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Cytochromes functionally associated to photochemical reaction centers in *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila*

Katsumi Matsuura and Keizo Shimada

Department of Biology, Faculty of Science, Tokyo Metropolitan University, Fukazawa 2-1-1, Setagaya-ku, Tokyo 158 (Japan)

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Electron donors to photo-oxidized reaction centers were studied in *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila* using whole cells, membrane preparations and reaction center-B880 complexes. In *Rps. palustris*, no hemes were tightly bound to the reaction center complex and cytochrome c_2 was presumed to be the direct electron donor to the photo-oxidized bacteriochlorophyll dimer in whole cells. Cytochrome c_2 was lost in the membrane preparation obtained after a French press disruption of cells and mammalian cytochrome *c* added externally was oxidized by illumination. On the other hand, in *Rps. acidophila*, the reaction center complex was tightly associated with four cytochrome hemes, two of which were identified as cytochrome *c*-553 ($E_{m7} = 110$ mV) and the other two as cytochrome *c*-555 ($E_{m7} = 360$ mV). When both cytochromes were reduced prior to illumination, cytochrome *c*-553 was rapidly oxidized by a flash. Cytochrome *c*-555 was the one oxidized by a flash when cytochrome *c*-553 was already oxidized before activation. Cytochrome c_2 was presumed to be the electron donor to the photo-oxidized cytochrome *c*-555 in whole cells. In the membrane preparation, a rapid electron transfer was observed from externally added mammalian cytochrome *c* to photooxidized cytochrome *c*-555. A possible phylogenetic correlation between the absence of the tightly bound cytochromes and a long class of cytochrome c_2 (Dickerson, R.E. (1980) Nature 283, 210–212) is discussed.

Introduction

In purple photosynthetic bacteria, two different types of photochemical reaction center complex have been found depending on species [1,2]. No cytochromes are tightly associated to reaction centers in one type. Reaction center complexes of the other type have strongly bound *c*-type cytochromes which donate electrons to the photo-oxidized bacteriochlorophyll dimer [$(BChl_2)_2^+$]. Except for the presence of the bound cytochrome *c*, the two types of reaction center complex are similar to each other with respect to pigments, redox centers and polypeptide compositions.

A 3-dimensional structure of a reaction center complex has recently been shown in *Rhodopseudomonas viridis* which has cytochrome *c* tightly associated to the complex [3,4]. According to the proposed model, a protein subunit of *c*-type cyto-

Abbreviations: BChl, bacteriochlorophyll; $(BChl)_2^+$, oxidized bacteriochlorophyll dimer in reaction centers; B-880, light-harvesting pigment-protein complex with an absorption peak of bacteriochlorophyll around 880 nm; RC, reaction center; Q_A, primary quinone acceptor of reaction centers; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; HNQ, 2-hydroxynaphthoquinone; PMS, *N*-methylphenazonium methosulfate; Mops, 4-morpholinepropanesulfonic acid.

Correspondence address: Department of Biology, Faculty of Science, Tokyo Metropolitan University, Fukazawa 2-1-1, Setagaya-ku, Tokyo 158, Japan.

chrome with four hemes is attached to L-M subunits in which bacteriochlorophylls are present. The cytochrome subunit is shown to be located at the periplasmic side of the membrane. Another extensively studied species which possess RC-bound cytochrome *c* is *Chromatium vinosum*. In these species, two of four hemes bound to one reaction center are distinguishable from the other two spectrophotometrically and potentiometrically [1].

Other species which have been suggested to have cytochromes tightly linked to the reaction centers are *Thiocapsa pfennigii* [5], *Ectothiorhodospira* sp. [6], *Rhodopseudomonas gelatinosa* [7,8], *Rhodomicrobium vanielli* [9] and *Erythrobacter* sp. (Och 114) [10]. These species are widely distributed among purple bacteria from non-sulfur to sulfur and from strict anaerobe to strict aerobe.

In *Rhodobacter sphaeroides* (formerly called *Rhodopseudomonas sphaeroides*), *Rhodopseudomonas capsulata* and *Rhodospirillum rubrum*, on the other hand, reaction centers do not have tightly bound cytochromes *c* [1,11]. Soluble cytochrome c_2 is an immediate electron donor to $(BChl)_2^+$. Although cytochrome c_2 has been suggested to be bound to the reaction center complex under some conditions [12–14], this cytochrome can be easily removed from the membrane by salt washing [12,15].

In *Chr. vinosum* cytochrome *c*-551, a water-soluble cytochrome which is suggested to be related to cytochrome c_2 evolutionally [16], was shown to donate electrons within milliseconds to photooxidized cytochrome *c*-555 [17]. Cytochrome c_2 in *Rps. viridis* [18] was also shown to be a donor to the membrane-bound cytochrome *c*-558 in a membrane preparation [19]. In this respect, the tightly bound cytochrome *c* does not seem to be an alternative to the water-soluble cytochrome *c*, but an additional component in the electron path from soluble cytochrome *c* to $(BChl)_2^+$.

Phylogenetic relationship among species of purple photosynthetic bacteria has been reconsidered recently based on structural similarities of proteins and nucleic acids [20–22]. The type of reaction center is a possible phylogenetically significant characteristic in purple photosynthetic bacteria. The loss or gain of cytochromes bound to reaction centers has probably not occurred so often in the course of evolution because it may have affected the efficiency of the photosynthetic electron transfer system. *Rb. sphaeroides*, *Rps. capsulata* and *R. rubrum*, which do not have cytochromes tightly associated to reaction centers as described above, share other common characteristics; they divide by binary fission and contain vesicular intracytoplasmic membranes [23], ubiquinone-10 [24] and cytochrome c_2 of a long type [20]. In *Rhodopseudomonas* which shows asymmetrical division and has lamellar-type intracytoplasmic membranes, only *Rps. viridis* has been proved to have the tightly bound cytochromes *c*. *Rps. palustris* and *Rps. acidophila* are other species in this group. *Rps. palustris* has been suggested to have the same type of reaction centers as *Rps. viridis* based on light-induced oxidation of cytochrome *c* at 77 K [1,2,25,26]. On the other hand, RC and RC-B880 complexes isolated from *Rps. palustris* have been reported to contain no polypeptide corresponding to a cytochrome subunit [10,27,28]. Dickerson [20] has suggested that the type of cytochrome c_2 of *Rps. palustris* is the same as that of *Rb. sphaeroides* but different from that of *Rps. viridis* or *Rps. acidophila*. Since cytochrome c_2 interacts directly to the reaction center complex, there may be correlation between the type of cytochrome c_2 and the type of the reaction center complex.

We studied cytochromes oxidized by flash-activated reaction centers in *Rps. palustris* and *Rps. acidophila* to determine the type of reaction centers and the role of soluble cytochrome c_2. The types of reaction center of the two species are different from each other, indicating a close relationship between the types of cytochrome c_2 and reaction centers.

Materials and Methods

Rps. palustris ATCC 17001 was grown anaerobically in the light in a medium described previously [29] with 30 mM malate as sole carbon source and supplemented with 0.5 mg/l *p*-aminobenzoic acid. *Rps. acidophila* ATCC 25092 (strain 7050) was grown anaerobically in the light in a medium similar to that for *Rps. palustris* but at pH 5.6 with 10 mM succinate as the carbon source and without vitamins. Cells were harvested

in the late exponential phase, washed in 100 mM KCl and 5 mM Mops-Na (pH 7.0) and suspended in the same buffer.

For the preparation of membranes, cells were disrupted by a passage through a French pressure cell at 1200 kg/cm^2. The supernatant of centrifugation at 10000 × *g* for 15 min was centrifuged at 100000 × *g* for 60 min. The sediment was washed once with 5 mM Mops-Na and 1 mM EDTA (pH 7.0) and used in the experiments as a membrane preparation.

RC-B880 pigment protein complexes were prepared by polyacrylamide gel electrophoresis after solubilization of the membranes with *β-n*-octylthioglucoside (Dotite, Japan) and Triton X-100. Membrane preparations were suspended in 25 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA to give an absorbance at 850 nm about 50. The suspension was cooled in ice and mixed with an equal volume of pre-cooled mixture of 2% *β-n*-octylthioglucoside/1% Triton X-100/1 M urea. The solubilized membranes were centrifuged at 150000 × *g* for 15 min and the supernatant was applied to polyacrylamide gel electrophoresis. The buffer system of the electrophoresis was according to Davis [30], except that the gel buffers contained 0.1% Triton X-100 at final concentration and the acrylamide concentration in the running gel was reduced to 5.7%. After electrophoresis, one of two major pigmented bands was extracted and concentrated [31] as RC-B880 complex.

Absorbance changes of cytochromes started with a xenon flash (5 μs half-maximal duration, Sugawara S-3AA) were measured with a single-beam spectrophotometer assembled by Dr. T. Satoh in this laboratory. Potentiometric titration was carried out as described by Dutton [32].

Results

Cytochrome oxidation in Rps. palustris

Fig. 1 shows flash-induced absorbance changes in the α band region of cytochromes in whole cells of *Rps. palustris*. The flash elicited a rapid (less than 0.5 ms) oxidation and a subsequent re-reduc-

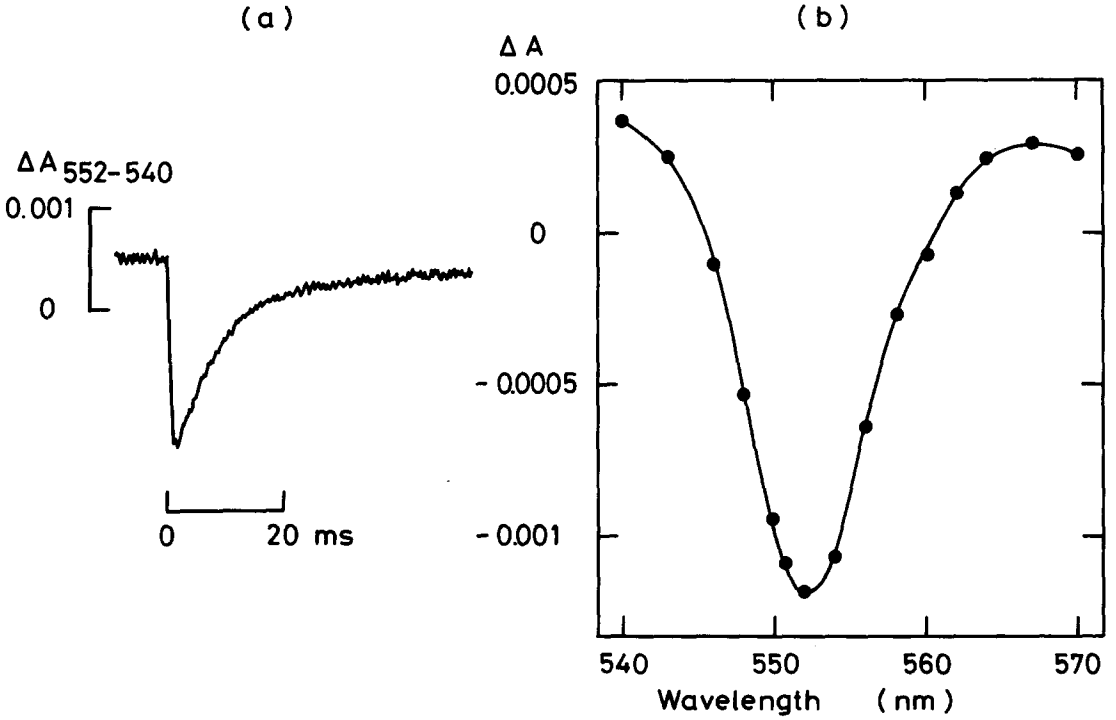

Fig. 1. Kinetics and spectrum of flash-induced absorbance changes in intact cells of *Rps. palustris*. Cells were suspended to 10 μM BChl in 100 mM KCl/5 mM Mops-Na/5 mM sodium succinate/5 mM sodium fumarate (pH 7.0), containing 20 μM CCCP. From kinetic traces as in (a), absorbance changes taken at 1.5 ms after the flash were plotted against wavelength in (b).

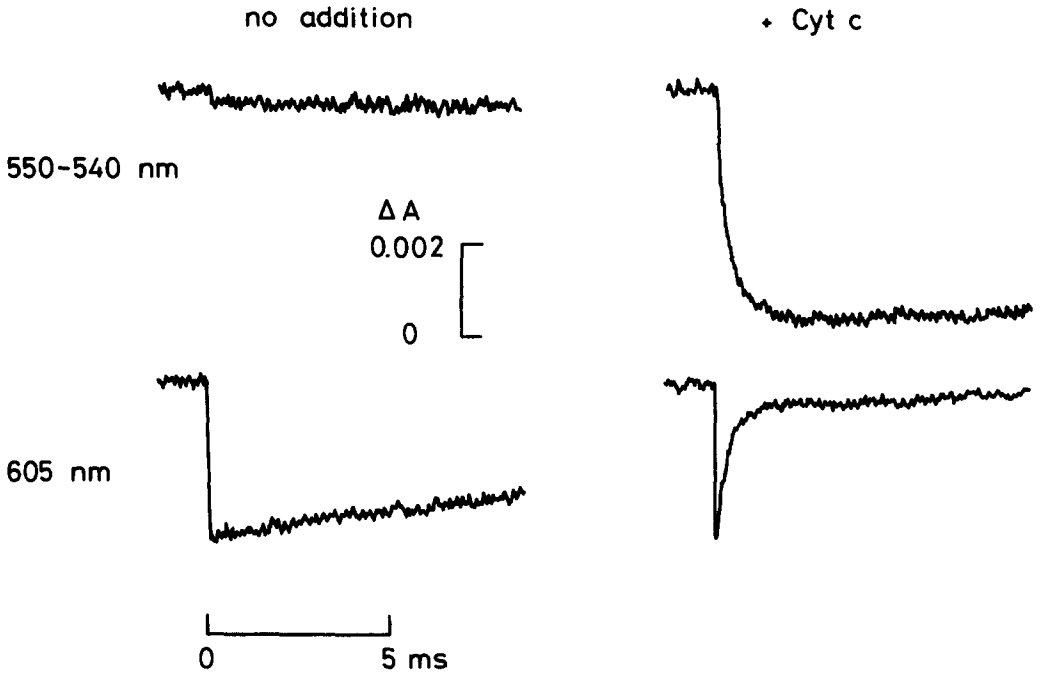

Fig. 2. Flash-induced absorbance changes of cytochrome *c* and $(BChl)_2$ in isolated membranes of *Rps. palustris* and the effect of externally added mammalian cytochrome *c*. The membranes were suspended to 20 μM BChl in 1 mM Mops-Na/1 mM sodium ascorbate (pH 7.0), containing 10 μM DAD and 10 μM CCCP. 8 μM horse heart cytochrome *c* were present in the suspension for the right traces.

tion of a component peaked at 552 nm. The rate of re-reduction was variable depending on preparations and on the period after suspending the cells, probably due to instability of the redox state of quinones in whole cells. Absorption changes of other cytochromes were not detected in kinetic traces at any wavelengths tested, except that small absorption decrease with a peak at 561 nm were sometimes observed after the re-reduction of the major component (data not shown). The peak wavelength of the major component is the same as that of cytochrome c_2 isolated from this species [18].

In the membrane preparation, on the other hand, flash-induced absorbance changes due to cytochromes were very small (Fig. 2, top left). The spectral profile of the small change (data not shown) was similar to that in whole cells, but the extent was about 10% of that in whole cells. When mammalian cytochrome *c* was added to the membrane suspension, cytochrome *c* was oxidized rapidly by a flash (Fig. 2, top right). With a similar time-course to that of the oxidation of cytochrome *c*, $(BChl)_2^+$ in the reaction center was re-reduced (Fig. 2, bottom right cf. bottom left). The half-time of the cytochrome oxidation and the $(BChl)_2$ re-reduction was about 270 μs under the conditions for Fig. 2. The rate of the electron transfer was dependent on the ionic strength in the medium as shown in Fig. 3 (open circles). Reciprocals of the half-time of cytochrome oxidation were plotted to indicate the rate of electron transfer. The rate was almost constant up to 20 mM KCl and decreased with increasing concentration of KCl above 20 mM. A similar observation has been reported in the electron transfer from soluble cytochrome *c* to $(BChl)_2^+$ in the reaction center of *Rb. sphaeroides* [33].

Fig. 4a shows the absorption spectrum of RC-B880 complex isolated from the membrane preparation and the dithionite-reduced minus ferricyanide-oxidized spectrum of the complex. The difference spectrum shows no absorbance changes of cytochromes in the α and β band region (Fig. 4a, inset). The minimal level of cytochrome detection in the difference spectrum in Fig. 4a is about

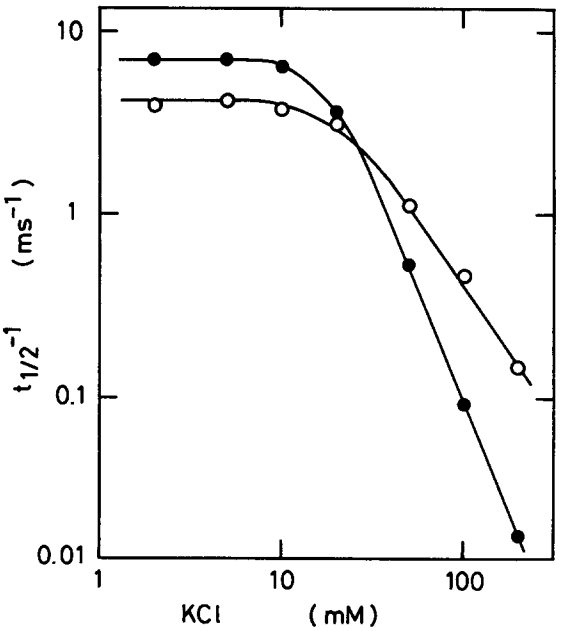

Fig. 3. Effect of KCl concentration on the oxidation rate of externally added cytochrome *c* induced by flash-activation in isolated membranes of *Rps. palustris* (○) and *Rps. acidophila* (●). The reciprocals of the half time of the oxidation were plotted against the concentration of KCl in the medium. Other conditions were the same as those in Fig. 2 for *Rps. palustris* and in Fig. 6 for *Rps. acidophila.*

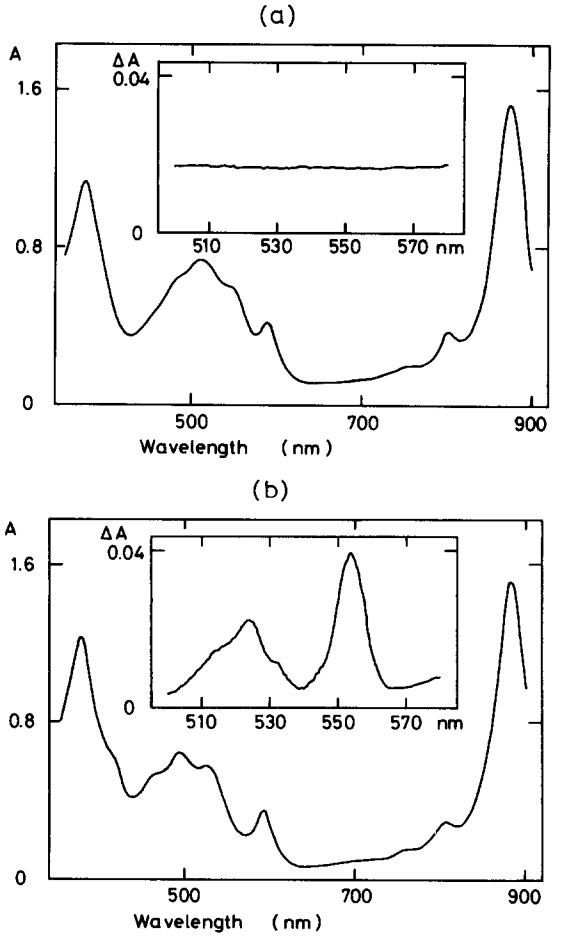

Fig. 4. Absorption spectra and reduced-minus-oxidized spectra of RC-B880 complexes isolated from *Rps palustris* (a) and *Rps. acidophila* (b). The complexes were dissolved in 10 mM sodium phosphate buffer (pH 7.4) containing 0.05% Triton X-100. The inset shows the difference spectrum recorded after the addition of a few grains of sodium dithionite by subtracting the spectrum in the presence of potassium ferricyanide (0.1 mM).

5% of the reaction center in the complex. Flash-induced oxidation of $(BChl)_2$ and a rapid electron transfer from horse-heart cytochrome *c* to $(BChl)_2^+$ were also observed in the isolated complex as in the membrane preparation (data not shown). From the extent of the flash-induced oxidation of cytochrome *c*, the content of RC in the RC-B880 complex was estimated to be one RC/30 BChl.

Cytochrome oxidations in Rps. acidophila

Fig. 5 shows flash-induced absorbance changes in the α band region of cytochromes in the whole cells of *Rps acidophila.* As shown in the spectrum taken at 0.5 ms after the flash (Fig. 5b, closed circles), a cytochrome peaked at 555 nm was oxidized rapidly. A subsequent change from 0.5 to 5 ms after the flash represented a shift-type spectrum to the shorter wavelength (Fig. 5b, open circles). This change was not affected by the presence of an uncoupler (CCCP) and a corresponding change was also observed in the Soret region. Therefore, this change does not represent an electrochromic bandshift but redox changes of cytochromes. From the analogy of the absorption changes in whole cells of *Chr. vinosum* [17], this change is presumed to be resulted from the electron transfer from a cytochrome peaked around 550 nm to cytochrome *c*-555. In the kinetic traces in Fig. 5a, reference wavelengths were chosen to observe the change of each cytochrome without interference from the other one, respectively. The absorbance decrease at 550 nm with a reference at 559.5 nm (Fig. 5a, bottom) was accompanied by the simultaneous absorbance increasse at 555 nm

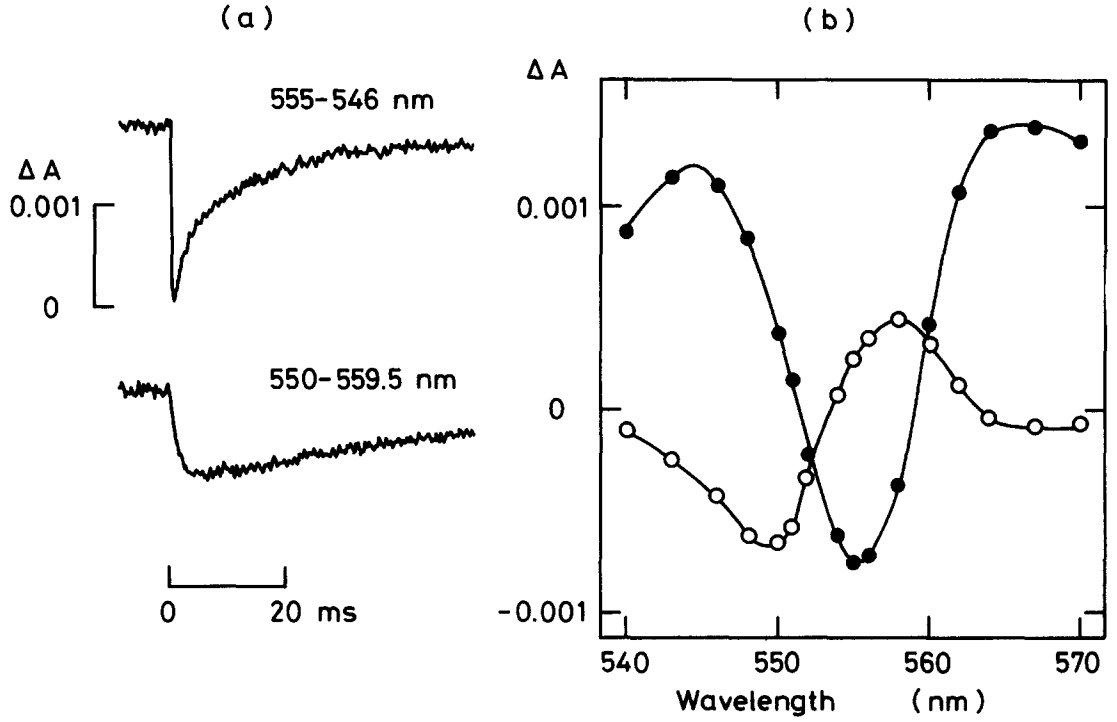

Fig. 5. Kinetics and spectra of flash-induced absorbance changes in intact cells of *Rps. acidophila*. Cells were suspended to 10 μM BChl in 100 mM KCl/5 mM Mops-Na/5 mM sodium succinate/5 mM sodium fumarate (pH 7.0), containing 20 μM CCCP. From kinetic traces as in (a), absorbance changes induced by the flash (0.5 ms after the flash, ●) and subsequent changes (from 0.5 ms to 5 ms after the flash, ○) were plotted against wavelength in (b).

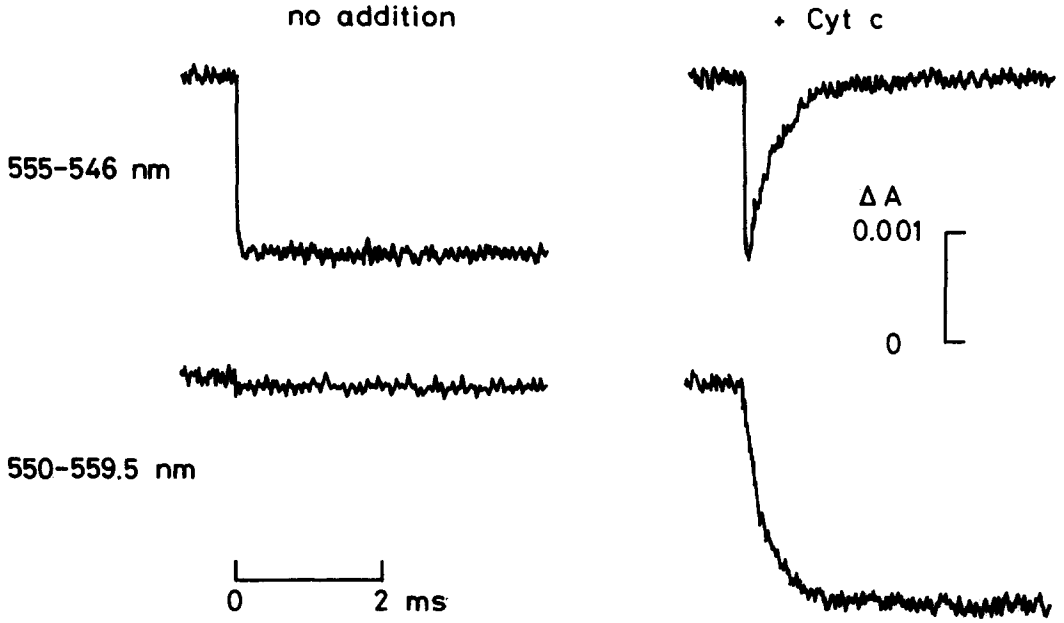

Fig. 6. Flash-induced absorbance changes of cytochrome *c*-555 in isolated membranes of *Rps. acidophila* and the effect of externally added mammalian cytochrome *c*. The membranes were suspended to 10 μM BChl in 20 mM KCl/1 mM Mops-Na/1 mM sodium ascorbate (pH 7.0), containing 10 μM DAD and 10 μM CCCP. 16 μM horse heart cytochrome *c* were present in the suspension for the right traces.

with a reference at 546 nm (Fig. 5a, top) up to 5 ms after the flash. This kinetic correspondence also suggests the secondary electron transfer between two *c*-type cytochromes. Cytochrome c_2 is a possible component of the secondary cytochrome that was oxidized by the flash-oxidized cytochrome *c*-555. The apparent half time of the oxidation was about 1.5 ms.

In the membrane preparation suspended in a medium with sodium ascorbate and DAD, the cytochrome peaked at 555 nm were also oxidized rapidly (Fig. 6, top left). No secondary electron transfer between cytochromes were observed in the membrane preparation itself. When horse heart cytochrome *c* was added to the suspension, cytochrome *c*-555 was re-reduced almost completely after the rapid oxidation (Fig. 6, top right). The re-reduction was accompanied by the oxidation of cytochrome *c* added (Fig. 6, bottom right cf. bottom left). The electron transfer was completed within 2 ms under the conditions of low ionic strength. By comparing the extent of the absorbance change of cytochrome *c*-555 with that of mammalian cytochrome *c* (Fig. 6), the extinction coefficient of cytochrome *c*-555 (reduced-minus-oxidized) was estimated to be 15.3 $mM^{-1} \cdot cm^{-1}$ at 555–540 nm, using 20.4 $mM^{-1} \cdot cm^{-1}$ at 550–540 nm for mammalian cytochrome *c* [12].

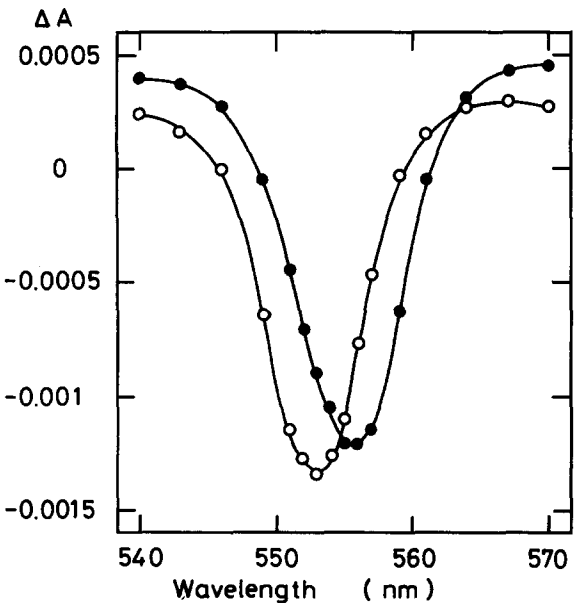

Fig. 7. Spectra of the flash-induced changes in isolated membranes of *Rps. acidophila* at ambient redox potentials of 280 mV (●) and 40 mV (○). The membranes were suspended anaerobically to 10 μM BChl in 100 mM KCl and 5 mM Mops-Na (pH 7.0), containing 10 μM CCCP/1 μM vlainomycin. Redox mediators used were 20 μM DAD/100 μM Fe-EDTA (1 mM)/5 μM PMS/10 μM HNQ. Absorbance changes taken at 5 ms after the flash were plotted against wavelength.

Fig. 3 (closed circles) shows the KCl-concentration dependence of the electron-transfer rate from cytochrome *c* to cytochrome *c*-555 in the membrane. The dependence is similar to that of the electron transfer from cytochrome *c* to $(BChl)_2^+$ in the membranes of *Rps palustris* (open circles), probably reflecting a similarity of electrostatic properties of the membranes around the reaction centers between the two species. The oxidation of cytochrome *c*-555 was not affected by the high concentrations of KCl (data not shown).

When ambient redox potential was decreased, another cytochrome in the membranes was oxidized by a flash, as observed in *Chr. vinosum* and *Rps viridis* [1]. Fig. 7 shows flash-induced spectral changes at two different ambient redox potentials. At 280 mV (closed circles), the oxidized cytochrome was *c*-555. At 40 mV, on the other hand, the spectrum (open circles) shows a peak at 553 nm. This component is probably cytochrome *c*-553 which is analogous to the low-potential cytochromes in the reaction center complexes of *Chr. vinosum* and *Rps. viridis*. E_h dependences of the flash-induced absorbance changes are shown in Fig. 8. The two wavelengths, 556 nm and 552 nm, were chosen for the predominant monitoring of cytochromes *c*-555 and *c*-553, respectively. As shown in the flash-induced spectra (Fig. 7), the change at 280 mV is due to cytochrome *c*-555 and that at 40 mV is due to cytochrome *c*-553. At E_h values around 500 mV, no oxidation of cytochromes was induced by a flash. With the apparent midpoint potential at 385 mV, the photo-oxidation of cytochrome *c*-555 became detectable with decreasing the ambient redox potential. The flash-oxidized component was changed from cytochrome *c*-555 to *c*-553 with an apparent midpoint potential at 130 mV. Cytochrome oxidation ceased at low potential following a curve with a midpoint potential of −45 mV probably due to the reduction of Q_A prior to activation. If the numbers of hemes of cytochromes *c*-555 and *c*-553 per reaction center are the same as those in *Chr. vinosum*

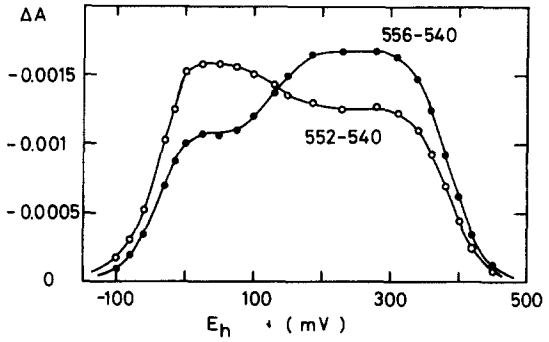

Fig. 8. Redox titration of flash-induced oxidation of cytochromes in isolated membranes of *Rps. acidophila.* Difference absorbance changes of the wavelength pair indicated were taken at 5 ms after the flash. Conditions were the same as those in Fig. 7.

or *Rps. viridis,* i.e., two hemes of cytochrome *c*-555 and two hemes of cytochrome *c*-553 per reaction center, the apparent midpoint potential of each cytochrome measured by the single-flash-induced oxidation is 23 mV higher than the real one of the cytochrome (see Ref. 34). If that is the case, the midpoint potentials of cytochrome *c*-555 and *c*-553 are estimated to be 362 mV and 107 mV, respectively. Fig. 9 shows the redox titration of cytochromes in the dark in the membrane preparation. Major cytochromes in the preparation were cytochromes *c*-555 and *c*-553 with almost the equal content. Midpoint potentials of these cytochromes were 355 mV and 115 mV, respectively, in the

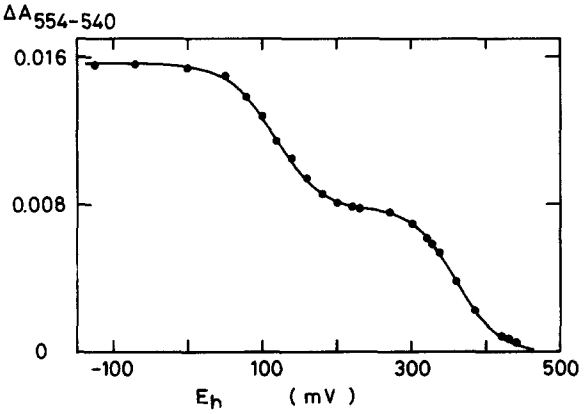

Fig. 9. Redox titration of cytochromes in the dark in isolated membranes of *Rps. acidophila.* The membranes were suspended anaerobically to 20 μM BChl in 100 mM KCl and 5 mM Mops-Na (pH 7.0), containing 10 μM CCCP. Redox mediators used were 50 μM DAD/100 μM Fe-EDTA (1 mM)/50 μM PMS/50 μM HNQ.

redox titration. Although it is possible that the observed values include small errors due to minor cytochrome components, the values are in good agreement with those obtained by the titration of flash-induced oxidation.

RC-B880 complex was also isolated from the membrane preparation of *Rps. acidophila.* Fig. 4b shows the absorption spectrum and the reduced-minus-oxidized spectrum of the complex. The absorption spectrum is similar to that of RC-B880 complex from *Rps. palustris* (Fig. 4a), but cytochromes were detected in the complex from *Rps. acidophila.* The difference spectrum shown in Fig. 4b (inset) has the α band maximum at 554 nm. Two cytochromes were distinguishable spectrophotometrically and potentiometrically; they were cytochromes *c*-555 and *c*-553 with midpoint potentials of 370 mV and 110 mV, respectively. Flash-induced oxidations of cytochromes *c*-555 and *c*-553 and electron transfer from mammalian cytochrome *c* to the flash-oxidized cytochrome *c*-555 were also observed in the isolated RC-B880 complex (data not shown). Content of cytochromes in the isolated complex was 1 per 6.7 BChl using the extinction coefficient of cytochrome *c*-555 estimated above. The amount of cytochrome *c*-555 oxidized by single flash in the isolated complex was 1 per 28.7 BChl. From these values, the number of cytochrome *c*-555 oxidized by the single flash was calculated to be 1 per 4.3 cytochromes in the complex. This suggests the presence of four hemes per reaction center in *Rps acidophila* as in *Chr. vinosum* and *Rps. viridis.*

Discussion

Contrary to the suggestions in the literature [1,2,25,26], the results of the present work clearly show that the immediate electron donor to $(BChl)_2^+$ in *Rps. palustris* is not a cytochrome tightly bound to the reaction center complex, but a soluble cytochrome *c* (Figs. 2–4). Cytochrome c_2 in this species is probably the one oxidized by $(BCHl)_2^+$ in whole cells (Fig. 1) as *Rb. sphaeroides, Rps. capsulata* and *R. rubrum.*

In *Rps. palustris,* however, the membrane preparation lacked the immediate electron donor in contrast with those species. In *Rb. sphaeroides,* chromatophores in which cytochrome c_2 is trapped

can be prepared with the same procedure [12,15]. After a flash excitation, about 90% of $(BChl)_2^+$ is re-reduced by the internal cytochrome. In *Rps. palustris,* on the other hand, most of the photo-oxidized $(BChl)_2$ was not re-reduced by internal components but rapidly reduced by cytochrome *c* added externally. This finding indicates that the periplasmic side of the reaction center is exposed to the bulk aqueous phase in the membrane preparation of *Rps. palustris.* The preparation is considered to consist of membrane fragments, sheets or right-side-out vesicles but not chromatophores, because chromatophores are inverted vesicles in which the periplasmic side of the membrane faces inside [15]. Carotenoid shifts which indicate light-induced membrane-potential changes [35] were measured in intact cells and membrane preparations to examine whether the membrane preparation was made of sealed vesicles or not. Only a small extent of uncoupler sensitive carotenoid shift (about one-seventh of that in whole cells, data not shown) was observed in the membrane preparation, which indicates that the membrane preparation is mostly composed of open fragments or sheets. In *Rps. palustris,* the structure of intracytoplasmic membrane is a lamellar type, but in *Rb. sphaeroides, Rps. capsulata* and *R. rubrum,* intracytoplamsic membranes have vesicular structure [23]. This difference in membrane structure in whole cells probably resulted in the difference in membrane preparations. *Rps. palustris* is the first species which has shown to have both the lamellar-type membrane and cytochrome c_2 as the immediate electron donor to $(BChl)_2^+$.

Rps. acidophila, on the other hand, has cytochromes tightly associated to the reaction center complex (Figs. 4–9), in spite of the similarity to *Rps. palustris* in some characteristics, i.e., the membrane structure of lamellar type [23], the multiplication by budding [22] and the length of isoprenoid side chain of quinones [24]. The bound cytochromes are similar to those of *Chr. vinosum* and *Rps. viridis.* Presumably four hemes are connected to a reaction center. Two of them are cytochrome *c*-555 with an $E_{m\,7}$ value about 360 mV and the other two are cytochrome *c*-553 with an $E_{m\,7}$ value about 110 mV.

The structure of the membrane preparation of *Rps. acidophila* seems similar to that of *Rps. palustris.* The measurements of flash-induced carotenoid shifts in whole cells and in the membrane preparation have indicated that the preparation are mostly composed of non-vesicular fragments or sheets. The rapid electron transfer from mammalian cytochrome *c* to cytochrome *c*-555 also suggests that the periplasmic side of the reaction center complex is exposed to the bulk aqueous phase and does not face to the inside of closed vesicles unlike the case of chromatophores from *Chr. vinosum* [17]. Shill and Wood [19] have shown that isolated membranes from *Rps. viridis* are not the form of sealed vesicles. This property of the isolated membranes seems a general characteristic in photosynthetic bacteria with lamellar-type membranes.

The role of soluble cytochrome *c* as the electron donor to the photo-oxidized membrane-bound cytochrome *c* has been shown in the whole cells of *Chr. vinosum* [17] and in the membrane preparation of *Rps. viridis* [19]. The present results indicate that a similar electron transfer also takes place in *Rps. acidophila,* in both the whole cells (Fig. 5) and the membrane preparation (Fig. 6). Cytochrome c_2 is probably the one oxidized by cytochrome *c*-555 in whole cells. The rate of the electron transfer from mammalian cytochrome *c* to cytochrome *c*-555 in the membrane preparation is as fast as that from cytochrome *c* to $(BChl)_2^+$ in the membrane preparation of *Rps. palustris.* The salt concentration dependences of the rates of both reactions are also similar to each other (Fig. 3). These similarities suggest that the rate of electron transfer from cytochrome *c* to cytochrome *c*-555 or $(BChl)_2^+$ depends mainly on electrostatic properties of cytochrome *c* and membranes, but not on the difference in electron acceptors. The slow reaction rate at high salt concentration indicates that net charges around the reaction sites of the soluble cytochrome and the membrane components are opposite; probably positive on cytochrome *c* and negative on the membrane [29].

According to Dickerson [20], cytochrome c_2 in purple photosynthetic bacteria can be classified into 3 classes. *Rps. palustris* has a long class cytochrome c_2 as *Rb. sphaeroides, Rps. capsulata* and *R. rubrum.* All the species in this group, examined so far, have the reaction centers without tightly bound cytochromes. *Rps. acidophila* has a

middle class cytochrome c_2 and the reaction center with bound cytochromes c as does *Rps. viridis*. *Rhodomicrobium vannielii,* another species with a middle class cytochrome c_2, has cytochrome c-553 tightly bound to the reaction center complex [9]. It is possible that the class of cytochrome c_2 has been changed in parallel with the type of the reaction center in the course of evolution. Dickerson [20] suggested that the long class cytochrome c_2 was derived from the middle class cytochrome c_2 by polypeptide insertions. The reaction center without tightly bound cytochromes c may also have arisen later from the *Rps. viridis*-type reaction centers by the loss of cytochromes. The molecular structure of the reaction center of *Rps. viridis* [4] seems to be consistent with this hypothesis, because the cytochrome subunit with four hemes is supposed to be located additionally at the periplasmic side of the reaction center complex. In a proposed phylogenetic tree, Dickerson [20] suggested that *Rps. palustris* is closer to *Rb. sphaeroides* than to *Rps. viridis* or *Rps. acidophila.* Imhoff et al. [22], on the other hand, placed *Rps. palustris* in the same group as *Rps. viridis* and *Rps. acidophila*. The present results of the type of reaction centers seem consistent with Dickerson's view. A phylogenetic tree based on 16S r-RNA sequence [21] is in disagreement with the present results, unless the bound cytochrome c has lost in both branches of *Rb. sphaeroides* and *Rps. palustris* independently or unless it has restored in the branch of *Rps. viridis* during the evolution of purple photosynthetic bacteria.

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