Biochimica et Biophysica Acta, 376 (1975) 549–560

BBA 46876

CYTOCHROME *b* AND PHOTOSYNTHETIC SULFUR BACTERIA

DAVID B. KNAFF and BOB B. BUCHANAN

Department of Cell Physiology, University of California, Berkeley, Calif. 94720 (U.S.A.)

(Received August 26th, 1974)

SUMMARY

Chromatophores isolated from the purple sulfur bacterium *Chromatium* and the green sulfur bacterium *Chlorobium* exhibit absorbance changes in the cytochrome α-band region consistent with the presence of a *b*-type cytochrome. Cytochrome content determined by reduced minus oxidized difference spectra and by heme analysis suggests that each bacterium contains one cytochrome *b* per molecule of photochemically active bacteriochlorophyll (reaction-center bacteriochlorophyll).

The *b*-type cytochrome in *Chromatium* has an α-band maximum at 560 nm and a midpoint oxidation-reduction potential of −5 mV at pH 8.0. The *b*-type cytochrome in *Chlorobium* has an α-band maximum at 564 nm and an apparent midpoint oxidation-reduction potential near −90 mV.

Chromatophores isolated from both *Chromatium* and *Chlorobium* cells catalyze a photoreduction of cytochrome *b* that is enhanced in the presence of antimycin A. Antimycin A and 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide inhibit endogenous (but not phenazine methosulfate-mediated) cyclic photophosphorylation in *Chromatium* chromatophores and non-cyclic electron flow from Na_2S to NADP in *Chlorobium* chromatophores. These observations suggest that *b*-type cytochromes may function in electron transport reactions in photosynthetic sulfur bacteria.

INTRODUCTION

The photosynthetic electron transport apparatus of all oxygen-evolving organisms (algae and higher plants) contains cytochromes of both the *b* and *c* types (see refs 1–3 for recent reviews). The distribution of cytochromes in photosynthetic bacteria, the other major group of photosynthetic organisms, is considered to be somewhat different. The occurrence of cytochrome *c* has been demonstrated for each of the three types of photosynthetic bacteria (purple non-sulfur bacteria, such as *Rhodospirillum rubrum*; purple sulfur bacteria, such as *Chromatium*; and green sulfur bacteria, such as *Chlorobium* [4–10]) but the occurrence of cytochrome *b* has been documented only for the purple non-sulfur group [4, 5, 7, 11]. There have been

Abbreviations: HOQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

proposals based on spectral evidence that photosynthetic sulfur bacteria contain cytochrome *b* [12, 13]; and small quantities of protoheme, the heme type found in *b*-type cytochromes (and of certain enzymes such as peroxidase), have recently been reported to occur in the soluble fraction in preparations from members of the purple sulfur group [14, 15]. By contrast, it was reported that the other major group, the green sulfur bacteria, do not contain protoheme [9, 16]. Furthermore, neither the oxidation-reduction properties nor the possible functions of membrane-bound *b*-type cytochromes have been described for a photosynthetic sulfur bacterium.

We therefore initiated an investigation to gain a greater understanding of the possible occurrence and function of cytochrome *b* in photosynthetic sulfur bacteria. We have obtained evidence based on absorbance measurements in the cytochrome α-band region and on heme analyses that suggests that chromatophores isolated from both the purple and green sulfur bacteria may contain a membrane-bound *b*-type cytochrome. Our data further suggest that the *b*-type cytochromes function in light-induced electron transfer reactions in these organisms. Preliminary accounts of these findings have been published [17, 18].

METHODS

Chromatium, Strain D, was grown with CO_2 as sole carbon source on the thiosulfate medium of Arnon et al. [19]. As indicated, thiosulfate was replaced by malate. *Chlorobium thiosulfatophilum* (Tassajara) was grown with CO_2 as sole carbon source on a modified *Chromatium* medium (40 mM Na_2CO_3) supplemented as indicated with 0.08 % sodium acetate [20]. *Ectothiorhodospira mobilis* was grown on Pfennig's medium supplemented with 5 % NaCl and 0.2 % sodium malate [21, 22].

The temperature for all steps of the preparative procedure was 4 °C. Chromatophores were isolated from freshly harvested *Chlorobium* and *Chromatium* cells and from frozen *E. mobilis* cells as described previously [23] except for omission of the DEAE-cellulose treatment and changes in preparative buffer, type of sonifier, and time of sonication. In the present study, *Chlorobium* chromatophores were prepared in 0.05 M potassium phosphate buffer (pH 6.5); *Chromatium* and *E. mobilis* chromatophores were prepared in the same buffer supplemented with 0.1 M NaCl. In all cases, cell suspensions were sonicated for 3 min with a Branson sonic probe (power setting 3) and the chromatophores were washed once with the preparative buffer solution prior to use. *Chlorobium* chlorophyll and bacteriochlorophyll content of the washed chromatophores was estimated as described by Stanier and Smith [24] and Cohen-Bazire et al. [25], respectively.

Oxidation-reduction titrations and absorbance measurements were performed under anaerobic conditions using previously described methods [26–28]. The sensitivity of the spectrophotometer was approx $1 \cdot 10^{-4}$ absorbance units. Light-induced reactions were measured using a 794-nm actinic beam (10-nm half-band width) with an intensity of $2 \cdot 10^4$ ergs/cm^2 per s. Photoreduction of NADP could be carried out anaerobically using *Chlorobium* chromatophores supplemented with either the native ferredoxin [29] and NAD(P) reductase [30–32] or the ferredoxin and ferredoxin-NADP reductase from spinach chloroplasts. Because of their greater stability, the chloroplast proteins were used routinely [29, 30]. NADP reduction was measured as described by McSwain and Arnon [33].

Heme analyses were conducted on chromatophores that had been freed of bacteriochlorophyll by extraction at 4 °C with 40 vol. of methanol and collected by centrifugation for 20 min at 39 000 × *g*. For estimation of total heme, the methanol-extracted chromatophores were suspended in water and analyzed directly. Protoheme was removed from the methanol-extracted chromatophores by further extraction with 10 vol. of acidic acetone (1 part 12 M HCl : 99 parts acetone) [34], the supernatant fraction was centrifuged off (5 min, 39 000 × *g*), and the resulting pellet was suspended in water and analyzed. This extraction removed from the chromatophores all of the protoheme and approx. 30 % of the heme *c*.

Heme *c* and protoheme were determined as their pyridine hemochrome derivatives according to Falk [34]. Potassium ferricyanide (2 mM) was added to the reference cuvette and crystalline sodium dithionite was added stepwise to the sample cuvette until the hemochrome was fully reduced. Heme concentrations were calculated from absorption spectra measured with a Cary model 14 spectrophotometer. Heme *c* was determined from the spectrum of the methanol-extracted chromatophores using a reduced minus oxidized extinction coefficient of 21.7 $mM^{-1} \cdot cm^{-1}$ (550 nm minus 580 nm). Protoheme was determined from the difference spectrum between the methanol-extracted (control) chromatophores and chromatophores that were extracted with acidic acetone following the methanol extraction by using a reduced minus oxidized extinction coefficient of 28.0 $mM^{-1} \cdot cm^{-1}$ (557 nm minus 580 nm). The difference extinction coefficients were determined using purified cytochrome *f* from spinach as a standard for heme *c* and liver cytochrome b_5 as a standard for protoheme (Wada, K., unpublished observations).

RESULTS

Experiments with Chromatium chromatophores

Fig. 1 shows the results of an oxidation-reduction titration experiment designed to test for *b*-type cytochromes in chromatophores from the purple sulfur bacterium

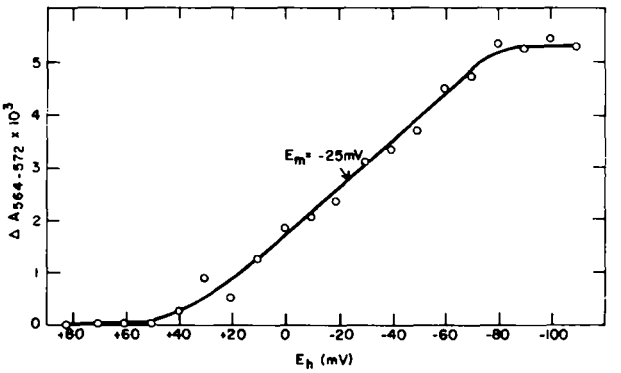


Fig. 1. Absorbance change at 564 nm as a function of oxidation-reduction potential in *Chromatium* chromatophores (reference wavelength, 572 nm). The reaction mixture contained (per 1.0 ml) *Chromatium* chromatophores (equivalent to 30 μg of bacteriochlorophyll) and the following: 50 μmol of Tris buffer (pH 8.0); 0.02 μmol of 2,5-dimethyl benzoquinone; 0.01 μmol of 1,2-naphthoquinone; 0.01 μmol of 1,4-naphthoquinone; 0.01 μmol of duroquinone; 0.01 μmol of 2-hydroxy-1,4-naphthoquinone; and 0.01 μmol of anthraquinone 1,5-disulfonate.

Chromatium by monitoring absorbance changes at 564 nm, a wavelength characteristic of α-bands of *b*-type cytochromes at which contributions from *c*-type cytochromes are minimal. As the oxidation-reduction potential was lowered below +50 mV, a component with a midpoint potential of −25 mV became reduced, leading to an increase in absorbance at 564 nm. Four titrations gave an average value of −5±20 mV for the midpoint potential (E_m) of this one-electron component at pH 8.0. In this experiment and in the experiments below, chromatophores were isolated from *Chromatium* cells grown in a thiosulfate medium; similar results were obtained with cells grown on a malate medium.

Fig. 2 shows the spectrum of *Chromatium* chromatophores in the cytochrome α-band region determined by lowering the oxidation-reduction potential from +20 mV to −140 mV. The spectrum shows two peaks, one at 553 nm corresponding to reduction of *Chromatium* cytochrome c_{553} (E_m = +10 mV [35–38]) and the other at 560 nm, corresponding to a reduction of an unknown component.

The midpoint oxidation-reduction potential of the 560-nm-absorbing component, unlike that of cytochrome c_{553} [36], was pH dependent. Four titrations of the 560-nm-absorbing component at pH 9.0 gave an average value of −70±15 mV, indicating the uptake of one proton per electron. At pH values less than 7.5, apparent *n* values greater than 1.0 were obtained, possibly because of poor equilibration with the oxidation-reduction mediators used.

An absorbance increase at 560 nm on reduction corresponds to the expected behavior of a *b*-type cytochrome. We therefore tentatively designated the component responsible for the 560-nm absorbance change cytochrome b_{560} and sought further evidence for its identity. Assuming a reduced minus oxidized extinction coefficient of

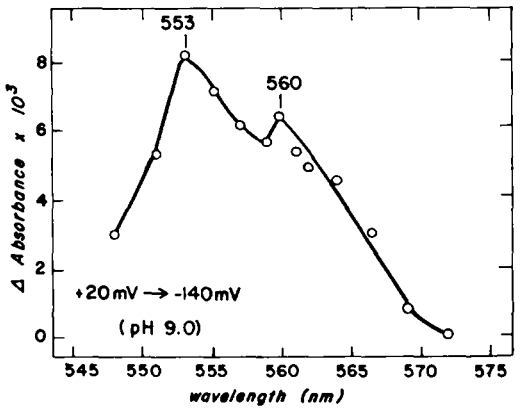


Fig. 2. Spectra of cytochromes *b* and *c* in *Chromatium* chromatophores reduced by lowering the oxidation-reduction potential from +20 mV to −140 mV (reference wavelength, 572 nm). The reaction mixture contained (per 1.0 ml) *Chromatium* chromatophores (equivalent to 30 μg of bacteriochlorophyll) and the following: 50 μmol of Tris buffer (pH 9.0); 0.02 μmol of 2,5-dimethyl benzoquinone; 0.01 μmol of 1,2-naphthoquinone; 0.0025 μmol of phenazine methosulfate; 0.01 μmol of phenazine ethosulfate; 0.005 μmol of 5-hydroxy-1,4-naphthoquinone; 0.01 μmol of duroquinone; 0.0025 μmol of pyocyanine; and 0.01 μmol of 2-hydroxy-1,4-naphthoquinone. The sample was poised at +20 mV and the absorbance at the indicated wavelength was measured. The oxidation-reduction potential was then lowered to −140 mV by the addition of dithionite and the absorbance was measured again. A fresh sample was used for each wavelength.

20 $mM^{-1} \cdot cm^{-1}$ (a value typical for *b*-type cytochromes [39]) and correcting for the absorbance of cytochrome c_{553} at 560 nm [38], we calculated that the amount of cytochrome b_{560} in *Chromatium* chromatophores corresponds to one cytochrome *b* per 140±20 bacteriochlorophyll molecules.

If the unknown component seen in Fig. 2 is indeed a *b*-type cytochrome, *Chromatium* chromatophores must contain a comparable amount of protoheme, the prosthetic group of *b*-type cytochromes. Fig. 3 shows the results of a heme analysis of methanol-extracted *Chromatium* chromatophores in which the pyridine derivatives were reduced stepwise with dithionite. In the presence of limiting dithionite, the spectrum showed a peak at 556 nm, corresponding to the 556–558-nm maximum characteristic of the pyridine hemochrome derivative of protoheme [10, 34]. On complete hemochrome reduction, total absorption increased markedly and the peak shifted from 556 nm to 549–550 nm, the wavelength characteristic of heme *c*, the prosthetic group of cytochrome *c* [10, 34].

Evidence for the occurrence of protoheme in *Chromatium* chromatophores was also provided by extraction with acidic acetone, a treatment known to remove the protoheme of *b*-type cytochromes [10, 34]. Addition of limiting amounts of dithionite to such chromatophore preparations did not give rise to an absorbance peak typical of protoheme (556 nm), as was observed in the control (methanol-extracted) chromatophores but, rather, gave a peak typical of heme *c* (550 nm) at all stages of reduction. Similar results were obtained with chromatophores from another purple sulfur bacterium, *E. mobilis*.

The protoheme and heme *c* contents of *Chromatium* chromatophores could

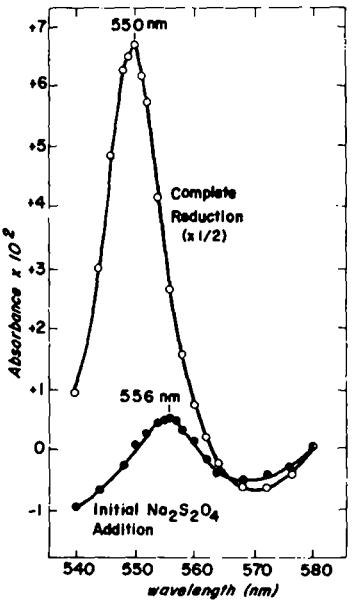


Fig. 3. Demonstration of protoheme in *Chromatium* chromatophores by reduction with a limiting concentration of dithionite. Hemochrome spectra were measured with methanol-extracted chromatophores equivalent to 0.16 mg/ml of bacteriochlorophyll.

be quantitated by a comparison of their pyridine hemochrome spectra before and after extraction with acidic acetone. The control minus acidic acetone-extracted difference spectrum for *Chromatium* chromatophores showed a pronounced shoulder at 558 nm, which corresponded to one protoheme per 165±25 bacteriochlorophyll molecules. That value is in good agreement with the above value of one cytochrome *b* per 140 bacteriochlorophyll molecules estimated from the reduced minus oxidized α-band spectra. These values indicate that cytochrome *b* is present in *Chromatium* chromatophores in amounts equimolar to the photochemically active bacteriochlorophyll, usually designated reaction-center bacteriochlorophyll, that corresponds to one molecule per 170–250 bacteriochlorophyll molecules [40, 41]. On a heme *c* basis, *Chromatium* chromatophores contained one protoheme per seven to eight heme *c*. Documentation that the protoheme found in the chromatophores was contributed by *Chromatium* cells rather than by a contaminating microorganism was provided by the finding that chromatophores of cells grown from a single colony isolated from the parent *Chromatium* strain showed a complement of cytochromes identical to that of the parent.

The above evidence suggesting that a *b*-type cytochrome was present in significant amounts in *Chromatium* chromatophores raised the possibility that the cytochrome could be involved in photosynthetic electron transport reactions. To

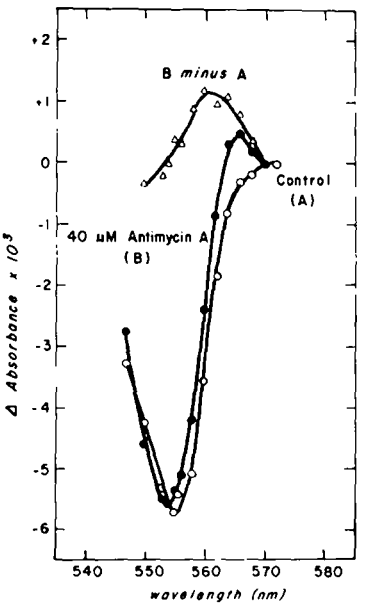


Fig. 4. Demonstration of photoreduction of cytochrome b_{560} in *Chromatium* chromatophores in the presence of antimycin A (reference wavelength, 572 nm). Control (A) and antimycin A (B) spectra represent light minus dark difference spectra determined for each treatment. The spectrum of photoreduced cytochrome b_{560} (B minus A) represents the absorbance difference: antimycin A treatment (B) minus control treatment (A). The control reaction mixture contained (per 1.0 ml) *Chromatium* chromatophores (equivalent to 30 μg of bacteriochlorophyll) and the following: 50 μmol of Tris buffer (pH 8.0); 0.10 μmol of benzoquinone; and 0.02 μmol of 2,5-dimethylbenzoquinone. Antimycin A ($4 \cdot 10^{-5}$ M) was added as indicated.

answer this question, we investigated the effect of light on cytochromes of *Chromatium* chromatophores poised (with and without antimycin A) at +200 mV prior to illumination (Fig. 4). The control sample showed light-induced absorbance changes characteristic of the photooxidation of cytochrome c_{555} (E_m = +330 mV [35–37]) but no substantial absorbance change in the cytochrome *b* region. A change in that region, however, was observed on the addition of antimycin A, an inhibitor that is known to block the oxidation of *b*-type cytochromes in other systems [42–45]. The difference spectrum (antimycin A treatment minus control) in Fig. 4 shows a single narrow band at 560 nm, indicating that cytochrome b_{560}, observed chemically by reduction with dithionite (Figs 1 and 2), could also be photoreduced and accumulate in reduced form in the presence of antimycin A. Antimycin A had no effect on photooxidation of cytochrome c_{555}.

The finding that antimycin A promoted the accumulation of reduced cytochrome b_{560} prompted us to test the effect of that inhibitor on cyclic photophosphorylation catalyzed by *Chromatium* chromatophores [46–48]. As shown in Table I, antimycin A at concentrations similar to that required to stimulate photoreduction of cytochrome b_{560} gave nearly complete inhibition of endogenous photophosphorylation but had no effect on photophosphorylation catalyzed by the nonphysiological cofactor phenazine methosulfate. HOQNO, another inhibitor that has been shown to inhibit the oxidation of *b*-type cytochromes [49, 50], showed an inhibition pattern similar to that of antimycin A.

TABLE I

EFFECT OF ANTIMYCIN A AND HOQNO ON ENDOGENOUS AND PHENAZINE METHOSULFATE CATALYZED CYCLIC PHOTOPHOSPHORYLATION IN *CHROMATIUM* CHROMATOPHORES

The reaction was carried out in Warburg vessels that contained (per 1.0 ml final volume) *Chromatium* chromatophores (equivalent to 100 μg of bacteriochlorophyll) added to the side-arm and the following added to the main compartment: 100 μmol of Tris buffer (pH 8.2); 5 μmol of $MgCl_2$; 5 μmol of $K_2H^{32}PO_4$; 5 μmol of ADP. Where indicated, phenazine methosulfate was present at a concentration of $5 \cdot 10^{-5}$ M. Vessels were equilibrated for 5 min with N_2; chromatophores were added from the side-arm and incubated for an additional 5 min in the dark prior to illumination. Reaction time, 10 min; temperature, 30 °C; light intensity, 20 000 lux.

Treatment	Q_{ATP}*	% of control**
Endogenous photophosphorylation		
Dark	4	–
Light	68	100
$2 \cdot 10^{-5}$ M antimycin A	8	12
$2 \cdot 10^{-5}$ M HOQNO	16	24
Phenazine methosulfate-catalyzed photophosphorylation		
Dark	0	–
Light	92	100
$2 \cdot 10^{-5}$ M antimycin A	80	87
$2 \cdot 10^{-5}$ M HOQNO	92	100

* ATP formed, μmol/h per mg of bacteriochlorophyll.

** Values corrected for ATP formed in dark control.

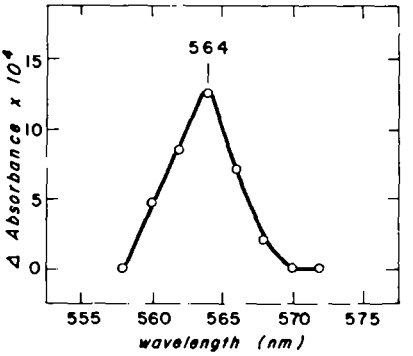
Experiments with Chlorobium chromatophores

The evidence suggesting the presence of a *b*-type cytochrome in purple sulfur bacteria raised the question whether the other major group of photosynthetic sulfur bacteria, the green bacteria, contain such a cytochrome. To test this point, we examined chromatophores isolated from *Chlorobium* cells for a light-induced absorbance change in the region from 558 nm to 572 nm and observed a change consistent with the photoreduction of a *b*-type cytochrome (maximum at 564 nm) (Fig. 5). The previously described absorbance changes in the 540–555-nm region due to the photooxidation of a *c*-type cytochrome were also observed [28].

We further investigated the nature of the light-induced absorbance change at 564 nm which, for the reasons discussed above for cytochrome b_{560} in *Chromatium*, was tentatively ascribed to cytochrome b_{564}. Photoreduction of cytochrome b_{564} was dependent on the oxidation-reduction potential of the chromatophore suspension; photoreduction decreased to zero as the potential was lowered from -20 mV to -150 mV. The average of four titrations gave a value of -90 ± 20 mV and an n value of 1.0. Because of the large drift in the instrument baseline observed with *Chlorobium* chromatophores over the 1-h-long period required to perform an anaerobic titration, it was not possible to titrate cytochrome b_{564} directly.

Fig. 6 shows the effect of antimycin A on *Chlorobium* cytochrome b_{564} reduction in the light and reoxidation in the dark. Antimycin inhibited the dark oxidation of photoreduced cytochrome b_{564} but, by contrast, stimulated the reduction. The light minus dark difference spectrum observed in the presence of antimycin A has the same shape and wavelength maximum as that shown in Fig. 5. Cytochrome *c* photooxidation [28] was not affected by antimycin A.

Assuming a reduced minus oxidized extinction coefficient of 20 $mM^{-1} \cdot cm^{-1}$



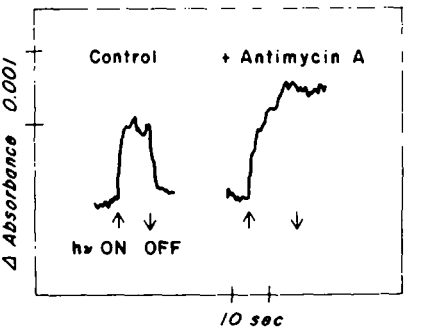


Fig. 5. Spectrum of photoreduced cytochrome b_{564} in *Chlorobium* chromatophores (reference wavelength, 540 nm). The reaction mixture contained (per 1.0 ml) chromatophores (equivalent to 150 μg of *Chlorobium* chlorophyll) and the following: 50 μmol of Tris buffer (pH 7.2); 0.02 μmol of 2,5-dimethylbenzoquinone; 0.01 μmol of 1,2-naphthoquinone; 0.01 μmol of 1,4-naphthoquinone; 0.01 μmol of duroquinone; 0.01 μmol of 2-hydroxy-1,4-naphthoquinone; 0.01 μmol of anthraquinone-1,5-disulfonate; and 0.01 μmol of anthraquinone 2-sulfonate. The oxidation-reduction potential prior to illumination was -20 mV.

Fig. 6. Effect of antimycin A on *Chlorobium* cytochrome b_{564} reduction in the light and oxidation in the dark (wavelength, 564 nm minus 540 nm). Antimycin A at a concentration of $2 \cdot 10^{-4}$ M was present where indicated. Other experimental conditions as in Fig. 5.

TABLE II

EFFECT OF ANTIMYCIN A AND HOQNO ON THE FERREDOXIN-DEPENDENT PHOTOREDUCTION OF NADP BY *CHLOROBIUM* CHROMATOPHORES

The reaction mixture contained (per 1.0 ml) *Chlorobium* chromatophores (equivalent to 200 μg of *Chlorobium* chlorophyll), spinach chloroplast ferredoxin-NADP reductase (70 μg), and the following: 100 μmol of Tris buffer (pH 8.5); 2 μmol of NADP; 300 μg of spinach chloroplast ferredoxin; and, as indicated, 12.5 μmol of Na_2S or 2-mercaptoethanol. Actinic light, 650 nm; light intensity, $2 \cdot 10^5$ ergs/cm^2 per s; temperature, 25 °C.

Treatment	Q_{NADP}*	% of control
Na_2S		
Control	8.4	100
+ $1 \cdot 10^{-4}$ M antimycin A	1.9	23
+ $2 \cdot 10^{-4}$ M antimycin A	0.7	8
+ $1 \cdot 10^{-4}$ M HOQNO	0.9	11
+ $2 \cdot 10^{-4}$ M HOQNO	0.5	6
Mercaptoethanol		
Control	1.3	100
+ $2 \cdot 10^{-4}$ M antimycin A	1.4	108
+ $2 \cdot 10^{-4}$ M HOQNO	1.4	108

* NADP reduced, μmol/mg *Chlorobium* chlorophyll per h.

for cytochrome b_{564}, the amount of cytochrome b_{564} photoreduced in the presence of antimycin A was estimated to be one cytochrome b_{564} per 1600 *Chlorobium* chlorophyll molecules. That value is in reasonable agreement with the content of protoheme (one protoheme per 1300 ± 200 *Chlorobium* chlorophyll molecules; ratio of heme *c*: heme *b* = 5) which was determined as described for *Chromatium*. The *b*-type cytochrome in *Chlorobium*, as in *Chromatium*, appears to be present at a concentration equimolar to the reaction-center bacteriochlorophyll: one molecule per 1000–1500 *Chlorobium* chlorophyll molecules [51]. Neither the properties nor the content of cytochrome *b* in *Chlorobium* chromatophores was altered by growth in a medium supplemented with acetate.

To test the possible role of cytochrome b_{564} in photosynthesis, we determined the effect of inhibitors of cytochrome *b* oxidation on the ferredoxin-dependent photoreduction of NADP by *Chlorobium* chromatophores [30, 31]. As shown in Table II, photoreduction of NADP (in the presence of spinach chloroplast ferredoxin and ferredoxin-NADP reductase) with the physiological electron donor Na_2S was sensitive to antimycin A and HOQNO. NADP photoreduction with the non-physiological electron donor 2-mercaptoethanol was insensitive to these inhibitors. In agreement with previous observations [31], NADP reduction with either donor was unaffected by such uncouplers of bacterial photophosphorylation as gramicidin D, desaspidin, and CCCP. NADP photoreduction was also unaffected by *o*-phenanthroline ($5 \cdot 10^{-3}$ M), an inhibitor that blocks electron transfer in other photosynthetic bacteria [52].

DISCUSSION

Reduced minus oxidized difference spectra obtained with chromatophores from the purple sulfur bacterium *Chromatium* indicate the presence of a previously undetected membrane-bound component which, because of its spectral properties and response to antimycin A, has been tentatively designated cytochrome b_{560}. This newly discovered component can, by its oxidation-reduction and spectral properties, be distinguished from cytochrome *c'*, a component of *Chromatium* chromatophores that also absorbs in this spectral region. Cytochrome b_{560} has a sharp α-band at 560 nm, whereas cytochrome *c'* has a broad α-band with maxima at 547 nm and 565 nm [38]. Although soluble *Chromatium* cytochrome *c'* has a midpoint potential (−5 mV [38]) identical to that of cytochrome b_{560}, the membrane-bound form of cytochrome *c'* has a much more oxidizing midpoint potential (+180 mV to +250 mV [35, 37]).

Evidence consistent with the presence of a *b*-type cytochrome in *Chromatium* chromatophores was also provided by their content of protoheme, the prosthetic group of *b*-type cytochromes, in an amount indicating the presence of one cytochrome *b* molecule per reaction center (one per 165 bacteriochlorophyll molecules). The protoheme spectrum obtained in the pyridine hemochrome assay cannot originate from the previously known membrane-bound cytochromes in *Chromatium*, including cytochrome *c'*, which show typical heme *c* pyridine hemochrome spectra [38]. Kamen and colleagues recently reported that protoheme is present in *Chromatium* and in another photosynthetic purple sulfur bacterium but that protoheme occurred in the soluble fraction rather than in the chromatophore fraction [14, 15]. Earlier investigators did not detect membrane-bound protoheme in *Chromatium* [8, 53].

In the present study, we have also presented evidence that a *b*-type cytochrome, tentatively designated cytochrome b_{564}, is bound to the chromatophore membranes of the green sulfur bacterium, *Chlorobium*, a member of the third major group of photosynthetic bacteria. Technical problems have prevented a direct determination of the oxidation-reduction potential of *Chlorobium* cytochrome b_{564}, but photoreduction measurements at defined potentials suggest that as the potential is lowered through a −90-mV midpoint the cytochrome becomes reduced. Accordingly, cytochrome b_{564} has been tentatively assigned a midpoint oxidation-reduction potential of −90 mV (pH 7.2). Direct titrations of cytochrome b_{564} are needed to confirm this value.

Although conclusive evidence regarding the function of the two newly discovered *b*-type cytochromes awaits further study, the possibility that they function in photosynthetic electron transport is suggested by experiments with antimycin A and HOQNO. The sensitivity of endogenous (and insensitivity of phenazine methosulfate-catalyzed) cyclic photophosphorylation in *Chromatium* chromatophores to these inhibitors resembles the pattern observed with chromatophores from *R. rubrum* [54–56] which has been shown to involve cytochrome *b* [43, 44]. Similarly, the sensitivity to antimycin A and HOQNO of NADP photoreduction by *Chlorobium* chromatophores with Na_2S (but not 2-mercaptoethanol) as electron donor is compatible with the involvement of a *b*-type cytochrome. The observation that antimycin A inhibits cytochrome b_{564} oxidation and electron transfer from Na_2S to NADP at similar concentrations suggests that the cytochrome may function in this pathway.

However, if cytochrome b_{564} functioned solely in electron transfer from Na_2S to NADP, one would expect, contrary to present findings, that the cytochrome would be oxidized by light instead of being reduced by light. The relation of the observed photoreduction of cytochrome b_{564} to its apparent function in non-cyclic electron transport in *Chlorobium* therefore remains to be elucidated.

In summary, we have presented three lines of evidence that suggest that chromatophores of photosynthetic sulfur bacteria contain membrane-bound cytochrome *b*: (i) Spectral measurements in the α-band region. (ii) The presence of protoheme, the prosthetic group of cytochrome *b*. (iii) Inhibition of both cytochrome *b* oxidation and photosynthetic electron transport reactions by antimycin A and HOQNO. These findings constitute support for the view that photosynthetic sulfur bacteria resemble other types of photosynthetic cells in containing cytochrome *b*.

ADDENDUM

After the submission of this manuscript, a paper appeared [57] reporting the presence of a *b*-type cytochrome in photosynthetic green sulfur bacteria.

ACKNOWLEDGEMENTS

We would like to thank Mr Richard Chain for preparation of spinach ferredoxin and ferredoxin-NADP reductase and Mr William Ufert for expert technical assistance. This investigation was supported in part by National Science Foundation Grant GB-40634 to D.B.K.

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