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Δψ-Mediated Signalling in the Bacteriorhodopsin-Dependent Photoresponse

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It has been shown previously that the proton-pumping activity of bacteriorhodopsin from *Halobacterium salinarium* can transmit an attractant signal to the bacterial flagella upon an increase in light intensity over a wide range of wavelengths. Here, we studied the effect of blue light on phototactic responses by the mutant strain Pho81-B4, which lacks both sensory rhodopsins but has the ability to synthesize bacteriorhodopsin. Under conditions in which bacteriorhodopsin was largely accumulated as the M_{412} bacteriorhodopsin photocycle intermediate, halobacterial cells responded to blue light as a repellent. This response was pronounced when the membrane electric potential level was high in the presence of arginine, active oxygen consumption, or high-background long-wavelength light intensity but was inhibited by an uncoupler of oxidative phosphorylation (carbonyl cyanide 3-chlorophenylhydrazone) and was inverted in a background of low long-wavelength light intensity. The response to changes in the intensity of blue light under high background light was asymmetric, since removal of blue light did not produce an expected suppression of reversals. Addition of ammonium acetate, which is known to reduce the pH gradient changes across the membrane, did not inhibit the repellent effect of blue light, while the discharge of the membrane electric potential by tetraphenylphosphonium ions inhibited this sensory reaction. We conclude that the primary signal from bacteriorhodopsin to the sensory pathway involves changes in membrane potential.

Halobacterium salinarium cells swim by means of bipolar flagellar tufts. Smooth swimming is occasionally interrupted by spontaneous cell reversals which are produced by changes in the direction of flagellar rotation. The frequency of spontaneous reversals varies depending on the culture conditions and the particular strain (2, 31, 38, 40, 41).

An important behavior of halobacteria is phototaxis, which these bacteria use to find the optimal light conditions for photosynthesis and to avoid the harmful effect of UV light. Changes in light intensity are sensed by retinal-containing proteins which control the direction of flagellar rotation through a system of sensory proteins. Increases in UV or blue light intensity or decreases in red or orange light are interpreted by the cells as unfavorable changes of environmental conditions and cause reversal of swimming direction. Decreases in UV-blue or increases in red-orange light suppress reversals. Two specialized photosensory systems have been identified in halobacteria. Sensory rhodopsins I and II (sRI [11, 38, 39] and sRII [24, 37, 42–45], respectively) serve as receptors for light with maximal absorbance at 578 and 487 nm, respectively. Photoexcitation of sRI generates a long-lived sensory rhodopsin intermediate, S_{373}, which triggers an attractant response by the cell (11). At the same time, S_{373} operates as a third specialized photoreceptor. While attractant light is detected by the ground form of the sRI molecule, repellent light is detected by sRII and the S_{373} intermediate of sRI (38).

Recently, we demonstrated that the light-driven proton pump bacteriorhodopsin is also able to act as a photoreceptor in halobacteria. This function is related to the proton-translocating activity of bacteriorhodopsin (7–10), and it has been suggested that receptor function is mediated by a device that monitors electrochemical gradient of hydrogen ions across the membrane ($\Delta\tilde{\mu}_{H^+}$) called a protometer (5, 6, 10, 15, 35). This supports other data on the photoreceptory function of bacteriorhodopsin which also attributed the effects of bacteriorhodopsin on swimming behavior to a secondary consequence of electrogenic proton pumping on metabolic or signal-transducing pathways rather than to primary sensory signaling such as that mediated by sRI (46). According to the original hypothesis (15, 35), increases in $\Delta\tilde{\mu}_{H^+}$ levels induce attractant responses, while decreases induce repellent responses in cells. At low $\Delta\tilde{\mu}_{H^+}$ levels, reversals are completely suppressed (8, 20). This hypothesis correlates well with the results from studies of aerotaxis and phototaxis in a range of species (1, 3, 17, 23, 27, 32–34), although no specific protein responsible for the $\Delta\tilde{\mu}_{H^+}$ reception has yet been identified. In our experiments, we have used the *H. salinarium* strain Pho81-B4 (7–9) constructed by transformation with a bacteriorhodopsin-encoding plasmid of the parental strain Pho81 (40), which is incapable of photoreception because it lacks all of the retinal-containing proteins. The Pho81-B4 strain is thus useful for investigations into the mechanism of the proton pump mediated taxis.

In principle, any change in the cell which is dependent on proton pump activity may be involved in the induction of a sensory response if it is somehow coupled to the taxis system. The response could be mediated by $\Delta\tilde{\mu}_{H^+}$ or only one of its components, i.e., membrane electric potential ($\Delta\psi$) or pH gradient (ΔpH). Internal pH changes dependent on the transmembrane proton transport could also be involved in the excitation of the sensory system as has been described for *Escherichia coli* (21, 29). In the present work, we show that $\Delta\psi$ alone is competent in signal transduction of the bacteriorhodopsin-mediated light signal.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The following strains of *H. salinarium* were used: Flx15 (bacteriorhodopsin negative [bR^-] halorhodopsin negative [hR^-] sRI^+ $sRII^+$) (36), Pho81-B4 (bR^+ hR^- sRI^- $sRII^-$), and Pho81-D96N-8 ($bR[D96N]^+$, hR^- sRI^- $sRII^-$) (7, 8). Strain Pho81-B4 was constructed by S. Bibikov; strain Pho81-D96N-8 with point mutation D96N in bacteriorhodopsin was a gift from D. Oesterhelt. Both were constructed by transformation of strain Pho81 (derived from Flx15 and deficient in the synthesis of all retinal proteins and the methyl-accepting protein involved in processing the signal sent from sRI [40]) with a plasmid carrying the bacterio-opsin gene according to the method described by Cline et al. (12) with the modifications of Ni et al. (28). The bacterio-opsin gene in transformant Pho81-D96N-8 carried the site-specific mutation of Asp-96 to Asn. The cells were grown and selected for motility under the conditions described by Bibikov et al. (8). The medium for selection of strains Pho81-B4 and Pho81-D96N-8 contained 20 μM mevinoline. The cells were harvested and washed once with basal salt solution (4.27 M NaCl, 27 mM KCl, 81 mM $MgSO_4$, 25 mM morpholinepropanesulfonic acid, 25 mM morpholineethanesulfonic acid, 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES]; all as sodium salts [pH 7.0 unless specifically mentioned]) by centrifugation at 3,500 × *g* for 10 min and were resuspended in this solution to a concentration of 10^7 to 10^8 cells per ml.

Phototactic reactions. Phototactic reactions were measured in a temporal gradient as previously described (7–9). Phototactic reactions in *H. salinarium* were monitored with a Univar (Reichert) photomicroscope at room temperature after changes in light intensity. To estimate the responses at very low intensities of monitoring light, we used an RCA videocamera with an autocontrasting device connected to a Panasonic monitor. To register the bacterial responses to short-wavelength light stimuli in a background of long-wavelength light, we applied the light stimulus through the objective of the microscope and the monitoring light through the condenser of the microscope fitted with an orange narrow-band pass filter (λ = 572 ± 10 nm). The stimulating light applied through the objective was passed through the blue band pass filter (λ = 450 ± 20 nm). A 200-W mercury lamp was the source of the background light, and a 40-W tungsten lamp was the source of the stimulating light. The times between photostimulus and reversal for each cell were measured, and the percentages of the population that reversed either at given times after stimulation or within a set period were plotted.

Photoelectric measurements. Purple membrane sheets containing bacteriorhodopsin were introduced into one of the compartments of a Teflon cell. The compartments were separated by a Teflon wall with a 3-mm hole. A collodium film impregnated with an asolectin solution in decane was inserted between the two compartments, and photovoltage data were obtained as described by Drachev et al. (14).

Measurement of light-dependent changes of pH in bacterial suspensions. Light-dependent changes of pH were measured with a PHM 92 Radiometer pH meter in a 4.5-ml polystyrene Kartell cell. The cells were suspended in basal salts solution without buffering at 2×10^9 cells per ml. The suspension of *H. salinarium* cells was aerated with a flow of oxygen-free nitrogen. The fiber optic MFO-90 (Microtec Fiber Optic) was used for illumination.

Measurements of changes of Δψ. Changes of Δψ were measured with the laboratory-made tetraphenylphosphonium (TPP^+)-sensitive electrode (16). This method is based on the fact that distribution of the membrane-permeable cation TPP^+ between the extracellular solution and cytoplasm depends on the level of Δψ in the cells. The concentration of cells in suspension was 2×10^9 cells per ml, and the concentration of TPP^+ bromide in suspension was 2 μM.

RESULTS

Photoelectric response of the bacteriorhodopsin D96N to blue light. To study the role of Δψ in bacteriorhodopsin-mediated photoreception, we took advantage of the ability of the bacteriorhodopsin proton pump to allow transient changes in Δψ across the membrane.

It was originally shown by Karvali and Dancshazy (19) and Dancshazy et al. (13) that blue light absorbed by M_{412}, a key intermediate of the bacteriorhodopsin photochemical cycle, returns M_{412} to the bR_{568} ground form. Upon formation of M_{412}, deprotonation of the Schiff base linking retinal and Lys-216 takes place with the consequent release of a proton to the extracellular space. The sequence of intermediates which follows the dark decay of M_{412} leads to the regeneration of bR_{568}, with binding of a proton from the intracellular space to the Schiff base. The blue-light-induced conversion of the M_{412} intermediate to the bR_{568} ground form is accompanied by the backward translocation of a proton from the extracellular space to reprotonate the Schiff base. The proton translocated in this process is moved through only half of the membrane thickness.

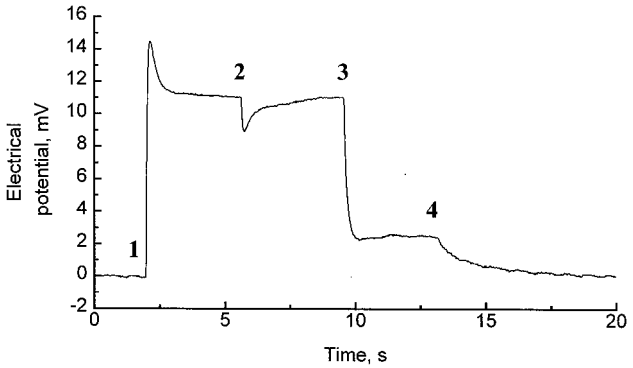

FIG. 1. Generation of electric potential by the mutant bacteriorhodopsin D96N in a purple membrane sheet-collodium film system. 1, orange light turned on; 2, blue light turned on; 3, orange light turned off; 4, blue light turned off.

In the first series of experiments, we used mutant D96N bacteriorhodopsin sheets adsorbed on collodium film impregnated with asolectin solution in decane. The D96N mutation in bacteriorhodopsin affects the decay of the M_{412} intermediate during the bacteriorhodopsin photocycle. As a result, bacteriorhodopsin is readily accumulated in the M_{412} form under long-wavelength light. A fast and transient decrease in Δψ was registered after switching on the blue light in the presence of the long-wavelength background light (Fig. 1). Under our experimental conditions, accumulation of the M_{412} intermediate in this mutant was observed at relatively low $\Delta\tilde{\mu}_{H^+}$ levels. Figure 1 demonstrates that orange light caused the generation of Δψ by bacteriorhodopsin. The additional illumination by blue light evoked a fast but transient decrease of the electric potential. Most probably, this transient decrease in the electric potential was due to a massive proton movement and capture accompanying the reprotonation of the Schiff base in bacteriorhodopsin by the blue-light-induced $M_{412} \rightarrow bR_{568}$ conversion. This process is known to be >10 times faster than the rise in the photopotential following the formation of M_{412}, just as blue-light-induced reconversion of M_{412} into bR_{568} is much faster than its formation (it is important to note that the decreasing effect of the blue light on Δψ begins with a very fast decrease [$\tau < 5$ μs] in the photopotential) (13). The transiency of the decrease in Δψ decrease is probably the result of the contribution of blue light to the generation of the normally oriented membrane potential, since bR_{568} also absorbs blue light to a limited extent.

The reversal response to blue light mediated by bacteriorhodopsin and the effect of Δψ. The intramembranous shift in charge evoked by blue light should be able to induce a tactic response in halobacterial cells if they can detect changes in Δψ. Thus, if changes in Δψ are sufficient for excitation, then Pho81-B4 cells containing bacteriorhodopsin but lacking both sRs should be able to respond to blue light as a repellent under conditions providing high stationary concentrations of the M_{412} form.

It is known that high Δψ arrests the bacteriorhodopsin photochemical cycle at the stage of conversion of M_{412} to the N_{520} form and causes the accumulation of the M_{412} intermediate under conditions of constant long-wavelength light intensity (18). Therefore, the reversal reaction to short-wavelength light would have to be stimulated by dark $\Delta\tilde{\mu}_{H^+}$-generating mechanisms as well as by the increase in the long-wavelength back-

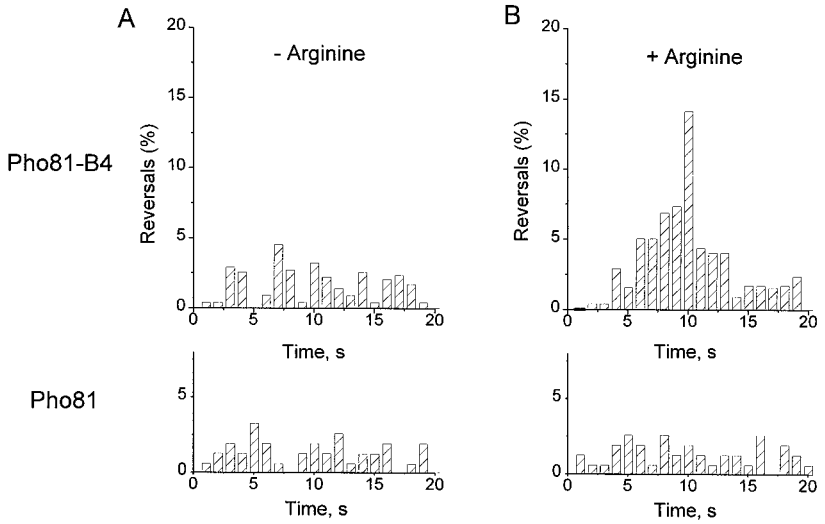

FIG. 2. Effect of arginine on the photosensitivity of Pho81-B4 and Pho81 control to blue light (450 ± 20 nm, 3.67×10^{15} quanta mm^{-2} s^{-1}). Background illumination was orange light (572 ± 10 nm, 3.8×10^{14} quanta mm^{-2} s^{-1}). (A) Without arginine; (B) 29 mM arginine. Bottom row demonstrates the effect of blue light on Pho81 cells in the absence (bottom graph in panel A) and presence (bottom graph in panel B) of arginine. Data for ≥140 cells were collected for each graph.

ground illumination. The responses of Pho81-B4 cells to the blue light stimuli confirmed these suggestions.

Measurement of the tactic response of the sR-deficient Pho81-B4 strain to blue light in the presence of low background light with maximal absorbance at 572 nm showed that the cells were repelled by the blue light despite the absence of sRs. This reversal response was very pronounced in the first minutes of monitoring after sample preparation, when there was sufficient oxygen in the samples to support respiration. The addition of arginine, a fermentation substrate for *H. salinarium*, stimulated the light-independent generation of $\Delta\tilde{\mu}_{H^+}$ and sensitized the reversal response to blue light (Fig. 2). It is noteworthy that oxygen respiration and arginine, as well as some other factors providing a bacteriorhodopsin-independent maintenance of high $\Delta\tilde{\mu}_{H^+}$ levels, were all potent suppressors of the attractant effect of light mediated by the proton-pumping function of bacteriorhodopsin (7–10). Addition of the protonophorous uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP) at a concentration which eliminated the suppression of the photoresponse in the presence of very bright light (7, 8) suppressed the sensitivity of the blue light reversal response. In the presence of CCCP, a higher background light intensity was needed to support the reversal response to blue light (Fig. 3). The blue-light-induced reversal response was still observed when a high $\Delta\tilde{\mu}_{H^+}$ was maintained. These conditions not only reduce the relative contribution of bacteriorhodopsin pumping to total cellular $\Delta\tilde{\mu}_{H^+}$ but also suppress the $\Delta\tilde{\mu}_{H^+}$-generating activity of bacteriorhodopsin, resulting in inhibition of the bacteriorhodopsin-mediated attractant response to light. The reversal response to blue light is unlikely to be the result of a decrease in $\Delta\tilde{\mu}_{H^+}$ because the effective single-photon photocycle of bacteriorhodopsin becomes a futile two-photon photocycle but is more possibly caused by the enforced proton translocation leading to transient generation of a reversed $\Delta\psi$.

As we have shown earlier, cells of the Pho81-D96N strain, which differ from Pho81-B4 by a point mutation (D96N) in bacteriorhodopsin, demonstrated a strong reversal response to blue light. The D96N mutation in bacteriorhodopsin affects the decay of the M_{412} intermediate during the bacteriorhodopsin photocycle. This leads to the accumulation of bacteriorhodopsin in the M_{412} form under long-wavelength background light. The intensities of blue light necessary to induce the reversal response in Pho81-D96N cells were lower than those for Pho81-B4 cells (Fig. 4), another indication of a role for M_{412} in this photoreception.

The effect of background illumination on the bacteriorhodopsin-mediated reversal response to blue light. As we mentioned before, in low-intensity background light Pho81-B4 cells displayed a reversal response when the blue stimulating light was switched off, thus demonstrating a pattern of behavior typical for a response to a decrease in attractant light (Fig. 5A). Under these conditions, Pho81-B4 cells sense the blue light as an attractant stimulus, unlike the wild-type cells with efficient sRs. This result supported our previous data on black-white photoreception by bacteriorhodopsin (8, 9). Here, we show that a high intensity of background orange light suppressed and even inverted this response. We observed an increase in the number of cells displaying a reversal response when the blue light was switched on (Fig. 5B). This effect may be considered a reciprocal change in the proportion of receptors

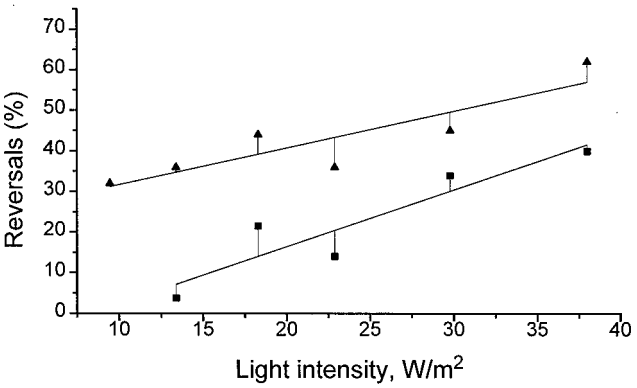

FIG. 3. The effect of CCCP on the sensitivity of Pho81-B4 to blue light (450 ± 20 nm, 3.67×10^{15} quanta mm^{-2} s^{-1}) as a function of the background long-wavelength light intensity (572 ± 10 nm) in the presence of 29 mM arginine. ▲, without CCCP; ■, 2.5 μM CCCP. Percentages of cells that reversed within 9 s of stimulation are given. Data for 75 cells were collected for each point.

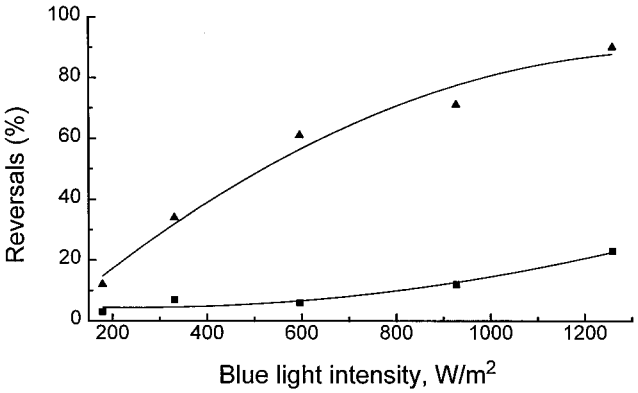

FIG. 4. The blue light reversal response in Pho81-B4 (■) and Pho81-D96N (▲) transformant cells under orange background illumination (572 ± 10 nm, 3.8 × 10^{14} quanta mm^{-2} s^{-1}). Blue light of various intensities (see abscissa) was added under conditions of constant background orange illumination. Percentages of cells that reversed within 6 s of stimulation are given. Data for 75 cells were collected for each point.

exhibiting the reversal suppression in response to light in black-white mode compared with receptors exhibiting the normal reversal response to short-wavelength light. In both cases, the receptor is represented by the bacteriorhodopsin molecule either in the bR_{568} ground form or in the M_{412} form, respectively.

Behavioral pessimism in the bacteriorhodopsin-mediated repellent response to blue light. The implied mechanism for the reversal response to blue light mediated by bacteriorhodopsin under conditions of long-wavelength background illumination in sR-deficient cells suggests that the decrease in blue light intensity should not induce a reversal suppression. The repellent response to blue light must, therefore, be asymmetric, unlike the photoreactions of cells containing sRs, such that increases in blue light intensity produce a reversal response and decreases in blue light intensity produce a reversal suppression (the sR-dependent response in Flx15 strain cells to blue light stimulation is presented in Fig. 5D as an example). The data presented in Fig. 5 show that this is the case. Cells did not demonstrate a suppression of reversal in response to a decrease in blue light intensity despite the strongly expressed reversal response to an increase in blue light intensity (Fig. 5B). Analogous behavior is seen in the strain possessing D96N mutant bacteriorhodopsin (Fig. 5C), suggesting the same mechanism of induction of the behavioral reaction to blue light for both wild-type and D96N bacteriorhodopsin. These results indicate that the transient change in $\Delta\psi$ elicited by blue light in bacteriorhodopsin-containing cells was sufficient to produce a tactic response. However, an alternative explanation is not excluded; according to this, the response could be induced by a transient local alkalinization of the extracellular space because of the proton uptake accompanying reprotonation of the Schiff base in a blue-light-induced $M_{412} \rightarrow bR_{568}$ conversion.

The effect of ammonium acetate on the bacteriorhodopsin-mediated photoresponse. Since acetate in its protonated form or ammonium in its deprotonated form is able to diffuse across the membrane, these species should strongly reduce ΔpH changes across the membrane. We observed that ammonium acetate (500 mM) had no effect on the bacteriorhodopsin-mediated response, confirming that changes in $\Delta\psi$, rather than in ΔpH, caused excitation (Fig. 6).

The effect of TPP^+ on the bacteriorhodopsin-mediated photoresponse. To study whether bacteriorhodopsin-induced pH changes contribute to the induction of phototactic responses, we used TPP^+ to discharge $\Delta\psi$ and thus convert $\Delta\psi$ to ΔpH. TPP^+ should dramatically increase light-dependent changes both in ΔpH and in the intracellular pH.

Illumination of *H. salinarium* Pho81-B4 caused the alkalinization, instead of acidification, of the media in spite of the pumping of protons by bacteriorhodopsin from the inner to the outer space. This is the result of the activity of the halobacterial Na^+/H^+ antiporter, converting $\Delta\tilde{\mu}_{H^+}$ to ΔpNa^+ (22, 25, 26). The light-dependent changes in pH had a biphasic pattern: the first phase showed an increase and the second phase showed a decrease in pH. Figure 7 shows that TPP^+ abolished the alkalinization but caused the acidification of media in response to illumination. TPP^+ had a significant inhibitory effect on the motility of halobacteria, with the almost complete inhibition of motility at a concentration of approximately 100 μM. Nevertheless, the analysis of the effect of TPP^+ on photophobic reaction to blue light in Flx15 cells mediated by sensory rhodopsins revealed the absence of any effect of 90

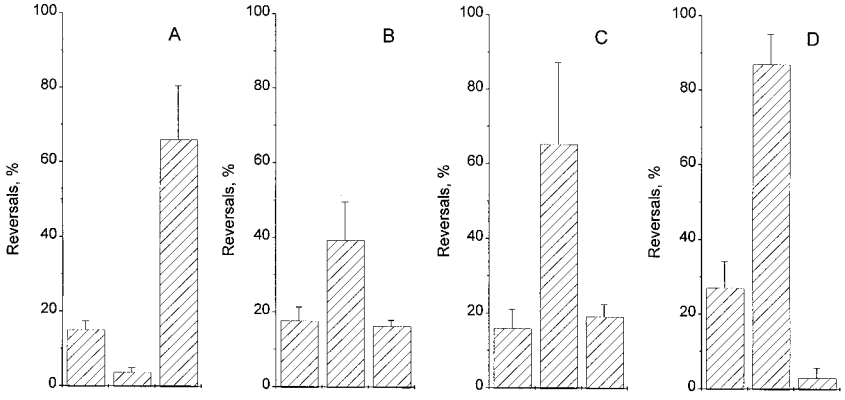

FIG. 5. The responses of halobacterial cells to blue light stimuli (450 ± 20 nm, 3.67 × 10^{15} quanta mm^{-2} s^{-1}). (A) Pho81-B4 cells under weak long-wavelength background illumination (572 ± 10 nm, 5.9 × 10^{12} quanta mm^{-2} s^{-1}); (B) Pho81-B4 cells under strong long-wavelength background illumination (572 ± 10 nm, 3.8 × 10^{14} quanta mm^{-2} s^{-1}); (C) Pho81-D96N cells; (D) Flx15 cells. In panels C and D, background light was 572 ± 10 nm, 3.8 × 10^{14} quanta mm^{-2} s^{-1}. In every diagram, the first column shows spontaneous reversals, the second column shows the response upon blue light stimulus, and the third column presents the response to removal of blue light. Percentages of cells that reversed within 6 s of stimulation were estimated, and the means and standard deviations of estimates for three populations of cells are shown. Data for 100 cells were collected for the Pho81-B4 strain, data for 60 cells were collected for the Pho81-D96N strain, and data for 90 cells were collected for the Flx15 strain for each population.

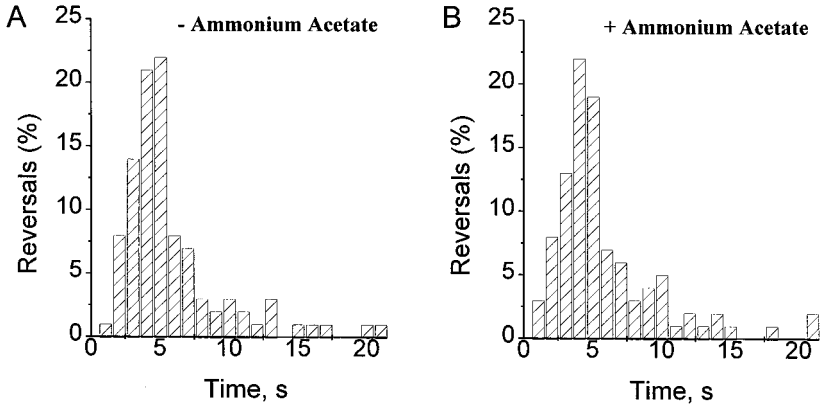

FIG. 6. The responses of Pho81-B4 cells to orange (572 ± 10 nm) light decrease from 3.8×10^{14} to 4.75×10^{13} quanta mm^{-2} s^{-1}. (A) Control; (B) 500 mM ammonium acetate. Data for 90 cells were collected for each graph.

μM TPP^+ on the taxis of motile cells. If halobacterial cells are able to respond to changes in ΔpH as well as in Δψ, the addition of TPP^+ in concentration sufficient to convert Δψ to ΔpH should not affect bacteriorhodopsin-mediated photoreception. We found that TPP^+ had an inhibitory effect on the response to a decrease in light intensity shown by Pho81-B4 (Fig. 8). If there is a role for ΔpH in the presence of TPP^+, then the response should be sensitive to ammonium acetate. However, the addition of ammonium acetate (350 mM) did not inhibit the response of cells in the presence of TPP^+ (Fig. 8), suggesting that Δψ was the only significant factor mediating the photoresponse.

The pH dependence of the bacteriorhodopsin-mediated photoresponse in *H. salinarium*. The contributions of ΔpH and Δψ to $\Delta\tilde{\mu}_{H^+}$ vary with pH. Shioi and Taylor (33) showed that the overall change in $\Delta\tilde{\mu}_{H^+}$ when anaerobic *Salmonella typhimurium* was aerated was greater at pH 5.5 than at pH 7.5. Despite data showing that changes in Δψ were smaller at more-acidic pHs, the aerotactic response was more pronounced at pH 5.5 than at pH 7.5. This result suggested that *S. typhimurium* cells could respond to changes in total $\Delta\tilde{\mu}_{H^+}$. Estimation of the light-induced changes in Δψ in *H. salinarium* cells at different pHs revealed that although Δψ increased with the increase in extracellular pH, the light-dependent change in Δψ was maximal at pH 6.5 and was lower at both higher and lower pHs (Table 1). At lower pHs, this may be a result of an increase in the ΔpH constituent of $\Delta\tilde{\mu}_{H^+}$. Under alkaline conditions, changes in the activities of the potassium-transporting system (4) and electrogenic Na^+/H^+ antiporter (22) might be involved. If only Δψ plays a role in bacteriorhodopsin-mediated signal transduction, then the photoresponse at pH 6.5 should be higher than that at pH 6.0 or pH 7.0. Table 1 shows that this was the case.

DISCUSSION

We have shown previously that in addition to the two specific photosensory systems sRI and sRII, *H. salinarium* has a

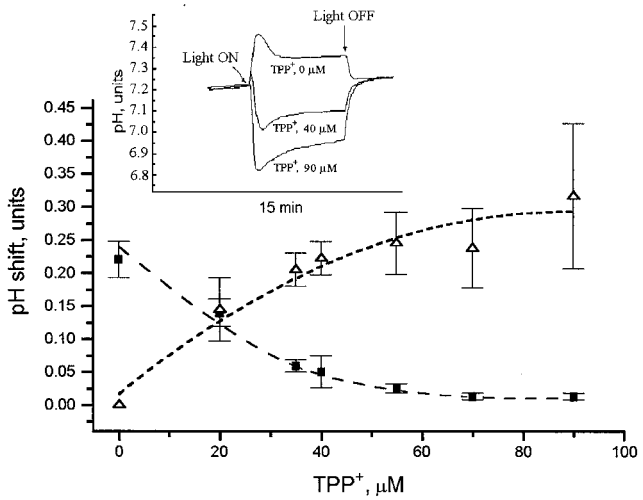

FIG. 7. The dependence of the light-induced shift in pH in the suspension of Pho81-B4 cells on the TPP^+ concentration in the medium. ■, alkalinization; △, acidification in units of pH. Means of six measurements and standard deviations are presented. Acidification and alkalinization at the same concentration of TPP^+ were estimated as the peaks of pH changes versus the initial value of pH 7.2. Inset, pattern of pH changes in response to illumination in the presence of different concentrations of TPP^+.

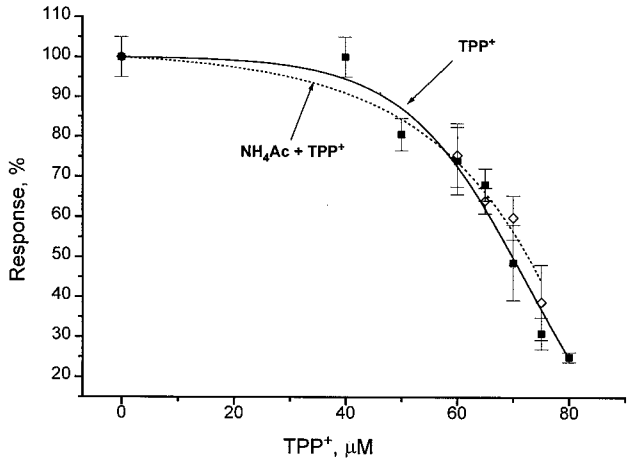

FIG. 8. The effect of TPP^+ on the photoresponses of Pho81-B4 cells. ■, absence of ammonium acetate; ◇, response in the presence of 350 mM ammonium acetate. The stimulus was an orange (572 ± 10 nm) light decrease from 3.8×10^{14} to 4.75×10^{13} quanta mm^{-2} s^{-1}. Reversals were estimated for a period of 8 s after a stimulus application. Photoresponses in the absence of TPP^+ were measured and assigned a value of 100%. The photoresponse values shown were calculated as percentages of photoresponses in the absence of TPP^+. The means and standard errors of the means for responses from three estimates for three cell populations (>75 cells per population) are shown.

TABLE 1. Effect of pH on phototaxis and light-induced Δψ changes in Pho81-B4 cells[a]

pH	Phototaxis (reversal [%])	Membrane potential changes (ΔTPP^+ [μM])
6.0	47.20 ± 6.60	0.10 ± 0.01
6.5	75.53 ± 2.74	0.15 ± 0.02
7.0	48.63 ± 3.77	0.09 ± 0.01

[a] Phototaxis was measured as percentages of reversals in a 5-s period after switching off white light (see also Materials and Methods). Relative changes of membrane potential were estimated by monitoring changes in TPP^+ concentrations. Means and standard errors of the means from three estimates for three cell populations (>75 cells per population) are shown.

third photosensory pathway in which photoreception is mediated by the proton-pumping activity of bacteriorhodopsin (7–10).

Our data suggest that a change in Δψ is essential for mediating the signal from bacteriorhodopsin to the taxis system in *H. salinarium*. This result is further supported by our observation that strain Pho81-HR, containing overexpressed Cl^- pump halorhodopsin as the only retinal protein, showed a reversal response when the light was switched off, in the same way as did the bacteriorhodopsin-containing strain Pho81-B4 (9a). Halorhodopsin pumps Cl^- anions into the cell, which results in the generation of a Δψ and, as a result of Δψ-dependent influx of protons, in acidification of the cytoplasm. The Δψ decrease in the Pho81-HR cells, as well as the disappearance of the oppositely oriented ΔpH, should follow the switching off the light.

Thus, the taxis system of the halobacterial cell is able to sense Δψ as an effector which is advantageous, since it is a fast message from the $\Delta\tilde{\mu}_{H^+}$ generators indicating unfavorable conditions leading to energy loss. It would allow cells to sense, nonspecifically, a wide range of factors affecting bacterial metabolism.

The receptor system involved in Δψ sensing is still unknown but recent results show that the methylation/demethylation of some sensory transducer(s) is involved in the signal transduction in both the bacteriorhodopsin mediated photoresponse (9a) and the aerotactic response (43a) in halobacteria. Aerotaxis in *E. coli* and *S. typhimurium*, which may be mediated by total $\Delta\tilde{\mu}_{H^+}$, is independent of methyl-accepting chemotaxis proteins (34), which implies that mechanisms in question are different in members of the *Enterobacteriaceae* and *Halobacteriaceae*. The CheA component of the halobacterial signal transduction chain seems to be essential for the bacteriorhodopsin-mediated response because its deletion from the chromosome blocks all known tactic reactions in halobacteria (30). Because all of the components of the basal body switching complex are still present in the CheA deletion mutant, Δψ sensing may not rely on the flagellar apparatus and membrane transmission, but probably use cytoplasmic components of the signal transduction chain. The data described here provide important evidence for the existence of a voltage receptor in halobacteria transmitting the signal through the conventional sensory cytoplasmic cascade.

It is interesting that the bacteriorhodopsin ground form bR_{568} may be considered a photochemical analog of the sRI ground form sR_{587}, whereas the M_{412} intermediate of bacteriorhodopsin serves as an analog of the S_{373} intermediate in sRI. The tactic responses which are produced as a result of photoexcitation of these analogous forms of rhodopsins are the same, despite the different mechanisms of signal generation in these retinal-containing proteins. Obviously, the role of bacteriorhodopsin as a functional receptor of blue light is unlikely, because it would be completely masked by sensory rhodopsins under natural conditions. Nevertheless, the similarity of the responses caused by analogous intermediates of these halobacterial rhodopsins suggests important evolutionary relationships.

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