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Articles

Membrane Potential Modulates Photocycling Rates of Bacterial Rhodopsins†

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ABSTRACT: Effects of membrane potential on photochemical reactions of three retinal-containing chromoproteins in *Halobacterium halobium*, sensory rhodopsin I (sR-I), bacteriorhodopsin, and halorhodopsin, are described. Each of the three exhibits a decreased rate of thermal decay of its principal intermediate when photoactivated in an artificially energized compared to a deenergized membrane. The similar response of the three pigments suggests a voltage-dependent conformational change common to their respective photocycles. Spectral and kinetic properties of the sR-I photochemical reaction cycle were measured in phototactic *H. halobium* cells, and differences from in vitro photocycle kinetics were attributable to the electrical membrane potential present in vivo. In vivo sR-I photocycling rates were reproduced in envelope vesicle preparations in the presence of a valinomycin-induced potassium diffusion potential.

Four retinal-containing proteins have been found in *Halobacterium halobium* membranes. Bacteriorhodopsin (bR, λ_{max} = 568 nm) and halorhodopsin (hR, λ_{max} = 578 nm) are light-driven ion pumps (for H^+ and Cl^-, respectively) which hyperpolarize the membrane, contributing energy to the cell in the form of transmembrane electrochemical potential [for reviews, see Stoeckenius (1980), Stoeckenius and Bogomolni (1982), and Lanyi (1986)]. In addition, the cells exhibit light-induced motility responses mediated by at least two phototaxis receptors, sensory rhodopsins I and II (sR-I, λ_{max} = 587 nm, and sR-II, λ_{max} = 480 nm) [for recent reviews, see Takahashi et al. (1987) and Spudich and Bogomolni (1988)]. Like bR and hR, the sensory rhodopsins undergo cyclic photochemical reactions, which, however, do not result in membrane hyperpolarization. Both sR-I and sR-II have long-lived photointermediates with absorption maxima in the near-UV. $SR\text{-}I_{587}$ is an attractant light receptor which allows cells to migrate into regions optimal for their light-driven ion pumps. Its long-lived intermediate (S_{373}) and $sR\text{-}II_{480}$ are repellent light receptors.

Biochemical and photochemical properties of sR-I and sR-II have been obtained almost exclusively from measurements using membranes prepared from *H. halobium* cells (Bogomolni & Spudich, 1982; Spudich & Bogomolni, 1983). Recently, a strain has been isolated [Flx5R (Spudich et al., 1986)] which produces the sR-I apoprotein in sufficient quantity to permit investigation of this pigment's photocycle in vivo. In this paper, we describe photocycling of sR-I in intact Flx5R cells under conditions in which they exhibit phototaxis and in sonicated cell envelopes in the presence or absence of electrical potential across the membrane. These results are compared to those obtained with similar vesicles containing bR and hR.

Our studies reveal a modulation of the sR-I photocycle by membrane potential in vivo. Such an effect on sR-I was suggested earlier by the observation that the sR-I photocycle was retarded by photoactivation of hR in the same membrane and that this effect was partially reversed by proton ionophore (R. Bogomolni, personal communication). This result and a similar effect on hR, reported here, generalize the membrane potential modulation of the bR photocycle noted previously from the effects of illumination on bR (Westerhoff & Dancshazy, 1984).

Materials and Methods

Chemicals. 3,3′-Dipentyloxadicarbocyanine iodide [$diOC_5(3)$][1] was purchased from Molecular Probes (Eugene,

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[1] Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; TPB, tetraphenylboron; DNP, 2,4-dinitrophenol; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; TRIS, tris(hydroxymethyl)aminomethane; $diOC_5(3)$, 3,3′-dipentyloxadicarbocyanine iodide; $diOC_2(3)$, 3,3′-diethyloxacarbocyanine iodide; $\Delta\Psi$, transmembrane electrical potential; $\Delta\Psi_{K^+}$, valinomycin-induced potassium diffusion potential; $t_{50\%}$, time for recovery of 50% of the initial absorbance change; $t_{1/2}$, half-time for a process obtained from curve fitting of the data to first-order kinetics.

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OR). Valinomycin, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 2,4-dinitrophenol, tetraphenylboron (TPB), and gramicidin were from Sigma (St. Louis, MO). All other chemicals were reagent grade; all solutions were prepared in distilled, deionized water.

Strains and Culture Conditions. H. halobium strains were grown aerobically in complex medium (Lanyi & MacDonald, 1979) in the dark. Three strains were used to obtain preparations of predominantly one pigment absorbing in the spectral region of the actinic flash used ($\lambda > 600$ nm). Flx5R regenerated with *all-trans*-retinal contains no bR or hR, but high concentrations of sR-I (Spudich et al., 1986). S9 contains at least 10-fold greater concentrations of bR than of any of the other pigments (Bogomolni & Spudich, 1982). OD2W, a carotenoid-deficient isolate from OD2 (Spudich & Spudich, 1982), contains sR-I and hR but not bR. SR-II absorption is negligible beyond 600 nm and therefore does not interfere with our flash-induced absorbance measurements (Spudich et al., 1986).

Vesicle Preparation. Cell envelope vesicles were prepared from stationary-phase cells (3.5–4.5-days growth) by a modification of the method of MacDonald and Lanyi (1975). Cells were pelleted (8000*g*, 30 min) and washed once with one-tenth their original volume of 4 M NaCl/25 mM Tris (pH 6.8). Following resuspension at one-tenth the original volume of the same solution, cells were sonicated 4 times for 15 s followed each time by 45-s cooling in ice water (probe sonicator, Heat Systems Ultrasonics, NY; 100-W output). Cellular debris and unbroken cells were pelleted (8000*g*, 30 min), and the vesicles were pelleted from this supernatant (150000*g*, 1 h), resuspended in $^1/_{20}$th the original volume of 4 M NaCl, pelleted again, and resuspended at 6–10 mg/mL in 4 M NaCl. All operations were performed at 4 °C. Sensory rhodopsin I activity was generated in Flx5R vesicles by addition of 3–5 μL of 0.7 mM *all-trans*-retinal in ethanol per milliliter of vesicles at 8 mg of protein/mL. Typically, A_{590} generated by retinal addition was 0.05–0.1 in the vesicles.

Vesicles from strain S9 were prepared as above, and the vesicular fraction was further purified by a Ficoll/CsCl/NaCl step gradient as described (Groma et al., 1984). Only the vesicular fraction from this gradient exhibited $\Delta\Psi$-dependent retardation of the bR photocycle.

Bleaching of sR-I. OD2W vesicles at 4–5 mg of protein/mL in 4 M NaCl were stirred at 37 °C under orange illumination from a 300-W tungsten lamp (type ENG,GE) filtered through 2.5 cm of water, a heat-absorbing filter, and a Corning 3-69 filter. Freshly prepared 2.0 M NH_2OH in 4 M NaCl, pH 9.0, was added to a final concentration of 0.2 M. After 1 h, the vesicles were pelleted by ultracentrifugation and resuspended in 4 M NaCl/25 mM Tris, pH 6.8, and repelleted twice with final resuspension in the original volume of 4 M NaCl; >70% of sR-I was bleached by this procedure while hR activity was essentially unaffected, as assessed by flash photolysis.

Diffusion Potential in Vesicles. Potassium diffusion potentials were induced in native vesicles as described (Renthal & Lanyi, 1976). Vesicles in 4 M NaCl were diluted 1:10 into 3 M KCl, washed 3 times in this solution, resuspended at 40–50 mg of protein/mL in 3 M KCl, and sonicated 5 s on ice [50-W output, microtip probe sonicator (Heat Systems Ultrasonics, Plainview, NY)]. For the K^+ diffusion potential experiments, these vesicles were diluted 30–40-fold into 2 mL of 3 M salt at various ratios of KCl/NaCl concentrations. Valinomycin was added from a 0.1 mM ethanolic stock to a final concentration of 0.4 μM. Potassium diffusion potentials were calculated by using the Nernst equation (Hodgkin & Katz, 1949; Kashket, 1985):

$$-\Delta\Psi_{mV} = 59 \log ([K^+]_{in}/[K^+]_{out})$$

where $[K^+]_{in} = 3$ M and $[K^+]_{out}$ is the K^+ concentration in the resuspension solution corrected for the carry-over of K^+ by the addition of vesicles, assuming negligible internal volume.

Fluorescence Measurements of Potassium Diffusion Potential. The potential-sensitive fluorescence of the cyanine dye $diOC_5(3)$ was used to monitor valinomycin-induced potentials (Renthal & Lanyi, 1976; Waggoner, 1979). Dye concentration was calculated from the extinction coefficient given by Sims et al. (1974) for $diOC_2(3)$ in ethanol. Twelve microliters of vesicles loaded with 3 M KCl was diluted into 3 mL of 3 M salt at various ratios of KCl/NaCl concentrations, 35 μL of a 47 μM ethanolic solution of dye added, and the suspension gently vortexed and placed in a quartz cuvette (1-cm path length) in a spectrofluorometer (Perkin-Elmer Model 650-40; excitation 450 ± 2 nm, emission 500 ± 2 nm). After a stable base line was achieved, 12 μL of 0.1 mM valinomycin in ethanol was added while the sample's fluorescence was monitored. When vesicles loaded with 3 M KCl were resuspended in isotonic media containing lower concentrations of K^+, the valinomycin-induced quenching of the dye fluorescence was proportional to the logarithm of the ratio $[K^+]_{in}/[K^+]_{out}$ and linear in the range $-\Delta\Psi = 40$–100 mV.

Flash Spectroscopy. (A) In Vitro Measurements. Flash-induced absorbance changes were measured in vesicles using an experimental system described previously (Spudich et al., 1986) based on photochemical properties of the pigments established earlier (Bogomolni & Spudich, 1982, 1987). For all experiments, the actinic flash was filtered with a $\lambda > 600$ nm filter (Ditric Optics, Hudson, MA) except where noted in Figure 2. Monitoring wavelengths for sR-I were 570 nm ($sR\text{-}I_{587}$) and 380 nm (S_{373}), 500 nm for hR (hR_{520}), and 410 nm for bR (M_{412}). The photomultiplier tube was protected by a filter transmitting the monitoring wavelength ±5 nm and $\lambda < 600$ nm short-pass filter (Ditric Optics, Hudson, MA). All measurements were made at 25 °C. For K^+ diffusion potentials, approximately 30 flashes were averaged and stored for each data set, valinomycin was added, and a second data set was recorded after mixing. The measurement was completed in less than 5 min after the addition, determined as a period of constant potential by the fluorescence measurements. Each trace was acquired as 3968 data points, with acquisition rates of 1 or 2 ms/point, 50 μs/point, and 100 μs/point for sR-I, hR, and bR, respectively. Data were converted to absorbance units and analyzed graphically and by computer. Rate constants and amplitudes were obtained by fitting the absorbance data to a single exponential [$A_t = B \exp(-kt)$] or a double exponential [$A_t = C \exp(-k_1 t) + D \exp(-k_2 t)$], using the least-squares method (Bevington, 1967) on an AT&T 6300 personal computer with a commercial software package (Asystant, Macmillan Software Inc., New York, NY). Rate constants and amplitudes are based on 250 data points linearly distributed over approximately 80% of the decay. Deciding whether a best fit was obtained by one or two exponential terms was based on the shape of the residual function, the sum of squares, and the standard deviation of the data from the fitted curve.

(B) In Vivo Measurements. Twenty milliliters of stationary-phase Flx5R cells was pelleted by centrifugation (4500*g*, 15 °C, 20 min), resuspended in basal salt (Weber et al., 1982) containing 1% arginine (pH 7.0), repelleted, and resuspended in the same solution at 2×10^{10} cells/mL. One microliter from a 2.0 mM ethanolic solution of *all-trans*-retinal was added per

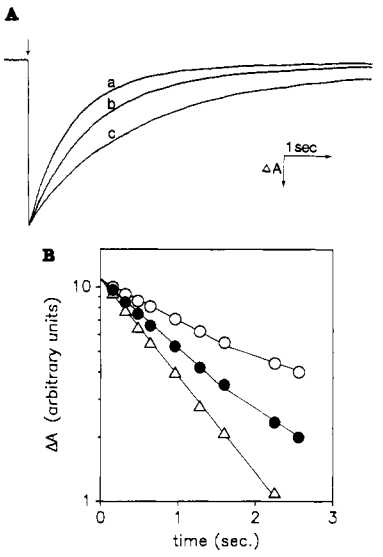

FIGURE 1: (A) Flash-induced absorbance changes at 570 nm. Flx5R cells and envelope vesicles were prepared and incubated with *all-trans*-retinal as described under Materials and Methods. (a) Vesicles (0.7 mg of protein/mL); (c) cells (20 mg of protein/mL); (b) cells, 5 min after addition of 10 μM CCCP. Actinic flash, λ > 600 nm at arrow, 1-cm path length, 25 °C. Initial absorbance changes (2 ms after the flash) were 0.006, 0.040, and 0.035 for vesicles, cells, and cells with CCCP, respectively, and are plotted normalized to the same arbitrary unit. (B) Semilogarithmic plot of the data in (A): (○) cells; (●) cells + CCCP; (△) vesicles.

milliliter of cell suspension, which saturated the sR-I apoprotein in Flx5R as determined by flash photolysis. For flash spectroscopy, 2 mL of the cell suspension in a 1-cm path-length cuvette was purged with argon for 20 s, and the cuvette was sealed with parafilm. Cells prepared in a similar manner have been observed to remain motile and phototactic for several hours (Wolff et al., 1986; W. Stoeckenius, personal communication). Confirming this, Flx5R cells prepared in this manner and placed in a chamber sealed with paraffin for microscopic examination remained motile and responsive to repellent light stimuli for over 5 h.

Protein concentrations were determined by the Lowry assay as modified for membrane proteins (Peterson, 1977).

RESULTS

SR-I Photochemical Reactions in Vivo. In sonicated vesicle preparations, flash-induced absorbance changes of strain Flx5R exhibited a submillisecond depletion at 590 nm and appearance of absorbance at 380 nm characteristic of the photoconversion of sR-I_{587} to S_{373} (Bogomolni & Spudich, 1987). The 380-nm absorbance change decayed with first-order kinetics concomitant with the reappearance of 570-nm absorbance ($t_{1/2}$ = 0.68 s), as expected from the thermal relaxation of S_{373} to sR-I_{587} (Bogomolni & Spudich, 1987). Flx5R cells, under conditions which maintain their phototactic activity (see Materials and Methods), exhibited similar absorbance changes at 570 and 380 nm (Figures 1A and 2). However, two differences are evident: (i) the overall rates of ΔA_{380} decay and ΔA_{570} recovery are much slower in vivo; and (ii) the absorbance changes in vivo deviate from a single first-order rate and are best fit by a sum of two exponential terms [Figure 1B, $t_{1/2}$(slow) = 1.98 s, $t_{1/2}$(fast) = 0.20 s]. A priori, these differences could be explained by different photocycle intermediates occurring in vitro and in vivo. However, when the absorbance changes immediately after the flash are monitored at various wavelengths, the resultant flash-induced difference spectra are indistinguishable (Figure 2), indicating that the same principal photointermediates occur in both conditions. Furthermore,

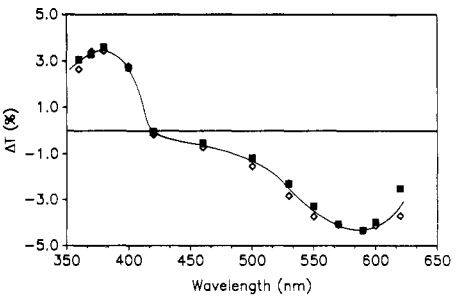

FIGURE 2: Flash-induced absorption difference spectra. Absorption changes (2 ms after photoexcitation) in retinal-regenerated Flx5R cells (◇) or vesicles (■) as in Figure 1 (except actinic flash λ > 640 nm), determined at various wavelengths. In vivo data were normalized to the vesicle value at 590 nm.

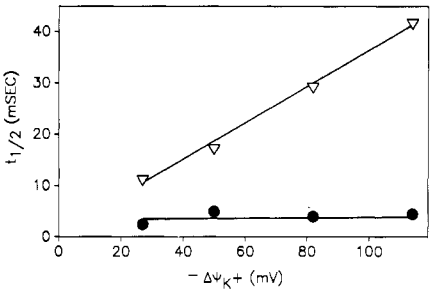

FIGURE 3: Dependence of bR photocycle on membrane potential. The lifetime of the flash-induced 410-nm absorbance change was monitored in S9 vesicles at different magnitudes of potassium diffusion potential. (▽) slow component, (●) fast component; 25 °C, pH 6.9.

Table I: Half-Life Values ($t_{1/2}$) and Ratios of Initial Amplitudes for Flash-Induced Recovery of sR-I_{587} in Different Preparations

preparation	$t_{1/2}$ (s)	$A_1/(A_1 + A_2)^a$
vesicles	0.68	1.00
cells	1.98, 0.20	0.75
cells + CCCP[b]	1.60, 0.59	0.44
cells + CCCP[c]	0.70	1.00
vesicles (energized)[d]	1.20, 0.30	0.75

[a] A_1 (A_2) is the initial amplitude of the slower (faster) component. [b] Five minutes after CCCP addition. [c] After storage at 4 °C for 14 days. [d] $\Delta\Psi_{K^+}$ = −96 mV.

the ratio of the fast and slow component amplitudes in the in vivo measurements is constant at 400 nm and across the depletion band (570–600 nm) (data not shown).

Addition of the proton ionophore CCCP to cells accelerates the overall photocycle (Figure 1, Table I) with changes in both rates and initial amplitudes of slow and fast components; incubation of CCCP-treated cells at 4 °C for 14 days yields a monophasic decay of absorbance change with a $t_{1/2}$ value similar to nonenergized vesicles. Other uncouplers (DNP and valinomycin/K^+) had similar effects (data not shown). Since these agents dissipate the transmembrane electrical potential ($\Delta\Psi$), this result suggested that the kinetic difference between cells and vesicles may be caused by modulation of the sR-I photocycle by $\Delta\Psi$ in the former. To test this possibility, we examined the photocycles of bR, sR-I, and hR in vesicles artificially energized with K^+ diffusion potential.

Photocycle Rates in the Presence of K^+ Diffusion Potential. (A) Bacteriorhodopsin. When bR-containing vesicles or cells are illuminated, a proton electrochemical gradient is generated across the membrane, and a marked decrease in the decay rate of the M_{412} photointermediate is observed (Quintanilha, 1980; Dancshazy et al., 1983; Groma et al., 1984; Westerhoff & Danchazy, 1984). We induced potassium diffusion potential across sonicated cell envelopes from strain S9 and monitored

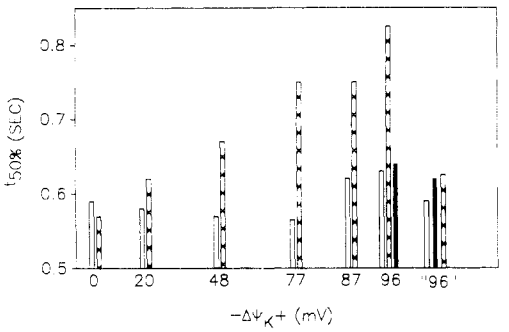

FIGURE 4: Dependence of sR-I photocycle rate on membrane potential. The recovery rate of flash-induced absorbance changes at 570 nm in retinal-regenerated Flx5R vesicles was monitored at different magnitudes of potassium diffusion potential. Time for 50% decay of the total absorption change before (open bars) and after (cross-hatched bars) addition of 0.4 μM valinomycin. (Solid bars) Time for 50% decay in the presence of 20 μM CCCP after (96 mV) or prior to ("96" mV) the addition of valinomycin.

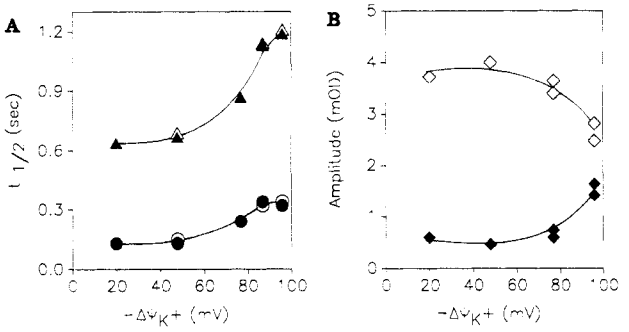

FIGURE 5: (A) Dependence of sR-I fast and slow component rates of S_{373} decay on membrane potential. Effect of potassium diffusion potential on the half-life of the fast (circles) and slow (triangles) components of the flash-induced absorbance changes in retinal-regenerated Flx5R vesicles at 570 nm (△, ○) and 380 nm (▲, ●). (B) Dependence of sR-I fast- and slow-component amplitudes of S_{373} generation on membrane potential. Effect of potassium diffusion potential on the initial amplitudes of the fast (♦) and slow (◇) components of the biphasic decay. Note that the fast- and slow-component amplitudes sum to a constant value independent of membrane potential.

the absorbance changes at 410 nm due to M_{412} formation and decay following an actinic red flash (Figure 3). The absorbance change data were best fit with biphasic kinetics, with one component ($t_{1/2}$ = 4.0 ms) not affected by $\Delta\Psi$ and a slower component ($t_{1/2}$ = 10–40 ms) retarded by increasing magnitudes of $\Delta\Psi$. These results matched the trend observed by Groma et al. (1984) using illumination-generated $\Delta\Psi$ in the same type of preparation.

(B) Sensory Rhodopsin I. The photocycle rate of sR-I, determined by the thermal decay of S_{373} to sR-I_{587}, was decreased by increasing magnitudes of membrane potential. This effect was reversed by CCCP (as well as by TPB or gramicidin) and prevented when CCCP was added before valinomycin (Figure 4), indicating that the increase in the lifetime is a response to membrane potential. The slight increase in $t_{50\%}$ at increasing K^+ gradients prior to the addition of valinomycin may result from a nonzero K^+ permeability. At symmetrical KCl solution ($[K^+]_{in} = [K^+]_{out}$ = 3 M), the data fit a single first-order process ($t_{1/2}$ = 0.68 s), while at large $\Delta\Psi$, the traces are clearly biphasic and better fit by the sum of two exponential decay terms (Table I). Both kinetic constants show a dependence on $\Delta\Psi$ in the range of −60 to −100 mV (Figure 5A). Identical results are obtained whether the absorbance change is monitored near the peak absorption of sR-I_{587} (570 nm) or near that of the principal photointermediate S_{373} (380 nm, Figure 5A). The amplitudes of the two processes are independent of membrane potential in the region of $\Delta\Psi > -80$ mV, above which the fast component increases at the expense of the slow component (Figure 5B). From Figure 5, it can be seen that the membrane potential value at which the fractional amplitudes change is about the same as the one where the rate constants are most affected.

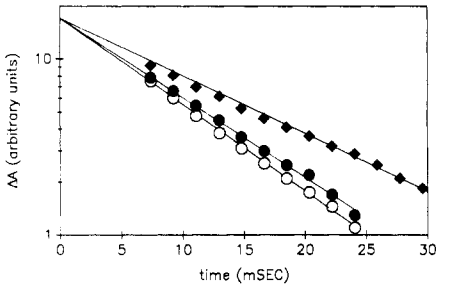

FIGURE 6: Halorhodopsin photocycle rates in the presence and absence of membrane potential. Semilog plot of the flash-induced absorbance change at 500 nm in OD2W vesicles (○), after the addition of 0.4 μM valinomycin [(♦) $\Delta\Psi_{K^+}$ = −87 mV], and the subsequent addition of 20.0 μM CCCP (●).

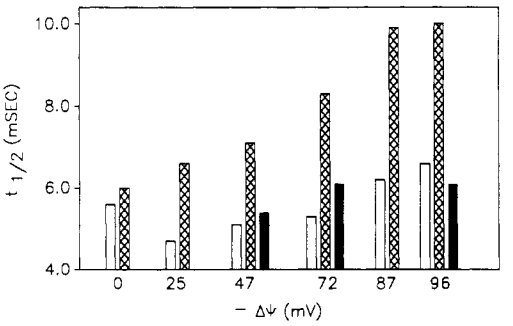

FIGURE 7: Dependence of hR photocycle rate on membrane potential. Effect of potassium diffusion potential on the half-life of the 500-nm absorbance change in OD2W vesicles before (open bars) and after (cross-hatched bars) the addition of 0.4 μM valinomycin, and (solid bars) after the subsequent addition of 20.0 μM CCCP.

To test whether pH changes or pH gradients, which could develop upon valinomycin addition, contribute significantly to the kinetic effects of $\Delta\Psi$, the measurements were repeated under conditions of strong buffering both inside and outside the vesicles (50 mM HEPES, pH 7.6). Both the retardation of the sR-I photocycle and the generation of a biphasic decay occur under these conditions (a single first-order $t_{1/2}$ of 0.58 s is split to biphasic $t_{1/2}$'s of 0.20 and 0.85 s at $\Delta\Psi$ = −91 mV).

(C) Halorhodopsin. OD2W vesicles, treated as described under Materials and Methods, exhibited a flash-induced absorbance increase at 500 nm as expected from the photoconversion of hR_{578} to hR_{520} (Lanyi, 1986). In the absence of membrane potential, the decay of hR_{520} was first order with $t_{1/2}$ = 5 ms (Figure 6). As with bR and sR-I, valinomycin-induced $\Delta\Psi$ retards the hR photocycle, and the effect is reversed by the addition of CCCP (Figures 6 and 7), gramicidin, or TPB. With hR, however, throughout the range of $\Delta\Psi$ values tested, data were well fit by single-exponential kinetics (Figure 6), and no improvement in the quality of the fit was achieved by fitting to two exponential terms. The small amount of sR-I remaining in this preparation (Materials and Methods) does not influence the data, since when the contribution of sR-I as measured in Flx5R vesicles under identical conditions is subtracted, the obtained kinetic values are not changed. Changes in pH evidently do not contribute significantly to the effect of membrane potential on hR in these measurements, since the same results were obtained in the

presence of 50 mM HEPES (pH 7.6) ($t_{1/2}$ changed from 5.8 to 9.0 ms upon induction of $\Delta\Psi = -91$ mV).

Discussion

The photochemical properties of sR-I which are important to color sensing by *H. halobium* have been determined previously in vesicle preparations. The observations reported here establish that these properties are essentially the same in vivo under conditions in which the cells exhibit phototaxis. However, two distinct differences are observed in the kinetics of the photocycle: (i) the thermal return of S_{373} to sR-I_{587} in vitro occurs at nearly twice the in vivo rate ($t_{50\%} = 1.20$ s in intact cells and 0.68 s in cell envelopes); and (ii) S_{373} decay in vivo is biphasic rather than monophasic as in vesicles. The effects of CCCP in vivo and study of artificially energized vesicles suggest that these kinetic differences arise from modulation of the photocycle by the membrane potential present in phototactically active cells. In view of this result, it is important to take into account the actual in vivo sR-I photocycling rates in behavioral analyses which relate sR-I photoreactions to the flagellar motor responses.

The decay of M_{412} was previously shown to be retarded by background illumination which drives electrogenic proton translocation by bR. This effect was interpreted as due to membrane potential, which our measurements of bR photocycling in artifically energized vesicles confirm. A "backpressure" effect (Westerhoff & Dancshazy, 1984), exerted on bR by its product (i.e., protonmotive force), was suggested to be responsible for the photocycle inhibition, much like the respiratory control observed in mitochondria (Harold, 1986). However, since the photochemical reaction cycle of sR-I does not result in electrogenic transport, this explanation would not hold for this pigment. An explanation which would apply to bR, sR-I, and hR is that intramolecular charge movements with a vectorial component normal to the plane of the membrane occur in the rate-determining thermal steps of each of the three pigments. Such movements are expected in the M_{412} decay during which protonation of the Schiff base occurs (Stoeckenius, 1980; Stoeckenius & Bogomolni, 1982), and similar protonation of S_{373} is strongly suggested on the basis of the close analogies between bR and sR-I photointermediates (Bogomolni & Spudich, 1987). The hR photocycle does not undergo Schiff base deprotonation, although charge displacements associated with the Schiff base have been suggested to play a role in Cl^- translocation by hR (Oesterhelt et al., 1986; Lanyi, 1986; Schobert et al., 1986). Regardless of the particular charge movements responsible for these effects, the fact that all three retinal pigments show a similar response to membrane potential suggests a voltage-dependent conformational change common to their respective photocycles. These conformational movements may be responsible for the electric signals associated with the photocycles of bR (Keszthelyi & Ormos, 1980; Keszthelyi, 1984) and hR (Der et al., 1987).

The existence of normal phototaxis responses in Flx mutant strains whose membrane potential is unaffected by light (Spudich & Spudich, 1982), the nonelectrogenic nature of sR-I (Spudich & Spudich, 1982; Bogomolni & Spudich, 1982; Ehrlich et al., 1984), and sR-II (unpublished results) and a report of localized flagellar motor responses in filamentous *H. halobium* (Oesterhelt & Marwan, 1987) demonstrate that membrane potential changes are not an obligate part of the sensory transduction pathway in *H. halobium* phototaxis. Membrane potential modulation of sR-I may, however, have influenced previous studies which were interpreted at the time as implicating membrane hyperpolarization in attractant signaling. Skulachev and co-workers observed a close correlation of the photoattractant response by *H. halobium* with the proton electrochemical potential generated by light (by bR and hR) (Baryshev et al., 1981, 1983), and peaks in *H. halobium* photoattraction action spectra have been reported near the absorption maxima of bR (Hildebrand & Dencher, 1975) and possibly hR (Traulich et al., 1983). Since the monitoring light used in these studies generates a photostationary-state mixture of sR-I_{587} and S_{373}, hyperpolarization by bR and hR would be expected to shift the mixture toward greater S_{373} concentration, producing an attractant response [Spudich & Bogomolni, 1984; see discussion in Spudich and Bogomolni (1988)]. Membrane potential changes may also signal taxis responses through the aerotaxis system in *H. halobium* (W. Stoeckenius and B. Taylor, personal communications), providing an additional indirect contribution of photoinduced polarization changes to motility responses.

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Electron-Transfer Events near the Reaction Center in O_2-Evolving Photosystem II Preparations†

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ABSTRACT: Time-resolved ESR has been used to study electron-transfer reactions in oxygen-evolving photosystem II membrane fragments. The exogenous acceptor dichlorobenzoquinone (DCBQ) is reduced by photosystem II; the ESR spectrum of the resulting DCBQ radical overlaps the center but not the wings of the ESR spectra of the endogenous tyrosine radicals Y_D^+ and Y_Z^+. Here Y_Z^+ denotes the species that is involved in electron transfer between the reaction center chlorophyll, P_{680}, and the manganese-containing, oxygen-evolving complex, and Y_D^+ denotes the stable photosystem II radical. By using appropriate magnetic fields, we recorded kinetic transients of Y_Z^+ under repetitive flash conditions with DCBQ present. We also used 1 mM $K_3Fe(CN)_6$ as an exogenous acceptor when recording kinetic traces of Y_Z^+, although at concentrations above 5 mm we observe an additional signal that could be due to P_{680}^+. The kinetic traces of Y_Z^+ obtained with DCBQ or 1 mM $K_3Fe(CN)_6$ are similar and show two phases. The slower phase has a half-time of 1.2 ms and corresponds to the reduction of Y_Z^+ by the S_3 state; the faster phase reflects reduction of Y_Z^+ by both S_1 and S_2. By using flowing, dark-adapted PSII membranes, we resolved the $Y_Z^+S_1$ reaction ($t_{1/2}$ = 100 μs) on the first flash and found it to be significantly faster than the $Y_Z^+S_2$ reaction ($t_{1/2}$ = 300 μs) which occurs on the second flash. A high-resolution ESR spectrum of Y_Z^+ in O_2-evolving PSII membranes was obtained with gated integration techniques and found to be similar to the spectra of Y_D^+ and of Y_Z^+ in inhibited membranes. Thus, the magnetic interaction between spins on Y_Z^+ and the manganese in the oxygen-evolving complex broadens the Y_Z^+ spectrum negligibly. These results support the idea that a single electron carrier, Y_Z, operates between P_{680}^+ and the manganese ensemble in the oxygen-evolving complex and functions on all four S-state transitions.

In the photosystem II reaction center, oxidizing equivalents generated by a specialized chlorophyll, P_{680},[1] are transferred to a manganese-containing, oxygen-evolving site by way of an intermediate electron donor, which has been called Z or D1 [for reviews, see Babcock (1987) and Ghanotokis and Yocum (1985)]. This species was first observed by use of electron spin resonance to study thylakoids that had lost the ability to evolve oxygen through manganese depletion. In these preparations,

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[1] Abbreviations: DCBQ, 2,5-dichlorobenzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ESR, electron spin resonance; OEC, oxygen-evolving complex; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; P_{680}, primary donor of PSII; P_{700}, primary donor of PSI; Q_A, primary acceptor of PSII; Q_B, secondary acceptor of PSII; Tris, tris(hydroxymethyl)aminomethane; Y_D^+, stable tyrosine radical of PSII; Y_Z, electron donor of PSII, formerly called Z.

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