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EFFECT OF OXIDATION–REDUCTION POTENTIAL ON LIGHT-INDUCED CYTOCHROME AND BACTERIOCHLOROPHYLL REACTIONS IN CHROMATOPHORES FROM THE PHOTOSYNTHETIC GREEN BACTERIUM *CHLOROBIUM*

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SUMMARY

The reaction center bacteriochlorophyll of *Chlorobium thiosulfatophilum* has a midpoint oxidation–reduction potential (E_m) of +330 mV. Its photooxidation is unaffected by oxidation–reduction potentials in the range from +260 mV to −70 mV but on further reduction is attenuated to zero in a one-electron transition with an E_m of −130 mV.

A *c*-type cytochrome with an E_m of +220 mV and absorption maxima at 551–552 nm (α-band) and 420 nm (γ-band) is present in *Chlorobium* chromatophores and undergoes photooxidation. Cytocrome *c* photooxidation is attenuated to zero in two 1-electron steps with E_m of +30 mV and −130 mV

Possible roles for +30 mV and −130 mV components in photosynthetic electron transport in *Chlorobium* are discussed.

INTRODUCTION

The response of light-induced electron transport reactions to chemically imposed oxidation–reduction potentials has been widely studied in photosynthetic bacteria[1–12]. These investigations have dealt mainly with the purple nonsulfur bacteria (*Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*) or the purple sulfur bacteria (*Chromatium*). Relatively little work has been done with the third major group, the photosynthetic green sulfur bacteria, which differ from their purple counterparts in both pigment composition and physiological characteristics. The single investigation[10] of the effect of oxidation–reduction potential on light-induced reactions in a green photosynthetic bacterium (*Chloropseudomonas ethylicum**) was confined to oxidation–reduction potentials more oxidizing than 0 V. Thus, no information was available concerning the effect of negative oxidation–reduction

* The organism referred to as *Chloropseudomonas ethylicum* has recently been shown to be a mixed culture of a photosynthetic green bacterium (*Chlorobium limicola*) and an unidentified nonphotosynthetic bacterium[13].

potentials on electron transport by chromatophores from this group of organisms which are of particular interest because of their ability to catalyze a light-dependent reduction of ferredoxin ($E_m = -420$ mV((ref. 14–17).

This paper describes the effect of oxidation–reduction potential over the range from +500 mV to −300 mV on the photooxidation of a *c*-type cytochrome and on the photooxidation of the reaction center bacteriochlorophyll at physiological temperatures in chromatophores of the green bacterium *Chlorobium thiosulfatophilum*. As the potential is lowered, photooxidation of both bacteriochlorophyll and cytochrome *c* is attenuated to zero in a manner suggesting the involvement of a one-electron transfer component with an E_m of −130 mV. The role of this component in electron transport reactions in *C. thiosulfatophilum* and its possible relation to the primary electron acceptor are discussed.

METHODS

C. thiosulfatophilum, strain Tassajara, was grown on a medium supplemented with acetate[14]. Once-washed chromatophores were prepared from freshly harvested cells as previously described[14,15] except that the DEAE-cellulose treatment was omitted. *Chlorobium* chlorophyll was determined as described by Stanier and Smith[18].

Light-induced absorbance changes were measured with a dual wavelength spectrophotometer (Phoenix Precision Instrument Co.) as previously described[19]. The half-band width of the measuring beam was 2.0 nm for measurements of the cytochrome α-band and 3.0 nm for measurements of the cytochrome γ-band or the bacteriochlorophyll 610-nm band. Monochromatic actinic light was provided using a Balzer 794-nm interference filter (10-nm half-band width) and had an intensity of $2 \cdot 10^4$ ergs$\cdot$cm$^{-2}\cdot$s^{-1}.

Absorbance measurements were made at 20 °C under anaerobic conditions using a cell similar to that described by Cramer and Butler[20] and by Dutton[7] as described previously[21]. The oxidation–reduction potential was monitored with a Radiometer PK 149 combination platinum–calomel electrode. The calomel reference electrode was calibrated against a saturated quinhydrone solution at pH 7.0.

The potential of the sample was lowered by addition of small aliquots of a solution containing 0.01 M $Na_2S_2O_4$ in 0.01 M NaOH. To facilitate equilibration between the chromatophore electron carriers and the platinum electrode, the following mediators were used: 100 μM potassium ferricyanide ($E_m = +430$ mV); 100 μM benzoquinone ($E_m = +290$ mV); 100 μM 2,5-dimethylbenzoquinone ($E_m = +180$ mV); 10 μM 1,2-naphthoquinone ($E_m = +145$ mV); 10 μM 1,4-naphtoquinone ($E_m = +60$ mV); 10 μM 5-hydroxy-1,4-naphthoquinone ($E_m = +30$ mV); 10 μM 2-hydroxy-1,4-naphthoquinone ($E_m = -145$ mV); 10 μM anthraquinone-1,5-disulfonate ($E_m = -170$ mV); and 10 μM anthraquinone-2-sulfonate ($E_m = -225$ mV).

RESULTS

Bacteriochlorophyll photooxidation

The most prominent absorbance change associated with the photooxidation of reaction center bacteriochlorophyll in photosynthetic green bacteria is a bleaching

centered near 840 nm (refs 10,22). In addition to the transition in the near-infrared, reaction center bacteriochlorophylls have been shown to possess an optical transition in the 600-nm region[2,7,8]. Fig. 1 shows the light *minus* dark difference spectrum of *Chlorobium* chromatophores poised at +240 mV. The bleaching with a minimum at 610 nm is similar to that observed with preparations from other photosynthetic bacteria[2,7,8].

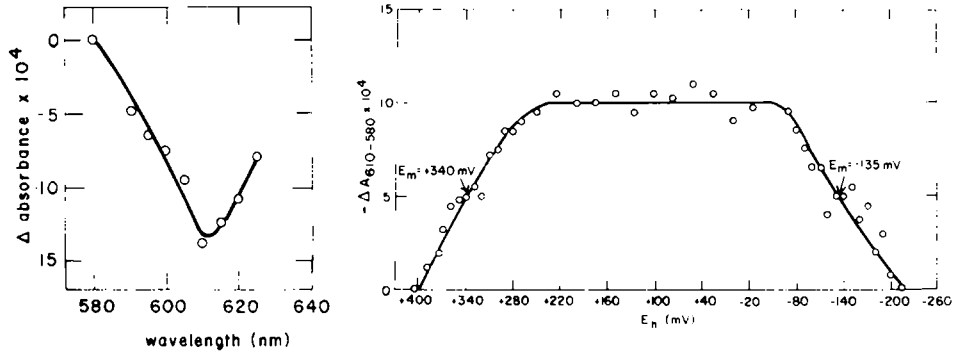


Fig. 1. Spectrum of bacteriochlorophyll photooxidation in *C. thiosulfatophilum* chromatophores. The reaction mixture contained (per 1.0 ml) *C. thiosulfatophilum* chromatophores (equivalent to 150 μg *Chlorobium* chlorophyll) and the following, in μmoles: potassium phosphate buffer (pH 6.5), 50; potassium ferricyanide, 0.1; and *p*-benzoquinone, 0.1. Reference wavelength, 580 nm. Oxidation-reduction potential, +240 mV.

Fig. 2. Oxidation-reduction potential dependence of bacteriochlorophyll photooxidation (610 nm *minus* 580 nm). Reaction mixture as in Fig. 1 except that all of the oxidation-reduction mediators listed in the Methods section were present.

Fig. 2 shows the oxidation-reduction potential profile of bacteriochlorophyll photooxidation in *Chlorobium* chromatophores monitored by the light-induced absorbance decrease at 610 nm *minus* 580 nm. No light-induced absorbance changes are observed at potentials more positive than +400 mV. At potenials between +400 mV and +240 mV the extent of the light-induced bleaching increases with decreasing potential, indicating that the reaction center bacteriochlorophyll becomes chemically reduced over this potential range and becomes capable of photooxidation. The response of bacteriochlorophyll photooxidation to change in potential follows that expected for a theoretical one-electron curve ($n = 1.0 \pm 0.1$) with an E_m of +330 mV $\pm$ 10mV (average of five experiments). This midpoint potential determined for the reaction center bacteriochlorophyll in chromatophores of *Chlorobium* differs from the value of +240 mV reported for P840 in a photochemically active subcellular fraction from the photosynthetic green bacterium *Chloropseudomonas ethylicum*[10].

From + 260 mV to −80 mV the extent of bacteriochlorophyll photooxidation remains constant (Fig. 2) and then decreases as the potential is lowered from −80 mV to −210 mV. No light-induced absorbance changes are observed in this spectral region at potentials more negative than −210 mV. The course of attenuation of bacteriochlorophyll photooxidation fits a one-electron curve ($n = 1.0 \pm 0.1$) with an E_m of −130 mV $\pm$ 10 mV (average of five experiments).

Cytochrome c photooxidation

Fig. 3 shows the oxidation–reduction profile of cytochrome *c* photooxidation (monitored by the decrease in absorbance at 551 nm *minus* 540 nm) in the region between +320 mV and +120 mV. Between potentials of +290 mV and +170 mV, the cytochrome becomes reduced and becomes available for photooxidation. The amount of cytochrome *c* photooxidized in the present preparation corresponds to one cytochrome per 1000–1200 *Chlorobium* chlorophyll molecules.

The profile fits that for a theoretical one-electron curve ($n=1.0\pm0.1$) with an E_m of +220 mV ±10 mV (average of five experiments). The midpoint potential for this *c*-type cytochrome in *Chlorobium* is more positive than the value of +170 mV found for the photooxidizable cytochrome c_{553} in a subcellular fraction from the green bacterium *Cps. ethylicum*[10]. Olson and Sybesma[23] have also reported the photooxidation of a *c*-type cytochrome in whole cells of green bacteria. The reported

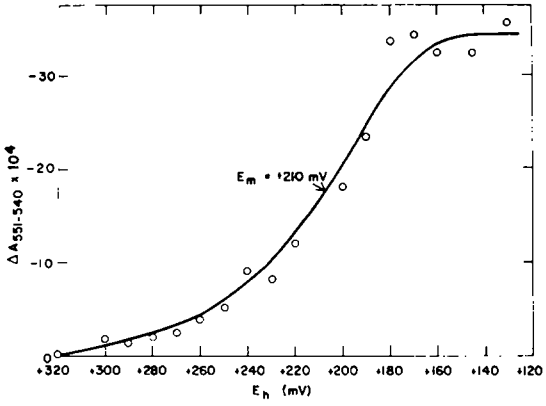


Fig. 3. Midpoint potential determination for the photooxidizable cytochrome *c* of *C. thiosulfatophilum* (551 nm *minus* 540 nm). Reaction mixture as in Fig. 1 except that 100 μM 2,5-dimethylbenzoquinone and 10 μM 1,2-naphthoquinone were also added.

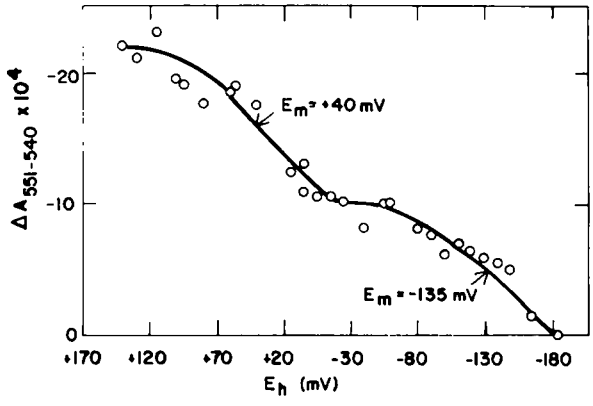


Fig. 4. Oxidation–reduction potential dependence of cytochrome *c* photooxidation (551 nm *minus* 540 nm). Reaction mixture as in Fig. 2 except that potassium ferricyanide and *p*-benzoquinone were omitted.

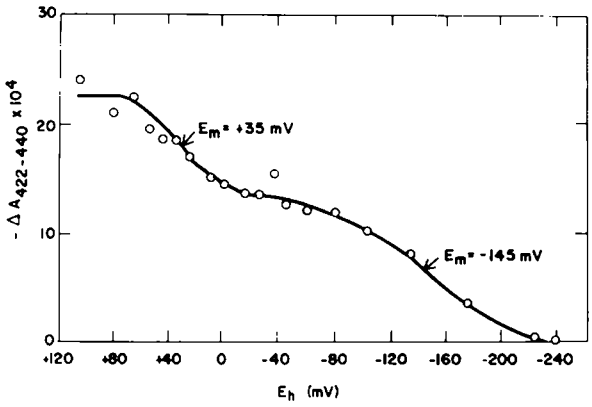


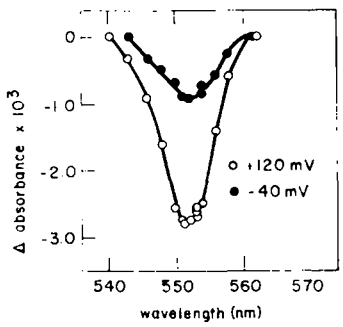
Fig. 5. Oxidation–reduction potential dependence of cytochrome *c* photooxidation (422 nm *minus* 440 nm). Reaction mixture as in Fig. 4 except that *C. thiosulfatophilum* chromatophores were present at a concentration of 35 μg *Chlorobium* chlorophyll per 1.0 ml.

α-band and γ-band maxima were 553 nm and 423 nm, respectively, but the midpoint potential was not reported.

The oxidation-reduction profile of cytochrome *c* photooxidation in *Chlorobium* chromatophores in the region between +140 mV and −180 mV monitored at the α-band (551 nm *minus* 540 nm) and between +110 mV and −240 mV monitored at the γ-band (422 nm *minus* 440 nm) are shown in Figs 4 and 5, respectively. Cytochrome photooxidation is attenuated in two steps, both of which correspond to theoretical curves for a one-electron transfer. No cytochrome photooxidation was observed at potentials more reducing than −230 mV.

The more positive of these curves has a midpoint potential of +30 mV ±10 mV and an *n* value of 1.2±0.1 (average of ten determinations) that is independent of pH in the range from pH 5.7 to pH 7.6. At pH 8.5, this curve shifts to an E_m of −15 mV ±10 mV (average of four determinations). This shift is approximately that expected for an oxidation–reduction reaction that involves one proton per electron. The more negative attenuation curve has a midpoint potential of −130 mV ±10 mV and an *n* value of 0.9±0.1 (average of ten determinations) that is independent of pH in the range from pH 6.5 to pH 8.5. Omitting the oxidation–reduction mediators 1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, or anthraquinone-1,5-disulfonate individually had no effect on the oxidation–reduction profile. Thus the attenuation of steady-state cytochrome *c* photooxidation in *Chlorobium* chromatophores does not appear to be due to rapid reduction of the photooxidized cytochrome by a reduced mediator.

Figs 6 and 7 show the α-band and γ-band absorption spectra of cytochrome photooxidation measured at the two plateaus in the oxidation–reduction profile. In both potential regions, the spectra indicate the photooxidization of a single *c*-type cytochrome with an α-band maximum at 551 nm to 552 nm (Fig. 6) and a γ-band maximum at 420 nm (Fig. 7). The only difference between the spectra in the two potential regions is the magnitude of the absorbance change. The extent of the light-induced absorbance decrease observed at +120 mV varied between two and three times that observed at −40 mV in both the α-band and γ-band spectral regions.



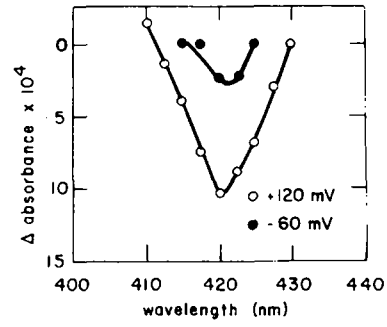


Fig. 6. α-Band spectrum of cytochrome *c* photooxidation. Reaction mixture as in Fig. 4. Reference wavelength, 540 nm.

Fig. 7. Soret (γ) band spectrum of cytochrome c photooxidation. Reaction mixture as in Fig. 5 Reference wavelength, 440 nm.

DISCUSSION

There is now general agreement that the primary electron acceptor in a variety of photosynthetic bacteria has a midpoint oxidation–reduction potential near −100 mV (refs 1–9, 11, 12). A major difficulty arises when one attempts to reconcile this value with the need of the cell to generate much more electronegative potentials for biosynthesis. For the purple photosynthetic bacteria, it has been suggested[24,25] that ATP (or a high-energy precursor) drives a reverse transfer of electrons from the reduced form of the primary acceptor ($E_m \approx -100$ mV) to a more electronegative component, such as NAD^+ ($E_m = -320$ mV).

There was no need to invoke such a mechanism for NAD^+ reduction in the green sulfur bacteria. Chromatophores from *Chlorobium* can photoreduce both ferredoxin[14,15,17] ($E_m = -420$ mV) and NAD^+ (refs 15, 16) in a reaction that is not coupled to photophosphorylation and is insensitive to inhibitors of reverse electron transfer[16]. Therefore, it appeared possible that the primary acceptor in *Chlorobium* might be more electronegative than in other photosynthetic bacteria. A difference in the midpoint potential of the primary acceptor of *Chlorobium* and other photosynthetic bacteria seemed possible in view of the significant difference in the midpoint potentials of the reaction-center bacteriochlorophylls. Values of +240 mV (ref. 10) and +330 mV (see above) have been obtained for the green bacteria *Cps. ethylicum* and *C. thiosulfatophilum*, respectively, while the reaction center bacteriochlorophylls in other types of photosynthetic bacteria have midpoint potentials near +480 mV (see refs 2 and 7, for example).

To test this possibility, the photooxidation of *Chlorobium* cytochromes and reaction center bacteriochlorophyll was measured as a function of oxidation–reduction potential. The technique is based on the premise that, as the primary acceptor becomes reduced, the photooxidation of chromatophore components is prevented[1,2,6,7,9,11]. The potential at which the photooxidation of chromatophore components is decreased by 50% would thus correspond to the midpoint potential of the primary acceptor.

The present evidence shows that in *Chlorobium* chromatophores the photo-

oxidation of neither reaction center bacteriochlorophyll nor cytochrome *c* was detected at potentials more negative than −200 mV. The photooxidation of both of these components was attenuated to zero as the potential was lowered, in agreement with a theoretical one-electron curve with an E_m of −130 mV. Such data might be taken as evidence that the primary electron acceptor in *Chlorobium* has an E_m of −130 mV and is a one-electron carrier.

One limitation must be applied to the above interpretation since the results were obtained from steady-state measurements at room temperature. These conditions may allow mutiple turnovers of the electron transport chain, and the possibility exists that the −130-mV component represents not the primary acceptor but a different molecule that receives electrons from the primary acceptor. It is also possible that *Chlorobium* has more than one photosystem and the components titrated in this series of experiments are not on the pathway involved in NAD^+ reduction. Rapid kinetic measurements or measurements at cryogenic temperature are needed before the role of the −130 mV component can be more definitely assessed.

Additional measurements are also required to explain why cytochrome *c* photooxidation is attenuated in two steps of +30 mV and −130 mV. Oxidation-reduction profiles for cytochrome photooxidation similar to the above have been reported for other photosynthetic bacteria[2,7,26] and have been explained on the basis of the photooxidation of two different *c*-type cytochromes. The possibility exists that a similar situation prevails in *Chlorobium* because of the presence of several *c*-type cytochromes in this organism[27].

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