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Photoresponses in *Rhodobacter sphaeroides*: Role of Photosynthetic Electron Transport

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Rhodobacter sphaeroides **responds to a decrease in light intensity by a transient stop followed by adaptation. There is no measurable response to increases in light intensity. We confirmed that photosynthetic electron transport is essential for a photoresponse, as (i) inhibitors of photosynthetic electron transport inhibit photoresponses, (ii) electron transport to oxidases in the presence of oxygen reduces the photoresponse, and (iii) the magnitude of the response is dependent on the photopigment content of the cells. The photoresponses of cells grown in high light, which have lower concentrations of light-harvesting photopigment and reaction centers, saturated at much higher light intensities than the photoresponses of cells grown in low light, which have high concentrations of light-harvesting pigments and reaction centers. We examined whether the primary sensory signal from the photosynthetic electron transport chain was a change in the electrochemical proton gradient or a change in the rate of electron transport itself (probably reflecting redox sensing).** ***R. sphaeroides*** **showed no response to the addition of the proton ionophore carbonyl cyanide 4-trifluoromethoxyphenylhydrazone, which decreased the electrochemical proton gradient, although a behavioral response was seen to a reduction in light intensity that caused an equivalent reduction in proton gradient. These results strongly suggest that (i) the photosynthetic apparatus is the primary photoreceptor, (ii) the primary signal is generated by a change in the rate of electron transport, (iii) the change in the electrochemical proton gradient is not the primary photosensory signal, and (iv) stimuli affecting electron transport rates integrate via the electron transport chain.**

Bacteria can sense and respond to a wide range of environmental stimuli, e.g., chemicals, oxygen, light, and changes in temperature and pH. Although the chemotactic system of *Escherichia coli* is understood in great detail, some other systems are extremely poorly understood, even at the level of the primary signal. There is good evidence that most sensory pathways are integrated before the flagellar motor, but the primary signals, for example, those of aerotaxis, phototaxis, and chemotaxis, may vary (1, 8).

The primary signal in *E. coli* chemosensing results from a conformational change in the membrane-spanning receptors, the change altering the phosphorylation activity of the histidine protein kinase that interacts with the cytoplasmic domain of the receptor (8, 12). Aerotaxis appears to depend on active respiratory electron transport (13, 15, 27), but whether the primary receptor senses a change in the electrochemical proton gradient (Δp) resulting from electron transport, or whether a change in the redox state of a electron transport component is the primary signal, is not understood. Similarly, the primary signal involved in photosensing in eubacteria has not been identified. In contrast, the archaebacterium *Halobacterium salinarium* is known to respond to changes in specific wavelengths of light because of light-driven conformational changes of the retinal-containing sensory rhodopsins (27). These changes probably alter the conformation of interacting membrane proteins homologous to the receptor proteins involved in *E. coli* chemotaxis. If these sensory rhodopsins are deleted, *H. salinarium* still exhibits a response to light as a result of bacteriorhodospin-dependent, light-driven changes in the membrane potential, but how these changes are sensed is unknown (14).

Data suggest that photosensing in eubacteria is linked to photosynthetic activity, with the action spectrum for phototaxis in *Rhodospirillum rubrum* being closely related to that for photosynthesis (7). Mutants in photosynthesis show impaired phototaxis, and inhibition of photosynthetic electron transport inhibits photoresponses. However, in these early studies the mutants were not characterized, and the effects of electron transport inhibitors on motility were not checked (3, 4, 29). Indirect evidence therefore suggests that active electron transport is required for phototaxis, but this possibility has not been investigated in detail, nor has the primary signal initiating the response been identified.

Electron transport can alter the size of Δp or the redox state of the electron transport components. Both of these processes have been suggested to be involved in sensory signalling. A change in Δp could be sensed via a "protometer" (4, 15, 16, 28), which could control many activities in the cell. In support of this interpretation, it has been shown that reducing Δp with proton ionophores can induce a repellent response in *E. coli*, *Azospirillum brasilense*, and *Bacillus subtilis* (16, 26, 28). On the other hand, changes in the redox state of components of the electron transport chain have been implicated in controlling the transcriptional activators of photosynthetic gene expression (31) and the photophobic responses of cyanobacteria (9). In practice, it is extremely difficult to separate the two phenomena in whole cells; a change in electron transport usually alters both Δp and the redox state.

The unusual pattern of behavioral responses of *Rhodobacter*

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sphaeroides, in which only a reduction in a stimulus results in a response, has allowed us to examine photoresponses under conditions where Δp is reduced but electron transport remains the same or even increases. In this study, we have confirmed that photosynthetic electron transport is required for a response to light and that the sensitivity of the response depends on the light-harvesting efficiency of the bacterium. We have also shown that although the primary sensory signal depends on electron transport, the signal is probably not a change in Δp.

MATERIALS AND METHODS

Growth media and conditions. The spontaneous nalidixic acid-resistant derivative of *R. sphaeroides* WS8 (WS8N) was used throughout this study. Cells were grown anaerobically with tungsten illumination in Sistrom succinate medium with 20 μg of nalidixic acid per ml as previously described (17). To induce dimethyl sulfoxide (DMSO) reductase, cells were grown in the presence of 40 mM DMSO. Cells were harvested in mid-log phase and washed once by centrifugation before being resuspended in 10 mM Na-HEPES buffer (pH 7.2).

Cells were grown under three different light intensities. High-light cells were grown with double-sided tungsten illumination at approximately 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$, normal-light cells were grown with single-sided illumination at about 58.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and low-light cells were grown at 11.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Intensities were estimated for the 400- to 900-nm region of the spectrum. The bacteriochlorophyll content of the cells was determined as previously described (30).

Carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP), nalidixic acid, glucose oxidase, catalase, antimycin A, and myxothiazol were all purchased from Sigma. Stigmatellin was purchased from Fluka, and other chemicals were purchased from BDH.

Photoresponses of tethered cells. Cells tethered by antiflagellin antibody were used to measure the response of *R. sphaeroides* grown at different light intensities, which lead to different photosynthetic efficiencies, to a step-down in light. Tethered cells were also used to measure the effects of FCCP on *R. sphaeroides* behavior and on the step-down response.

To measure the responses of cells grown at different light intensities, bacteria were resuspended in O_2-free 10 mM Na-HEPES (pH 7.2) containing chloramphenicol (50 μg/ml) to inhibit apoprotein synthesis and thus inhibit changes in pigment composition during the experiment. Bacteria were tethered to the walls of 0.2-mm-path-length optically flat capillaries sealed with grease. All operations were carried out in an anaerobic cabinet (Don Whitley Scientific, Shipley, United Kingdom). The capillary was then placed on the stage of a Nikon Optiphot microscope, and each population of tethered cells was equilibrated at 11 different specific light intensities (white tungsten light). The cells were then subjected to a 97% decrease in light intensity by the addition of neutral density filters into the light path. The response to the reduction in light intensity was measured for at least 10 cells of each population at each light intensity. The behavior of cells in response to the step-down in light intensity was recorded on videotape, and the stop probability of the cells was calculated. *R. sphaeroides* does not tumble but stops to change direction. *R. sphaeroides* shows great variability in swimming speed and stopping frequency, both within the population and within individual cells. The rotational behavior of cells was recorded every 0.06 s, and the probability of each of 10 cells being stopped was calculated for each time point; therefore, a stop probability of 1.0 means that all 10 cells were stopped, whereas a stop probability of 0.0 means that no cells were stopped. The stop probabilities were smoothed over 1.8-s intervals. The response of the population of cells grown at different intensities to the step-down in light was plotted as a single response magnitude value, which corresponds to the area under the response curve, with the baseline measured as the prestimulus stop probability.

To measure the effect of FCCP on unstimulated cells and on the photoresponse, a flow chamber was used to allow the incubation medium to be changed (5). Cells were tethered via antiflagellin antibody onto the coverslip of a flow chamber, as previously described, with the addition of 10 mM glucose, 20 μg of glucose oxidase per ml, and 10 μg of catalase per ml to remove any residual oxygen from the system, which could have an inhibitory effect on any response. High-light cells were used, since their photoresponses allowed a good video image to be obtained under conditions where the response was not saturated. The step-down stimulus was a decrease in light intensity (wavelength region, 530 to 600 nm) from 560 to 17.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When solutions were changed, 1 ml of new solution was flowed through the chamber, and the cells were incubated in the new medium for at least 20 min before application of a photostimulus. The behavior of cells to the step-down and step-up in light intensity was recorded on videotape.

The responses of the tethered cells to changes in light intensity were measured with the Seescan motion analysis system as described earlier (22), and the stop probability was calculated. Photostimuli were applied via a fiber-optic light guide through a filter with maximum transmission at 530 to 600 nm. Light with a wavelength of 650 ± 10 nm and intensity of 17.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was used to monitor the response. This wavelength coincides with the absorbance minimum of the photosynthetic pigments. For video recording, a 650-nm transmission filter was inserted in front of the video camera to avoid interference by the stimulating light.

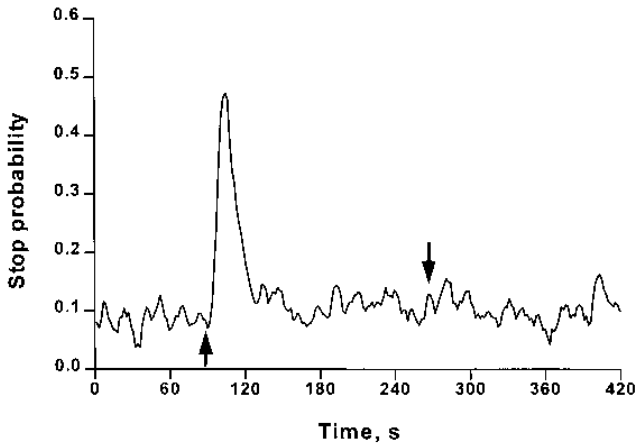

FIG. 1. Behavioral photoresponses of tethered *R. sphaeroides* WS8N cells to changes in light intensity. Cells were allowed to equilibrate in light at an intensity of 16.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and wavelength of 650 ± 10 nm. Stimuli were the changes in intensity of yellow-green light (wavelength region, 530 to 600 nm) from 560 to 17.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and back. Light was decreased at 90 s (↑) and increased after a 3-min interval (↓). The data are for 12 cells.

To measure the response of tethered cells to the addition of FCCP, 10 mM oxygen-free HEPES containing chloramphenicol and succinate was flowed over the cells and then replaced with the same buffer containing 500 nM FCCP. The behavior of tethered cells was recorded on videotape and analyzed as described above. Cells which became permanently nonmotile after the addition of FCCP were not monitored.

Photoresponse of free-swimming cells. Free-swimming cells in 10 mM Na-HEPES (pH 7.2) containing 40 μg of chloramphenicol per ml and 2 mM succinate were incubated in optically flat capillaries. Glucose, glucose oxidase, and catalase were also added where indicated. Inhibitors of photosynthetic electron transport were added at least 5 min before cells were drawn into the capillaries, and behavior was analyzed only after at least 2 min of incubation on the microscope stage. Free-swimming behavior, average run speed, and stopping probability were measured with the computerized motion analysis system (Hobson Tracking Systems Ltd., Sheffield, United Kingdom).

Photostimuli were applied via a fiber-optic light guide through either a 530- to 600-nm or near-infrared transmission filter (Kodak Wratten 88A). Light with a wavelength of 650 ± 10 nm and intensity of 17.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was used to monitor the response. For video recording, a 650-nm filter was inserted in front of the video camera to avoid interference by the stimulating light.

Measurement of $\Delta\psi$. The membrane potential-dependent change in the absorption spectrum of the light-harvesting carotenoid pigment was used to monitor changes in the $\Delta\psi$. Under the experimental conditions used here, Δp was mainly $\Delta\psi$ (17). The absorbance at 523 nm relative to that at 510 nm (3, 17) was measured with a DW-2000 dual-wavelength spectrophotometer (SLM-Aminco, Urbana, Ill.). Photosynthetic stimulation was achieved by illuminating the sample at a 90° angle with light at a wavelength of 850 ± 10 nm. The decrease in $\Delta\psi$ upon addition of FCCP was measured in a stirred cuvette under a stream of argon.

RESULTS

Phototactic response of tethered *R. sphaeroides* cells. Tethered cells responded to a decrease in light intensity by stopping transiently and then returning to their prestimulus behavior (Fig. 1). There was no measurable change in either stopping probability or velocity of rotation in response to an increase in light intensity (see also Fig. 5). This response to a decrease, but not an increase, in a light stimulus is very similar to the chemotactic response of *R. sphaeroides* (20). This "behavioral pessimism" is in contrast to enteric species, which show a greater response to the addition of an attractant than to its removal (25).

Figure 2 shows the photoresponses measured for cells grown under different light intensities. The cells grown under the three different light regimens, and therefore possessing differ-

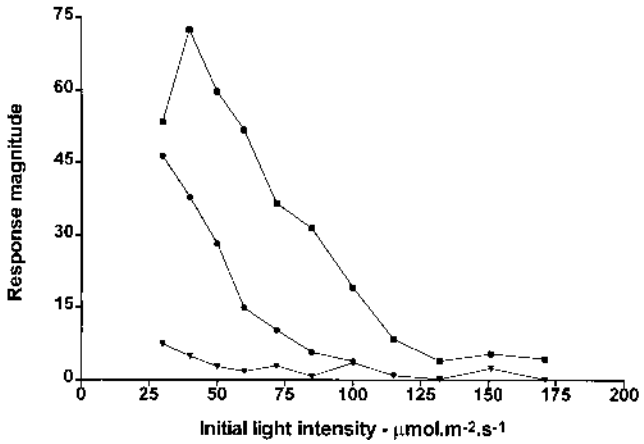

FIG. 2. Responses of cells incubated at different intensities to a step-down in light intensity. Cells were adapted to an initial intensity of white tungsten light, which was then reduced by 97.2% of this initial value. The response was evaluated as the area under the response curve as in Fig. 1, with the baseline set at the mean prestimulus stop probability. ■, cells grown under high light intensity (bacteriochlorophyll concentration, 3.1 nmol per 10^9 cells); ●, cells grown under intermediate light intensity (bacteriochlorophyll concentration, 10.51 nmol per 10^9 cells); ▼, cells grown under low light intensity (bacteriochlorophyll concentration, 17.5 nmol per 10^9 cells).

ent complements of light-harvesting pigments (23, 24), were incubated on a microscope slide at 11 different light intensities, and the light intensity was reduced rapidly by inserting a neutral-density filter into the light beam to cause an equal relative decrease (97%) in light intensity, whatever the starting intensity. In all cases, the photoresponse was smaller if the starting (and hence final) light intensity was high than if the starting/final light intensity was low. This result suggests that the photoreceptor system remains saturated if the final light intensity is over a certain value. *R. sphaeroides* does not, therefore, measure the relative size of a step-down in light intensity, although it is possible that changes in light intensity are measured only within a certain range of intensity.

Cells grown under low light intensity, which have a much greater efficiency of light harvesting, showed no response to a step-down in light that elicited a response from high-light cells with fewer light-harvesting II (LH-II) complexes. This finding suggests that whether or not a response is produced by a step-down in light depends on the capacity of the photosynthetic light-harvesting apparatus. Photosynthesis in low-light cells, which have more LH-II and reaction center complexes, remains saturated at much lower light intensities than that in high-light cells, and the cells therefore do not respond to a step-down in light, since photosynthesis remains saturated. The much lower concentrations of LH-II and reaction center complexes in high-light cells causes these cells to require much higher light intensities to saturate photosynthesis. These cells correspondingly show photoresponses to a much greater range of step-down intensities, showing that the photoresponse depends on the rate of photosynthesis, not on specific receptors responding to specific changes in light intensity.

Effect of oxygen on the phototactic response. The sensitivity of the photoresponse to oxygen was examined in free-swimming high-light cells. Figure 3 shows the response of such cells to a step-down in light intensity when they were incubated with and without glucose and glucose oxidase to deplete the buffer of any oxygen. The overall response to a step-down was much greater in the absence of oxygen than when oxygen was present. This finding suggests that the photosynthetic and respiratory electron transport systems, which share components in these organisms, interact and that electron transfer to oxygen can reduce the response to a signal requiring photosynthetic electron transport.

Effect of photosynthetic electron transport inhibitors on photoresponses. Antimycin A inhibits ubiquinone reduction at the *i* site of cytochrome *b* in the cytochrome bc_1 complex, whereas myxothiazol and stigmatellin block the oxidation of ubiquinol by cytochrome *b* at the *o* site. They therefore inhibit different stages of electron transfer through ubiquinone and cytochrome bc_1. Stigmatellin has also been shown to inhibit Q_B reduction in the photosynthetic reaction center (6).

Measurement of the $\Delta\psi$ by using the carotenoid band shift showed that both myxothiazol and stigmatellin (10 μM) inhibited the photosynthetic generation of the membrane potential, presumably by completely inhibiting photosynthetic electron transfer. Antimycin A, on the other hand, only partly inhibited photosynthesis at a concentration of 60 μM. Cells treated with myxothiazol or stigmatellin eventually became nonmotile if incubated anaerobically but recovered motility if given oxygen, showing that stigmatellin and myxathiazol inhibited photosyn-

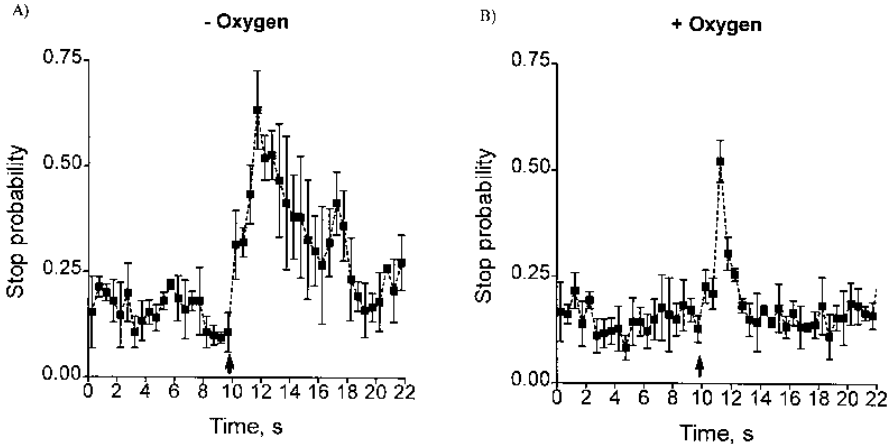

FIG. 3. Effect of oxygen on the response of free-swimming cells to a step-down in light intensity. (A) Response in the presence of a glucose-glucose oxidase oxygen depletion system; (B) response in the absence of glucose oxidase. Light was switched off after 10 s (arrow). Data for each graph were obtained from tracking of cells from three populations, 10 cells per population. Means of stop probabilities and standard errors are indicated.

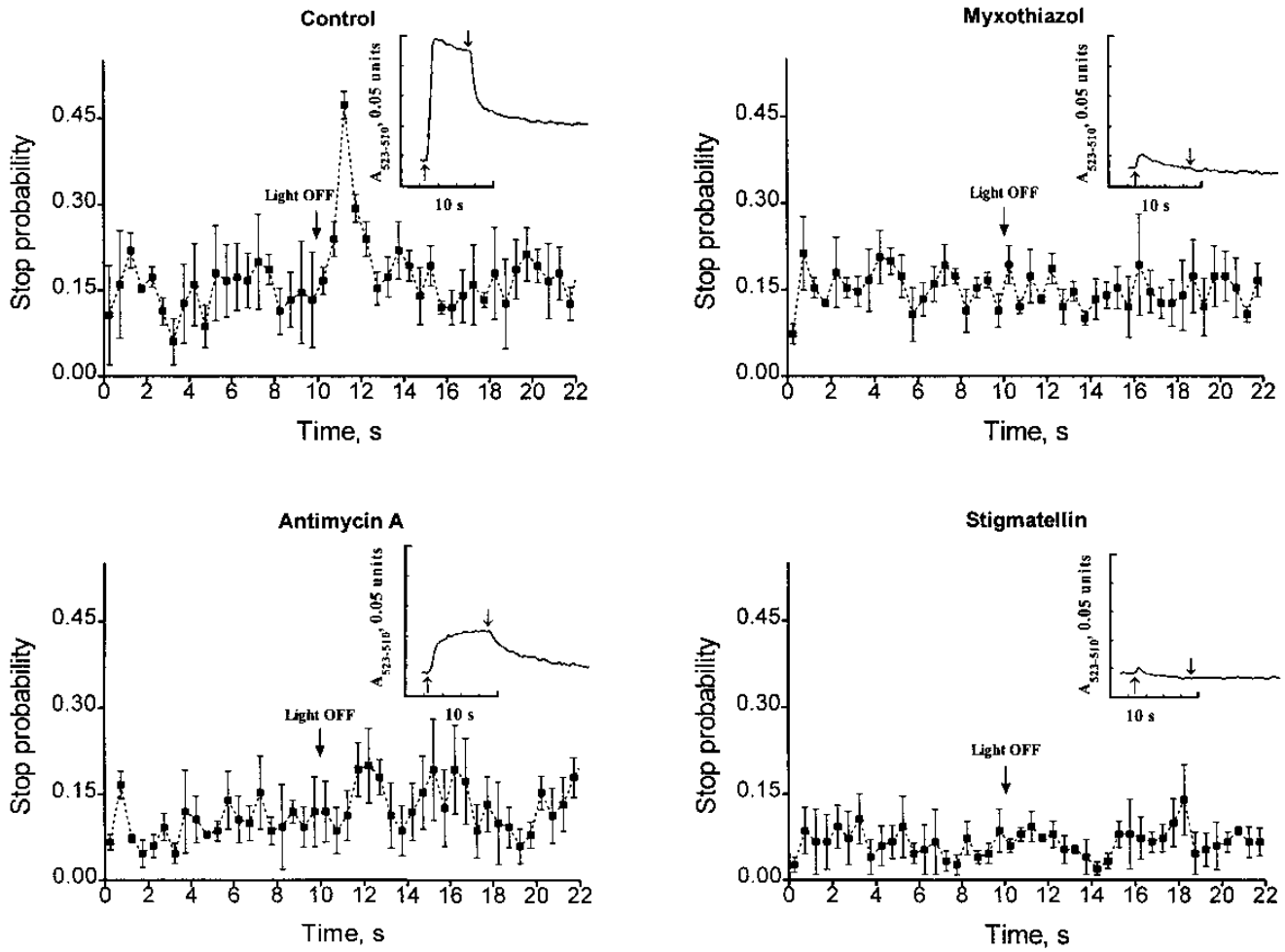

FIG. 4. Effects of antimycin A (50 μM), myxothiazole (30 μM), and stigmatellin (30 μM) on the photoresponse of free-swimming cells in the absence of glucose oxidase. Light (wavelength region, 530 to 600 nm; intensity, 560 μmol · m^{-2} · s^{-1}) was switched off at 10 s (↓). Background light was at a wavelength of 650 ± 10 nm and intensity of 17.4 μmol · m^{-2} · s^{-1}. Data for each graph were obtained by tracking cells from three populations, 10 cells per population. Means of stop probabilities and standard errors are shown. Insets, effects of antimycin A, myxothiazole, and stigmatellin on Δψ measured by the carotenoid band shift. ↑, light on; ↓, light off.

thetic electron transport completely. Cells treated with antimycin A continued to swim if incubated anaerobically in high light, indicating that generation of Δψ via photosynthetic electron transport was only partially inhibited (Fig. 4). Controls showed that cells incubated with low levels of oxygen still show a photoresponse to a step-down in light intensity (Fig. 3 and 4), but the cells treated with inhibitors of photosynthetic electron transport did not show any response to a step-down in light, indicating that photosynthetic electron transport is essential for photoresponses. Cells treated with antimycin A showed a greatly reduced response, reflecting the low level of electron transport continuing in these cells. To ensure that small responses were not being suppressed by oxygen, the experiments were repeated with cells grown on the alternative electron acceptor, DMSO. DMSO supports continued motility, but its presence does not inhibit photosynthetic electron transport. Electron transfer to DMSO reductase does not compete with photosynthetic electron transfer, as it is relatively slow and takes electrons directly from the quinone. Cells still showed no photoresponses in the presence of photosynthetic electron transport inhibitors when incubated with DMSO (results not shown).

Effect of FCCP on the photoresponse. The proton ionophore FCCP at concentrations higher than 50 nM reduced the rotation rate of tethered cells under low light, presumably because the electrochemical proton gradient was reduced. Cells, however, rotated normally in the presence of 25 nM FCCP. Interestingly, 47.6% ± 8.2% of cells responded to a step-down in light before the addition of FCCP, whereas 61.0% ± 2.4% responded to the same reduction in light after incubation with 25 nM FCCP (Fig. 5). Low levels of FCCP cause both a reduction in the baseline dark Δψ, and therefore the size of the Δψ change generated by switching the light on and off, and an increase in the rate of Δψ decay after the light was switched off (Fig. 6). Respiratory control also results in increased electron transport rates to compensate for the increased proton leakage in FCCP-treated cells.

The increase in the number of cells showing photoresponses in the presence of FCCP implies that Δψ may have a regulatory effect on photoresponses. The signal could be sensitive to the rate of change of Δψ or the rate of electron transfer, or there could be a critical value of Δψ. In the latter case, the increased number of cells responding to a step-down in light when incubated with FCCP would imply that cells with a Δψ value below

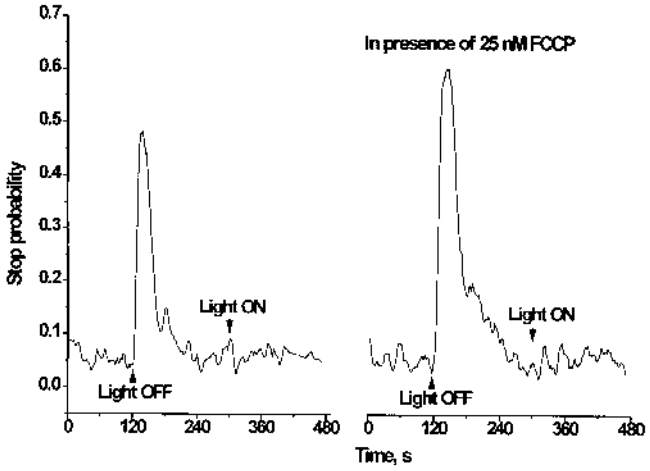

FIG. 5. Behavior of tethered *R. sphaeroides* cells in response to switching the light on and off, in the absence and in the presence of 25 nM FCCP. Stop probabilities in real time were calculated for populations of 24 cells. The stimulus was a decrease in intensity of light (wavelength region, 530 to 600 nm) from 560 to 17.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Background light was 650 ± at a wavelength of 10 nm and intensity of 16.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

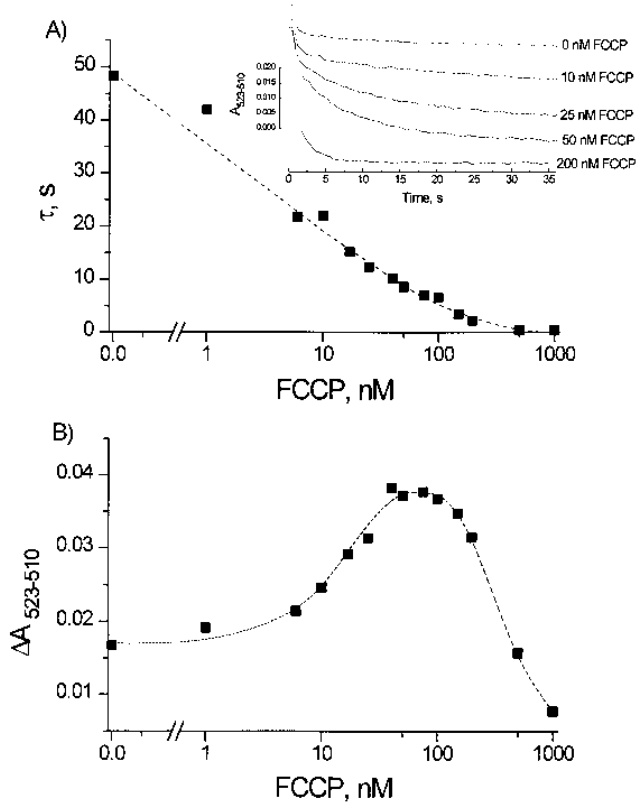

FIG. 6. Effect of FCCP on the kinetics and amplitude of Δψ decay in *R. sphaeroides* after the light was switched off. The inset shows the actual size and rate of fall in Δψ, as measured by the carotenoid band shift, when the light was switched off on FCCP-treated cells. (A) Increasing decay kinetics with increasing FCCP concentration; (B) size of the Δψ change upon switching off the light. At low concentrations of FCCP, cells can maintain a dark potential, but as the concentration increases, the potential in the dark is lost, hence the increase in size of Δψ change with increasing FCCP. The kinetics approximated to a two-phase decay.

a critical level respond, whereas those maintaining a Δψ above this level after a step-down in light show no response. This interpretation is consistent with the data from cells grown under high and low light.

Behavioral responses to FCCP. If the photoresponse is generated by a change in Δψ, then any stimulus causing a step-down in Δψ should cause a behavioral response. FCCP added alone causes a reduction in Δψ and a parallel increase in the rate of electron transfer. Tethered *R. sphaeroides* showed no response to the addition of up to 500 nM FCCP (Fig. 7B and D). (Higher concentrations significantly decreased motility.) Although the addition of FCCP caused no response, Δψ fell well below the value induced by switching off the light in the control cells, a treatment which did cause a response (Fig. 4, control). The first phase of the rate of fall of the Δψ with FCCP was, however, slower than that seen when the light was switched off in control cells. The effect of reducing the light intensity more slowly was therefore measured. Reducing the light intensity at a rate which caused the membrane potential to fall at a rate comparable to that caused by the addition of FCCP produced a step-down response (Fig. 7A and C). Interestingly, the increase in the stopping probability of the population of cells was slower than seen with a fast reduction in light intensity (Fig. 1), suggesting that the rate of change in electron transport is important in producing the signal. These data together suggest that although photosynthetic electron transport is essential for photoresponses, a change in Δψ is not the primary sensory signal.

DISCUSSION

These and previously published data suggest that the major response shown by anaerobically grown *R. sphaeroides* is to a reduction in a stimulus, whether that stimulus is a chemoattractant, oxygen, or light. This behavior is in contrast to that of enteric species, in which the major response is prolonged smooth swimming upon an increase in attractant, whereas the response to removal is a much briefer period of tumbling. This pessimistic behavior of *R. sphaeroides*, with cells responding primarily when moving down rather than up an attractant gradient, may be an intrinsic property of this species.

The photoresponse was sensitive to the presence of oxygen, which suggests that the activity of the electron transport chain is involved in generating the primary signal. Ubiquinone and the cytochrome bc_1 complex are common to both respiratory and photosynthetic electron transfer, and the presence of oxygen should alter the rate of light-driven electron flow through these components by competing for electrons. This reduction in the photoresponse by oxygen could be important physiologically, since it may prevent bacteria from moving up a light gradient into an aerobic environment in which toxic photooxidation products could be generated.

The photoresponse was also sensitive to a range of inhibitors of photosynthetic electron transport, conclusively showing that, as had been suggested earlier, active photosynthetic electron transport is essential for photoresponses in *R. sphaeroides*. The lack of any measurable response in antimycin A-treated cells, although they showed a small light-dependent increase in Δψ, suggests either that there is a threshold change in Δψ required to generate a signal or that electron transport through ubiquinone is essential for signaling.

If the primary photosignal is really generated by photosynthetic electron transfer, the responses of cells grown under

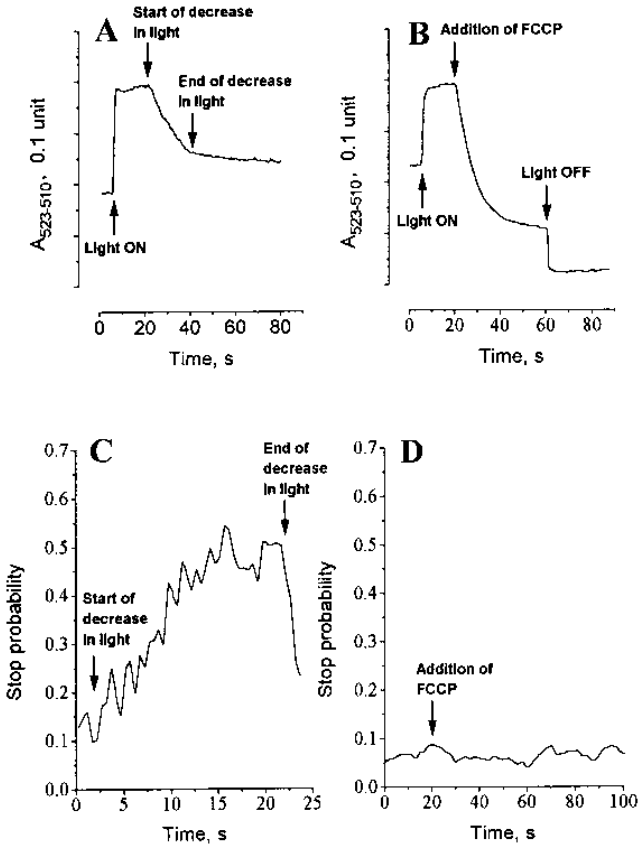

FIG. 7. Behavior of tethered *R. sphaeroides* cells in response to addition of 500 nM FCCP and the slow reduction in light intensity. Panel A shows the fall in $\Delta\psi$ caused by a slow reduction in light intensity, and panel C shows the stop response caused by this natural reduction in $\Delta\psi$. Panel B shows the effect on $\Delta\psi$ of the addition of 500 nM FCCP, causing a similar change in carotenoid absorption but no behavioral response (D). The stop probabilities calculated for a population of 26 cells are shown.

different light intensities, and thus with different photosynthetic capabilities, should be different. The light intensity under which cells are grown determines the number of light-harvesting complexes (LH-II) and reaction centers per cell; high-light cells have fewer light-harvesting complexes than low-light cells, although the turn over kinetics of the reaction centers is higher in high-light cells (10, 23, 24). The increased concentration of antenna complexes provides much higher light-harvesting capacity for the reaction centers in low-light cells compared to high-light cells. Therefore, in high-light cells, the photoreceptor should be saturated only by very high light intensities, when all reaction centers are active. Any decrease in light intensity will lead to a percentage of reaction centers becoming inactive and therefore to consequent changes in electron transfer, $\Delta\psi$, and photophosphorylation because of the high ratio of electron transfer components to reaction centers in these cells. On the other hand, low-light cells have a higher proportion of light-harvesting complexes and reaction centers to electron transfer intermediates. Under most light intensities, therefore, electron transfer is probably limited only by the concentration of electron transfer intermediates, not light-harvesting or reaction center turnover. In low-light cells, therefore, the photoreceptor would be saturated over a very wide range of intensities and consequently would show a photoresponse only when the light level after a step-down was very low. Photosynthesis-mediated photoresponses should, therefore, be saturated at lower light intensities in low-light cells than in high-light cells, and this was found to be the case (Fig. 2). In addition, the increase in the percentage of cells showing a photoresponse in the presence of low concentrations of uncoupler, which reduces the baseline $\Delta\psi$ and may also increase rates of electron transport, supports the idea of a critical threshold $\Delta\psi$ for a response.

We clearly show here that decreases in photosynthetic electron transport must generate the primary signal in the step-down photoresponse, but that does not identify the nature of the primary signal. Several species of bacteria have been shown to be repelled by proton ionophores, and *H. salinarium* has been shown to respond to changes in $\Delta\psi$. Indeed, early data on phototaxis and aerotaxis suggested that the primary signal may be the change in electrochemical proton gradient sensed through a protometer, which has even been suggested to be part of the flagellar motor (13, 28). The proton ionophore FCCP has been shown to decrease $\Delta\psi$ but to increase the rate of electron transfer and change the redox state of electron transfer components (oxygen will also decrease $\Delta\psi$ and change the redox state of photosynthetically growing cells).

Since *R. sphaeroides* shows a step-down response only under anaerobic conditions, we examined whether a response could be generated artificially by inducing a step-down in $\Delta\psi$ by addition of the proton ionophore FCCP. Addition of the ionophore did not cause any response, although the fall in $\Delta\psi$ was much greater than that seen in response to a step-down in light intensity. A reduction in light intensity causing a similar rate of fall in $\Delta\psi$ still induced a response, showing that the lack of a response to FCCP was not the result of slower kinetics (Fig. 7).

This response to a step-up in FCCP concentration correlates with previous results which showed that *R. sphaeroides* is not repelled by spatial gradients of protonophore but tends to be trapped in regions of high uncoupler concentration (19). Aerobically grown *R. sphaeroides* cells also respond to a decrease in the concentration of electron acceptors, such as oxygen, DMSO, trimethylamine *N*-oxide, and which also caused a decrease in $\Delta\psi$, but these cells also fail to respond to the addition of FCCP, responding only to its reduction by an increase in stopping (11).

Taken together, these data suggest that behavioral responses in *R. sphaeroides* to stimuli effecting electron transfer are not mediated by changes in $\Delta\psi$ being sensed directly by a $\Delta\psi$-sensitive receptor. They suggest instead that, in *R. sphaeroides*, there is a general sensing mechanism responding to the changes in the activity of the electron transport chain, possibly sensing the redox state of one of the electron transfer components. The data also suggest that *R. sphaeroides*, and possibly other eubacterial species, do not directly sense stimuli which affect electron transport but rather respond to changes in the rate of electron transport. Bacteria therefore bias their overall direction of swimming toward environments in which the rate of electron transport is maximal, rather than by sensing individual electron acceptors or light.

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