are derived from regression analysis.

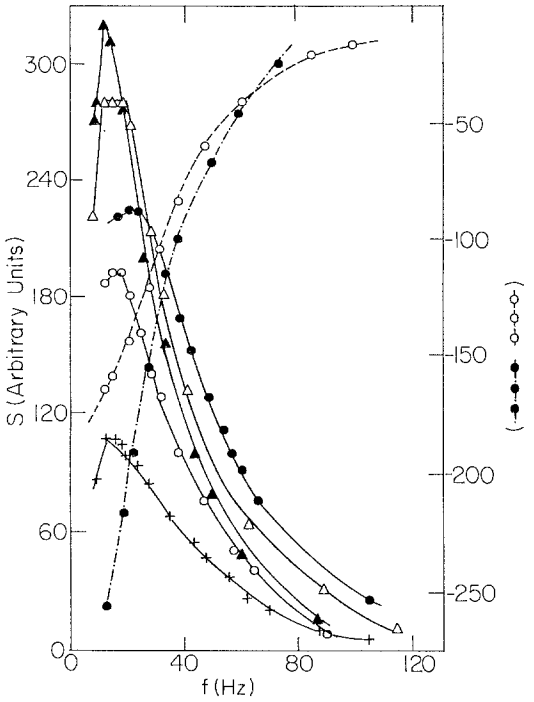

Fig. 9. Frequency spectra of the photoconductivity change (S) for freeze-dried PM suspensions in 2 M KCl at various pH values (T = 18°C). (+—+) pH 6.2, (○—○) pH 6.5, (▲—▲) pH 7.0, (△—△) pH 7.1; and (●—●) pH 7.5, (●···●) pH 7.1 and (○---○) pH 7.5 in the presence of valinomycin (note the scale change).

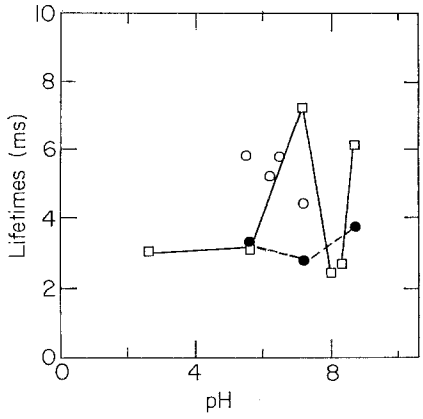

Fig. 10. The pH dependence of the lifetimes for PM fragments suspended in 4 M $MgCl_2$ solution (T = 18°C): (○) lifetime for freeze-dried PM fragments. Two lifetimes obtained in suspensions containing freshly prepared PM fragments: (□—□) t_1 and (●---●) t_2.

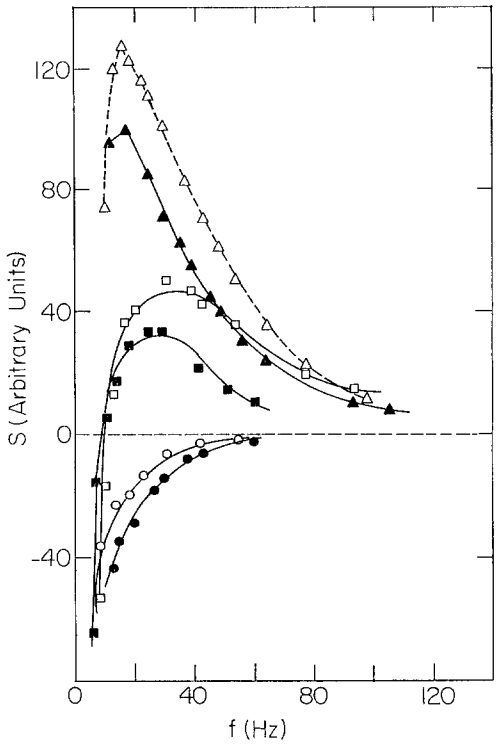

Fig. 11. The effect of the presence of valinomycin on the photoconductivity change (S) of PM fragments in 4 M NaCl solution (T = 18°C). (●—●) pH 1.8, (■—■) pH 5.9, (▲—▲) pH 6.4, (□—□) pH 7.6, and (○—○) pH 8.8; (△---△) pH 6.4 in the absence of valinomycin.

A summary of these results is given in Table III. The differences between fresh and freeze-dried material do not appear to be electrolyte dependent. The results in "unbuffered" solutions should be treated with caution owing to the possible presence of traces of contaminants as well as dissolved CO_2.

Discussion

The key problem in the above findings is to establish which of the relaxation processes assumed to take place upon illumination, if any, are responsible for the photoconductivity effects seen here. The relative conductivity changes measured probably reflect light-induced changes in the number of current carriers in the suspensions. Thus, it is natural to try to correlate them with light-induced protonation–deprotonation processes. Clearly, proton-pumping effects can be excluded, seeing that we are dealing with

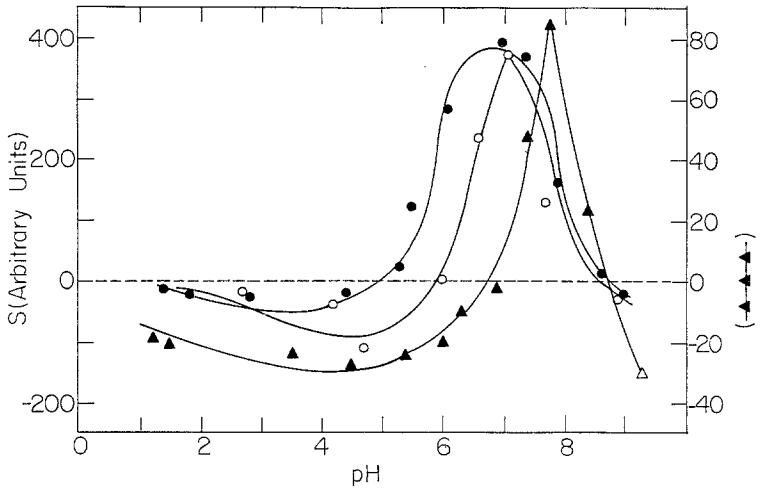

Fig. 12. Dependence of the photoconductivity change (S) on pH in PM suspensions (T = 18°C). (▲—▲) PM fragments (freeze-dried) suspended in 4 M NaCl (at 12.2 Hz) (scale at right), (●—●) centrifuged PM fragments resuspended in 4 M NaCl (at 16 Hz), and (○—○) PM fragments suspended in 2 M KCl (at 20 Hz).

membrane fragments rather than closed vesicles (Eisenbach *et al.*, 1978). Since illumination of PM suspensions energizes the photocycle of bR, any light-induced protonation–deprotonation processes will inevitably be coupled to the photocycle, and so to any conformational changes associated with it (Eisenbach and Caplan, 1979). The first-order losses of conductivity characterized by long lifetimes (50–100 ms) cannot be due to light-induced protonation or deprotonation. Their lifetimes are too long to result from the deprotonation of the Schiff base (Dencher and Wilms, 1975; Lozier *et al.*, 1976), but are of the order of pH changes measured in PM suspensions and attributed to conformational changes (Eisenbach *et al.*, 1978; Garty *et al.*, 1977; Trissl and Montal, 1977). The slow loss of conductivity characterized by an average lifetime of ~40–50 ms obtained for low pH values suggests that still other processes are involved. These may well be the result of light-induced changes in the surface charge of the membrane. (In buffer alone, where only a fast lifetime is observed, the effect must evidently be attributed to protonation–deprotonation—particularly since, as mentioned above, D_2O causes a fourfold increase in relaxation time. The longer lifetimes seen in the presence of strong electrolytes are presumably due to ion binding.) A much faster loss of conductivity characterized by an average lifetime of 18.8 ms is observed around neutral pH. Although PM is known to aggregate at high salt concentrations and low pH, we did not observe any indications of this in these experiments.

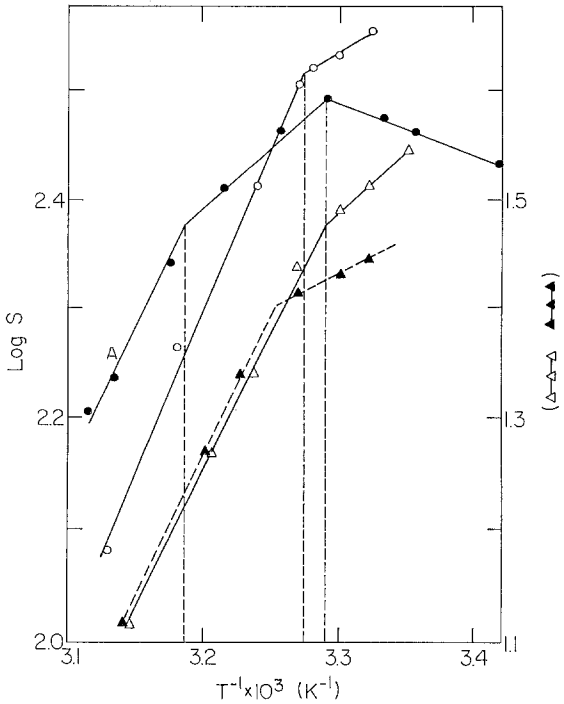

Fig. 13. Arrhenius plots of photoconductivity changes (S). PM fragments were suspended in 4 M NaCl solution. At each temperature, the suspension was allowed to equilibrate 20–30 min before conductivity measurements were performed. (●—●) pH 6.6 at 14.8 Hz (plot A), (○—○) pH 6.6 at 30.2 Hz, (△—△) pH 4.9 at 41 Hz (scale at right), and (▲---▲) pH 4.9 at 41 Hz (cooling from high temperatures, scale at right). In plot A, two apparent transitions are seen.

The fast gain in conductivity at higher pH's characterized by a short lifetime of ~ 2 ms may tentatively be attributed to pK shifts, possibly associated with conformational changes. The occurrence of conformational changes has been inferred from the observations reported by Bogomolni and co-workers (1978) on illumination-dependent changes in the intrinsic fluorescence of bR. These workers considered that the changes seen might reflect changes in the spatial disposition of tryptophan and other amino acid residues during the photocycle. If these are the processes responsible for the photoconductivity effect, the latter would be closely associated with the photocycle. Eisenbach *et al.* (1978) have suggested that illumination of light-adapted bR causes initial fast conformational changes that might well bring about a cascade of further changes with progressively longer time scales.

The temperature effects indicate that the processes involved are sensitive to the physical state of the purple membrane. The observations of break

Table II. Lifetimes of the Photoconductivity Effect for PM Fragments Suspended in 100-mM Phosphate Buffer Solution at Various pH's in the Presence of Different Concentrations of NaCl (for Signs and Amplitudes, See Figs)

pH	NaCl concentration (M)	Lifetimes (ms)	
		t_1	t_2
2.5	4.0	20.40 ± 0.78	4.56 ± 0.20
	2.0	18.86 ± 1.46	–
	1.0	21.22 ± 1.90	–
	0.1	16.96 ± 0.71	–
2.9	4.0	9.51 ± 0.37	2.34 ± 0.06
	2.0	16.43 ± 0.95	–
	1.0	15.14 ± 0.57	–
	0.1	13.75 ± 0.52	–
4.0	4.0	9.27 ± 0.22	–
	2.0	8.46 ± 0.39	–
	1.0	7.99 ± 0.26	–
	0.1	6.51 ± 0.14	–
5.6	4.0	10.21 ± 0.41	–
	2.0	7.10 ± 0.13	–
	1.0	5.97 ± 0.08	–
	0.1	5.66 ± 0.08	–
6.3	4.0	12.91 ± 0.75	2.03 ± 0.12
	2.0	8.16 ± 0.17	–
	1.0	7.58 ± 0.10	–
	0.1	5.32 ± 0.08	–
7.0	4.0	30.71 ± 2.50	3.22 ± 0.21
	2.0	27.15 ± 1.80	3.06 ± 0.21
	1.0	26.05 ± 1.50	3.47 ± 0.12
	0.1	27.77 ± 1.34	3.37 ± 0.09
8.0	4.0	26.70 ± 2.19	3.37 ± 0.19
	2.0	11.75 ± 0.66	3.14 ± 0.05
	1.0	10.55 ± 0.52	–
	0.1	6.42 ± 0.14	–

points at ~31°C in the Arrhenius curves may be due to an intrinsic property of the PM lipids. Chignell and Chignell (1975), using spin-labeled fatty acids as probes, found a reversible thermal transition at ~29° C which, however, they attributed to a change in the structure of bR. Similar transitions were obtained for measurements of microviscosity using fluorescent probes, and a phase transition at ~31°C was seen using a spin-labeled probe (Gupte *et al.*, 1976; Korenstein *et al.*, 1976). Proton nuclear magnetic resonance studies (Degani *et al.*, 1978) have also shown a break in the Arrhenius plot of relaxation times at 31°C (±4°C). Comparable effects are seen in chemical relaxation studies (Slifkin *et al.*, 1986a) and dielectric relaxation studies (Slifkin *et al.*, 1986b).

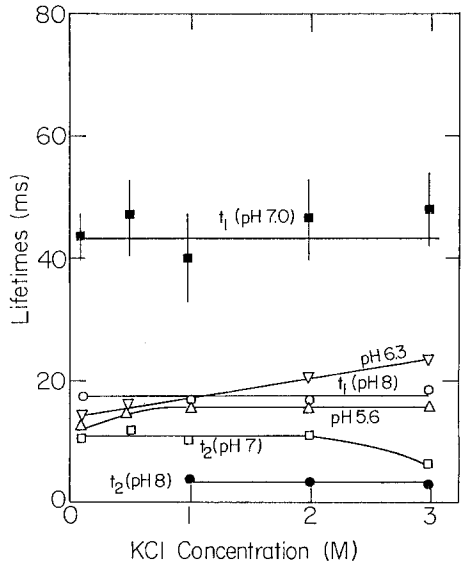

Fig. 14. Effect of the presence of KCl in buffered PM suspensions on the lifetimes of photoconductivity. PM fragments were suspended in 100 mM phosphate buffer solution (at different pH's) containing various concentrations of KCl (T = 18°C). The bars indicate standard errors derived from regression analyses.

In the presence of potassium, valinomycin has the effect of reversing the direction of the photoconductivity effect. Sherman *et al.* (1976), using flash photometry, showed that, in the presence of potassium, valinomycin inhibited and quenched the formation of the 660-nm transient with a concomitant increase in lifetime of the 410-nm transient and a delay in the recovery of the 570-nm state. In the photoconductivity behavior, valinomycin in the presence of potassium decreases the two lifetimes of the conductivity change by ~ 1 ms (at pH 7.5) and reverses the sign. This suggests a possible association between bR and the valinomycin–potassium complex.

Marinetti and Mauzerall (1983) have suggested that the independence of their signal with buffer concentration points to the involvement of other ions in the conductivity. Our results on the effect of adding different cations confirm this suggestion, and may well have a bearing on the specific cation-binding effects now known to be associated with the functioning of the proton pump (Ariki *et al.*, 1987; Ovchinnikov, 1987). Kimura *et al.* (1984) have examined the effects of pH and salt on the spectrum of bR and conclude similarly that ion-binding and protonation–deprotonation effects occur, accompanied by conformational changes of the protein.

While our studies were being prepared for publication, an important article by Marinetti (1987) appeared, demonstrating the abrupt onset of

Table III. Summary of Lifetimes Associated with the Photoconductivity Effect in Suspensions of Freeze-Dried PM Fragments (for Signs and Amplitudes, See Figs)

(A) Unbuffered Solutions

Ion	pH range	Lifetime range (ms) t_1	Lifetime range (ms) t_2
Na^+	2–6.5, 7.5–10	~10	20–30
	6.5–7.5	~2	~120
K^{+a}	3–6, 8–10	~20	–
	~7	~20	~40
Ca^{2+}	3–5, >8	10–20	30–50
	6.5–8	2–10	5–20
Mg^{2+}	3–9	3–8	–

[a]Valinomycin reverses sign of conductivity change.

(B) Buffered Solutions

Ion	pH range	Lifetime range (ms) t_1	Lifetime range (ms) t_2
Na^+	2.5–5	~3	10–20
	~7	~3	~25
K^+	2–5, >8	12–20	–
	~7	~10	~45

large-scale nonproton ion release by photo-excited purple membrane suspensions near neutral pH (using transient conductivity measurements following actinic flashes). Marinetti's study is in substantial agreement with our own, particularly as regards the singularity at pH 7 seen, for example, in Fig. 4.

References

Ariki, M., Magde, D., and Lanyi, J. K. (1987). *J. Biol. Chem.* **262**, 4947–4951.
Bogomolni, R. A., Stubbs, L., and Lanyi, J. K. (1978). *Biochemistry* **17**, 1037–1041.
Chignell, C. F., and Chignell, D. A. (1975). *Biochem. Biophys. Res. Commun.* **62**, 136–143.
Danon, A., and Stoeckenius, W. (1974). *Proc. Natl. Acad. Sci. USA* **71**, 1234–1238.
Degani, H., Bach, D., Danon, A., Garty, H., Eisenbach, M., and Caplan, S. R. (1978). In *Energetics and Structure of Halophilic Microorganisms* (Caplan, S. R., and Ginzburg, M., eds.), Elsevier, Amsterdam, pp. 223–232.
Dencher, N. A., and Wilms, M. (1975). *Biophys. Struct. Mech.* **1**, 259–271.
Eisenbach, M., and Caplan, S. R. (1979). In *Current Topics in Membranes and Transport* (Bronner, F., and Kleinzeller, A., eds.), Vol. 12, Academic Press, New York, pp. 165–248.
Eisenbach, M., Garty, H., Bakker, E. P., Klemperer, G., Rottenberg, H., and Caplan, S. R. (1978). *Biochemistry* **17**, 4691–4698.
Falk, G., and Fatt, P. (1968). *J. Physiol.* **198**, 647–699.

Garty, H., Klemperer, G., Eisenbach, M., and Caplan, S. R. (1977). *FEBS Lett.* **81**, 238–242.
Gupte, S. S., Haug, A., and El-Bayouni, M. A. (1976). *Biophys. J.* **16**, 103a.
Hara, R. (1963). *J. Gen. Physiol.* **47**, 241–264.
Kimura, Y., Ikegami, A., and Stoeckenius, W. (1984). *Photochem. Photobiol.* **40**, 641–646.
Korenstein, R., Sherman, W. V., and Caplan, S. R. (1976). *Biophys. Struct. Mech.* **2**, 267–276.
Lozier, R. H., Niederberger, W., Bogomolni, R. A., Hwang, S. R., and Stoeckenius, W. (1976). *Biochim. Biophys. Acta* **440**, 545–556.
Marinetti, T. (1987). *Biophys. J.* **51**, 875–881.
Marinetti, T., and Mauzerall, D. (1983). *Proc. Natl. Acad. Sci. USA* **80**, 178–180.
Oesterhelt, D., and Stoeckenius, W. (1971). *Nature (New Biol.)* **233**, 149–152.
Oesterhelt, D., and Stoeckenius, W. (1974). *Methods Enzymol.* **31**, 667–678.
Ovchinnikov, Yu. A. (1987). *Photochem. Photobiol.* **45**, 909–914.
Sherman, W. V., Slifkin, M. A., and Caplan, S. R. (1976). *Biochim. Biophys. Acta* **423**, 238–248.
Slifkin, M. A., and Ali, S. M. (1985). *J. Phys.* [*E*] **18**, 425–429.
Slifkin, M. A., and Darby, M. I. (1978). *J. Phys.* [*E*] **11**, 11–68.
Slifkin, M. A., and Darby, M. I. (1980). *J. Phys.* [*E*] **13**, 896–897.
Slifkin, M. A., Garty, H., Sherman, W. V., Vincent, M. F. P., and Caplan, S. R. (1979). *Biophys. Struct. Mech.* **5**, 313–320.
Slifkin, M. A., Bakarudin, I., and Ali, S. M. (1983). *J. Phys.* [*E*] **16**, 1194–1199.
Slifkin, M. A., Bakarudin, I., Corda, D., and Caplan, S. R. (1986a). *Biochim. Biophys. Acta* **883**, 169–171.
Slifkin, M. A., Smith, C. W., and Bakarudin, I. (1986b). *J. Biol. Phys.* **14**, 15–20.
Trissl, H.-W., and Montal, M. (1977). *Nature* **266**, 655–657.