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Direct and indirect electron transfer from cytochromes *c* and c_2 to the photosynthetic reaction center in pigment-protein complexes isolated from *Rhodocyclus gelatinosus*

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Electron transfer from soluble cytochromes *c* and c_2 to the photo-oxidized bacteriochlorophyll dimer was studied in photosynthetic reaction center (RC) complexes and RC-B870 complexes isolated from *Rhodocyclus gelatinosus*. In the RC-B870 complex which consists of 6 polypeptides including a bound cytochrome subunit, cytochrome *c* from horse heart or cytochrome c_2 from *R. gelatinosus* was oxidized by photo-oxidized bacteriochlorophyll dimer indirectly via cytochrome *c*-555 in the complex. In the RC complex which is composed of two polypeptides, the oxidized bacteriochlorophyll dimer was reduced directly by the soluble cytochromes. Although direct electron transfer from soluble cytochrome does not function in *R. gelatinosus* in vivo, it would replace indirect electron donation if the bound cytochrome were lost mutationally.

Bacterial photosynthesis; Reaction center; Cytochrome *c*; Electron transport; Bacteriochlorophyll; (*Rhodocyclus gelatinosus*)

1. INTRODUCTION

One of the functions of the photosynthetic electron-transfer system is to stabilize the charge separation that is driven by light energy. In purple photosynthetic bacteria, one contribution to this stabilization occurs when *c*-type cytochromes operate to transfer an electron to the photo-oxidized bacteriochlorophyll dimer in the reaction center (RC) [1-3]. Two kinds of cytochrome are known to serve as a reductant to the oxidized bacteriochlorophyll dimer. One is soluble cytochrome c_2 in *Rhodobacter sphaeroides, Rb. capsulatus, Rhodospirillum rubrum, Rhodopseudomonas palustris* and *Erythrobacter longus* [1-5]. The other is cytochrome *c* bound to the RC complex operative in many species of purple photosynthetic bacteria, such as *Rps. viridis, Rps. acidophila* and *Chromatium vinosum* [1-4]. In these species, soluble *c*-type cytochromes function as secondary electron donors by reducing the bound cytochromes [4,6,7]. These soluble cytochromes are members of the same family as the well-known mitochondrial cytochrome *c* [8].

Matsuura and Shimada [4] have recently suggested the possibility that species which do not contain the RC-bound cytochromes have evolved by mutational loss of the cytochrome subunit from those which do, since the distribution of species lacking in RC-bound cytochromes is restricted to one branch (or a few branches) of the phylogenetic tree of purple bacteria based on 16 S ribosomal RNA sequence [9-11]. This presents an interesting experimental question which has prompted us to examine the effect of the removal of the cytochrome subunit from the RC complex.

RC complexes isolated from species that possess the bound cytochromes usually retain the tightly bound cytochrome subunit [12]. An exception is *Rhodocyclus gelatinosus* in which the cytochrome subunit has been shown to be lost during the preparation of RCs [13-15]. Recently, we have

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isolated RC-B870 pigment-protein complexes from *R. gelatinosus* in which the cytochrome subunit was retained [15]. We have compared electron transfer from soluble *c*-type cytochromes to the RC-B870 complex and to the RC core complex in this species to examine the effect of removal of the bound cytochrome subunit on the electron transfer from soluble cytochromes.

2. MATERIALS AND METHODS

R. gelatinosus IL144 was grown anaerobically at 30°C in the light. RC-B870 pigment-protein complexes with and RC core complexes without bound cytochromes were prepared from membrane preparations by detergent solubilization and gel electrophoresis as described [15]. The RC-B870 complexes consist of 6 kinds of polypeptides, i.e. α- and β-subunits of B870 light-harvesting proteins and H, M, L and cytochrome subunits of the RC proteins. The cytochrome subunit contains two hemes for cytochrome *c*-555 (E_{m7} = 330 mV) and two hemes for cytochrome *c*-551 (E_{m7} = 90 mV). The RC core complexes consist of the L and M subunits of RC.

Cytochrome c_2 was prepared from the supernatant obtained by ultracentrifugation after French press disruption of cells. The fraction sedimenting between 50 and 100% saturation of ammonium sulfate was dissolved in 10 mM Mops-NaOH (pH 7.0) and applied on a Sephadex G-75 gel filtration column. The brown fraction was collected and adsorbed on a CM-Sephadex C-50 column equilibrated with Mops buffer, washed with 75 mM NaCl in Mops buffer and eluted with 100 mM NaCl in the buffer. After sedimentation at 100% saturation of ammonium sulfate, proteins were redissolved in a small volume of 10 mM Mops-NaOH (pH 7.0) and dialyzed vs 1 mM Mops-NaOH (pH 7.0). Horse heart cytochrome *c* was purchased from Sigma (type VI).

The absorbance changes of the cytochromes initiated by using a xenon flash were recorded on a single-beam spectrophotometer as in [4].

3. RESULTS

Fig.1 demonstrates the flash-induced absorbance changes in the α-band region of cytochromes in RC-B870 complexes (dashed lines) and the effect of addition (continuous traces) of horse heart cytochrome *c* or cytochrome c_2 of *R. gelatinosus*. Two measuring wavelengths were chosen in order to resolve changes in bound cytochrome *c*-555 and soluble cytochromes (absorbance maxima for cytochrome *c* and c_2 at 550 and 551 nm, respectively), both of which had been reduced by ascorbate. In the absence of soluble cytochromes (dashed lines), the flash-activated oxidation of cytochrome *c*-555 measured at 557 nm (spectrum shown in fig.2,

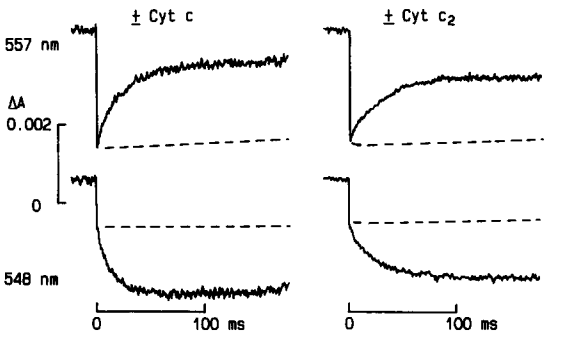

Fig.1. Effects of cytochrome *c* or c_2 on flash-induced absorbance changes in isolated RC-B870 complex from *R. gelatinosus*. RC-B870 complex was dissolved to A_{870} = 1.0 in 1 mM Mops-NaOH (pH 7.0) containing 0.5 mM sodium ascorbate and 10 μM each of ubiquinone-6, vitamin K-1 and diaminodurene (DAD). (– – –) Measurements before addition of soluble cytochromes. 8 μM horse heart cytochrome *c* (left) and 2 μM cytochrome c_2 from *R. gelatinosus* (right) were present for measurements shown by the continuous traces.

dashed line) took place at a rate which was not resolved on the time scale shown; also, the re-reduction was not observed during the period shown. However, when mammalian cytochrome *c* was present in the suspension of RC-B870 complexes (fig.1, left, continuous traces), additional, time-resolved absorbance changes were observed after the rapid oxidation of cytochrome *c*-555. The slow increase at 557 nm is caused by re-reduction of cytochrome *c*-555, the slow decrease at 548 nm resulting from oxidation of soluble cytochrome *c* as shown in the difference spectra (fig.2) taken at 0.5 (dashed line) and 400 ms (unbroken line) after flash excitation. Similar kinetics were observed when cytochrome c_2, from *R. gelatinosus* was used (fig.1, right). The kinetics of oxidation at 548 nm and of reduction at 557 nm were in good agreement. These slow changes indicate that the secondary electron transfer occurred from soluble cytochromes to the cytochrome *c*-555 bound to the RC-B870 complex. The second-order rate constants were calculated to be 8×10^6 and 3×10^7 $M^{-1} \cdot s^{-1}$ for mammalian cytochrome *c* and cytochrome c_2 of *R. gelatinosus*, respectively.

RC core complexes devoid of the cytochrome subunit, comprising the 2 polypeptides, were used for the experiments illustrated in figs 3 and 4. As expected, no changes due to cytochromes (at 550–542 nm) were observed in the absence of added soluble cytochrome *c* (fig.3, dashed lines). The

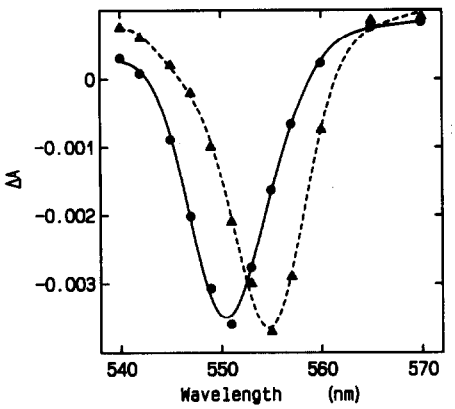

Fig.2. Spectra of flash-induced changes in isolated RC-B870 complex in the presence of cytochrome *c*. Absorbance changes taken at 1 ms (▲) and 100 ms (●) after the flash were plotted vs wavelength from measurements similar to those in fig.1 (left).

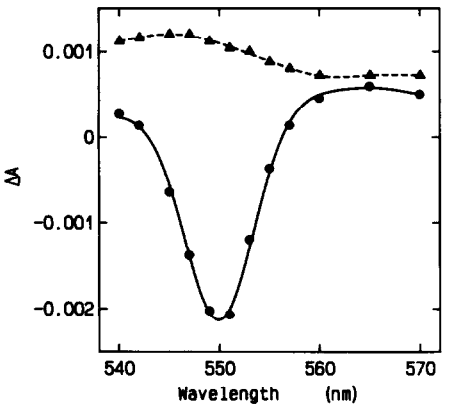

Fig.4. Spectra of flash-induced changes in isolated RC core complex in the presence of cytochrome *c*. Absorbance changes recorded 0.1 ms (▲) and 10 ms (●) after the flash were plotted vs wavelength for measurements similar to those in fig.3 (left).

absorbance increase at 542 nm is due to formation of the oxidized bacteriochlorophyll dimer. After the addition of soluble cytochrome *c* or c_2 in the reduced form (fig.3, continuous traces) electron transfer from the cytochrome to the photo-oxidized bacteriochlorophyll dimer was observed, as demonstrated by reduction of the dimer (absorbance decrease at 542 nm) and the corresponding oxidation of the cytochrome (decrease in the difference absorbance, 550–542 nm). The spectra before and after electron transfer are shown in fig.4, confirming the oxidation of added cytochrome *c*. The second-order rate constants were 1×10^8 and 4×10^7 $M^{-1} \cdot s^{-1}$ for electron transfer from cytochrome *c* to RC and from cytochrome c_2 to RC, respectively.

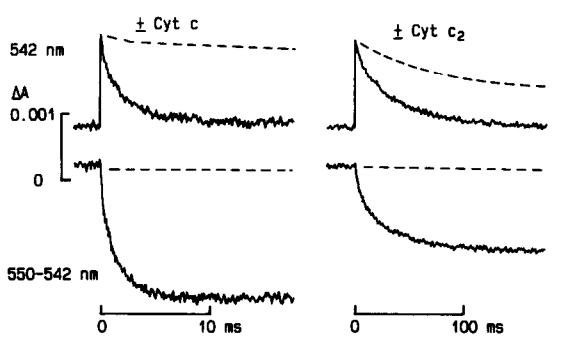

Fig.3. Effects of cytochrome *c* or c_2 on flash-induced absorbance changes in isolated RC core complex from *R. gelatinosus*. RC core complex was dissolved to $A_{800} = 0.12$ in buffer as described for fig.1. (– – –) Measurements before the addition of soluble cytochromes. 8 μM horse heart cytochrome *c* (left) and 2 μM cytochrome c_2 from *R. gelatinosus* (right) were present for measurements indicated by the unbroken lines.

Fig.5 shows the dependence of the oxidation rate of mammalian cytochrome *c* (A) and cytochrome c_2 from *R. gelaninosus* (B) on salt concentration by flash-oxidized cytochrome *c*-555 in RC-B870 complexes (▲) and by photo-oxidized bacteriochlorophyll dimer in RC core complexes (●). With increasing salt concentration, electron-transfer rates slowed in each case. This suggests that the electron-transfer reaction is influenced by the surface electrical charges on the reaction sites which have net charges of opposite sign [16,17]. Since the reaction site of the mammalian cytochrome *c* has positive charges [18], it is reasonable to postulate that the net charge is negative at the reaction sites on the RC-B870 complex and the RC core complex. The slope of the salt dependence is steeper for the reaction with the mammalian cytochrome (fig.5A) compared to the bacterial cytochrome (fig.5B). This suggests that the effective net charge on cytochrome c_2 of *R. gelatinosus* is less than that on the mammalian cytochrome although the sign is the same (positive) [16–18]. On the other hand, the slope was similar when the RC-B870 complex or the RC core complex was used (cf. ▲ and ●). This suggests that the effective net charges on the reaction sites of RC-B870 complexes and of RC core complexes are similar.

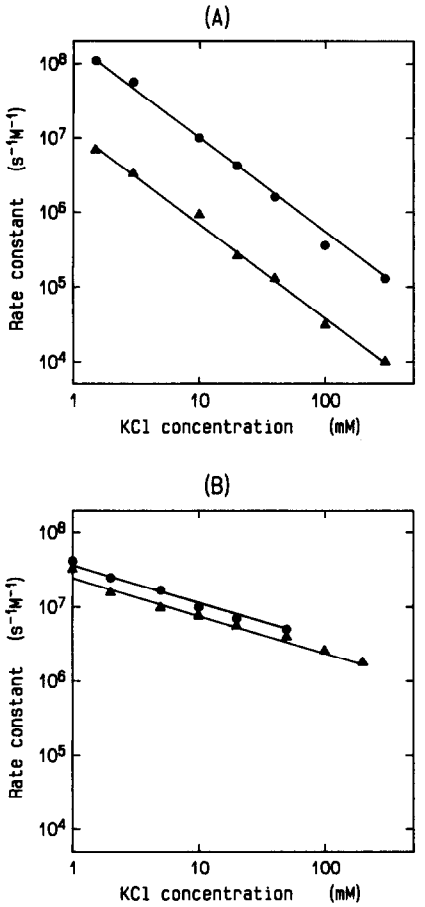

Fig.5. Effects of KCl concentration on the rate constant for oxidation of cytochrome *c* (A) and cytochrome c_2 (B) by flash-activated RC-B870 complex (▲) and RC core complex (●). Second-order rate constants obtained from measurements similar to those in figs 1,3 are plotted.

4. DISCUSSION

Two pathways of electron transfer from soluble cytochrome c_2 to the photo-oxidized bacteriochlorophyll dimer have been described in purple photosynthetic bacteria depending on the species [1–3]. One is the direct route, the other being indirect via other cytochrome *c* bound to the RC complex. *R. gelatinosus* contains the bound type of cytochrome but the interaction of the cytochrome subunit with the RC core complex is different from that of other purple bacteria [15]. RC core complexes isolated from the membranes of *R. gelatinosus* have no cytochromes [13–15] but whole cells, membrane preparations and RC-B870 complexes do have cytochromes bound to the photosynthetic RC [15]. Therefore, direct electron transfer from soluble cytochrome c_2 to the isolated RC core complex is probably not the physiological pathway but an artificial one. However, the rate of direct electron transfer to the oxidized bacteriochlorophyll dimer is as fast as that from the soluble cytochrome to the cytochrome *c*-555 bound to the RC-B870 complex. This suggests that the direct pathway from soluble cytochrome c_2 to the bacteriochlorophyll dimer would also be possible in vivo if the bound cytochrome subunit were lost by mutation.

The three-dimensional structure of an RC complex has been elucidated in the case of *Rps. viridis* [12]. In this structure, the bound cytochrome subunit is located at the periplasmic side of the membrane, attached to the L and M subunits without peptide chains penetrating into the hydrophobic region of the membrane bilayer. It seems reasonable to consider that, despite some interesting differences in properties, the location and function of the cytochrome subunit in *R. gelatinosus* are similar to those in *Rps. viridis*. Thus, if the results obtained with *R. gelatinosus* are general, then it can be suggested that for those species that contain the bound cytochrome *c*-RC structure, mutational loss of the bound cytochrome subunit from the RC would not result in serious damage to the chances of bacterial survival, since comparably efficient, direct electron transfer between cytochrome c_2 and the RC bacteriochlorophyll dimer is possible.

Rb. sphaeroides, Rb. capsulatus, Rs. rubrum and *Rps. palustris* possess RCs without tightly bound cytochromes [1–4]. We have suggested the possibility that the RCs of these species have evolved by the mutational loss of a bound cytochrome subunit [4]. The present results lend support to this hypothesis by showing that artificial removal of the bound cytochrome results in little change in the rate of electron transfer from cytochrome c_2 to the photo-oxidized bacteriochlorophyll dimer in RCs of *R. gelatinosus*.

What is the indispensable role of the bound cytochromes in the RC complexes in many species of purple photosynthetic bacteria? Although further studies are required to resolve this problem, we have shown that the bound cytochrome subunit is not necessary merely to enable electron transfer to

take place from cytochrome c_2 to the bacteriochlorophyll dimer in *R. gelatinosus*.

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