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Electron transfer between soluble cytochrome *c*-554 and purified reaction center from the green sulfur bacterium *Chlorobium tepidum*

M Itoh[1], D Seo[1], H Sakurai[1], P Sétif[2]

[1]*Department of Biology, School of Education and Division of Bioengineering, Graduate School of Science and Engineering, Waseda University, Nishiwaseda, Shinjuku, Tokyo, 169-8050, Japan. Fax, +81-3-3207-969.*
e-mail, sakurai@mn.waseda.ac.jp
[2]*CEA/DBCM SBE, Gif sur Yvette, 91191. France.*

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Introduction

Different cyt *c* serve as the immediate as well as the secondary electron donors to the oxidized primary donor in bacterial photochemical reaction centers (RCs). RC complexes of purple bacteria are very similar among different species both in amino acid sequences of the core polypeptides and in bound functional ligands, however, they show a rich variety of electron transfer pathways involving the primary donor and cyt *c* (Fig. 1). For example, the RC complex of *Rhodopseudomonas (Rp.) viridis* binds a tetraheme cyt *c*, while that of *Rhodobacter (Rb.) sphaeroides* does not. *Rb. sphaeroides* contains a soluble cyt *c* with *M*r of about 12 kDa (cyt c_2), which receives an electron from a cyt bc_1 complex and donates it directly to the primary donor. *Rp. viridis* also contains cyt c_2, which receives an electron from a cyt bc_1 complex and donates it indirectly to the primary donor via RC-bound tetraheme cyt *c*. The RC complex of *Rb. capsulatus* has no firmly bound cyt *c*, and the bacterium contains soluble cyt c_2 as well as a membrane-bound cyt *c* (cyt c_y). These two *c*-type cytochromes constitute alternative electron transfer pathways between the cyt bc_1 complex and the primary donor (Jenney et al. 1994).

The RCs of green sulfur bacteria (PS-C) belong to an Fe-S type RC like PSI, and the oxidized primary donor (P840) is reduced by RC-bound monoheme type cyt *c*–551 (Okkels et al. 1992). In addition to this, green sulfur bacteria contain a soluble cyt *c* (~10 kDa, α-peak maximum: 553-555 nm) presumed to donate an electron to PS-C (Meyer et al. 1968). Okumura et al. (1994) showed that a soluble cyt *c* is oxidized by membrane preparation from *Chlorobium tepidum*. It is not yet determined which of the two, the bound cyt *c*-551 and P840, is the direct electron acceptor of soluble cyt *c* (~10 kDa). Recently, Oh-oka et al. (1998) reported that RC bound cyt *c*-551 is directly reduced by a cyt bc_1 complex in a *C. tepidum* membrane preparation. In this paper, we characterize the soluble ~10 kDa cyt *c* and studied electron transfer kinetics between purified RC and purified soluble cyt *c* from *C. tepidum*.

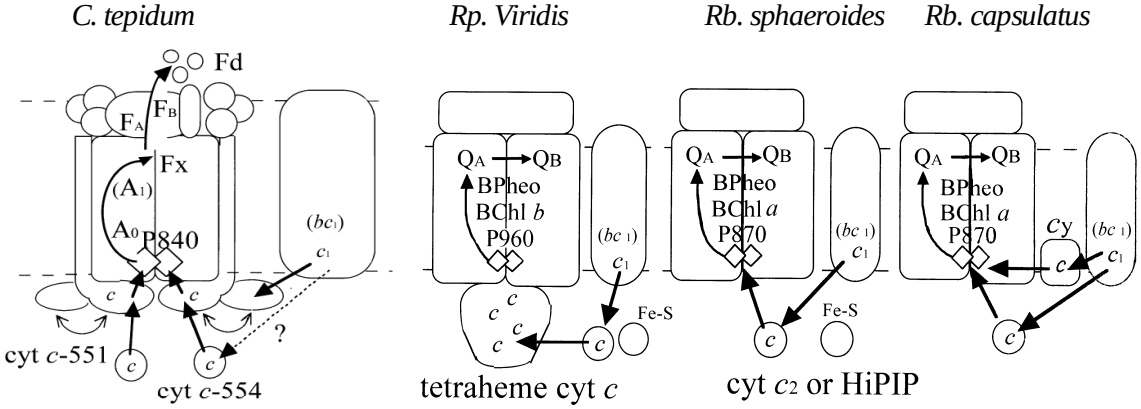

Fig. 1. Electron transfer pathways involving the special pair and cyt *c* in RCs of purple bacteria and *C. tepidum*. RC of *C. tepidum* binds two cyt *c*-551 per P840, and they function as direct electron donors to P840 in kinetically equivalent manner.

Materials and Methods

C. tepidum cells were cultured, harvested anaerobically, and disrupted by a French pressure cell (Seo et al. 2001). Soluble cyt *c* in the cell extract was purified as described by Itoh M. et al. (submitted to Photosynthesis Research): briefly, by ammonium sulfate fractionation and by DEAE-cellulose, gel-permeation, hydrophobic (Phenyl Superose) column chromatographies. Purification of PS-C and flash-induced absorbance change measurements were as described by Kusumoto et al. (1994 and 1999).

Results and Discussion

Properties of purified cyt c-554

The dithionite-reduced absorbance spectrum showed an α-peak maximum at 554 nm, and the cyt *c* will be referred to as cyt *c*-554. The α-band maximum of this cyt *c* from the same organism was previously reported to be either at 554 or at 553 nm. The shape of the α-peak is broad and asymmetric. This property of *C. tepidum* cyt *c* may be one of the causes of the slightly different α-peak reported by different researchers.

From redox titrations, the *E*h-dependence of the absorbance was fitted to an n = 1 Nernst's curve, and the $E_{m,7}$ was estimated to be +148 mV, a value close to the 135 mV reported by Selvaraj et al. (1998), but significantly more negative than the value of 195 mV reported by Okumura et al. (1994). Thus, cyt *c*-554 is very similar in $E_{m,7}$, in *M*r and in absorption spectrum to the majority of its counterparts found in green sulfur bacteria.

Function of cyt c-554

Effects of soluble cyt *c*-554 on the redox kinetics of photo-oxidized PS-C and of cyt *c*-551 were studied in the ms time range at 545 and 558 nm (Fig. 2A). In the absence of cyt *c*-554, A_{545} showed an initial rapid decrease upon flash excitation, which recovered slowly with a $t_{1/2}$ longer than 500 ms. The bleaching is ascribed to oxidation of cyt *c*-551 by $P840^+$ and the recovery phase to reduction of cyt *c*-551 by reductants (mainly ascorbate) present in the reaction mixture.

In the presence of soluble cyt *c*-554, the bleaching at 545 nm after the flash was followed by a faster recovery, the rate of which was accelerated by increasing the concentration of cyt *c*-554. At 558 nm, there were little absorbance changes in the absence of cyt *c*-554. In the presence of cyt *c*-554, A_{558} showed an initial decrease with kinetics similar to that of the faster recovery of A_{545}, followed by a very slow recovery. These data indicate that soluble cyt *c*-554 donates an electron to bound cyt *c*-551 and/or $P840^+$ as reported with a membrane preparation (Okumura et al. 1994).

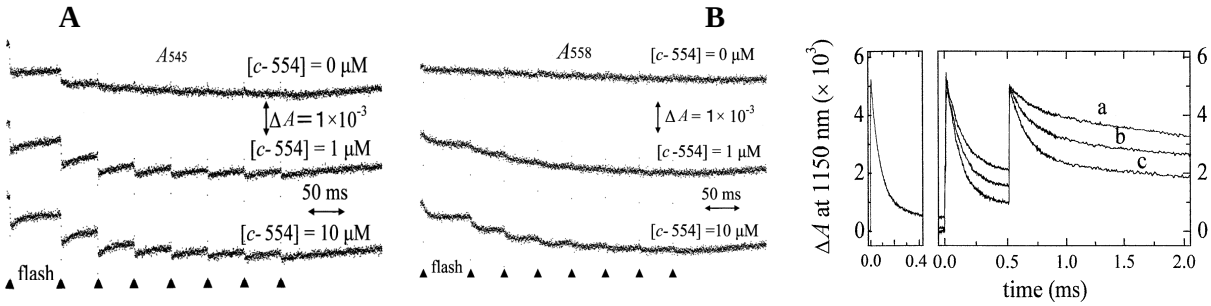

Fig. 2A. Multiple flash experiments monitored at 545 nm and at 558 nm. **B.** Decay kinetics of $P840^+$ at 1150 nm during a series of three flashes. Different kinetics observed in the presence of 3 μM cyt *c*-554 and variable delays between the first and the second flashes (0.5, 11 and 57 ms for curves **a**, **b**, **c**, respectively. The interval between the second and the third flash was 0.5 ms).

In order to determine whether cyt *c*-554 donates an electron to bound cyt $c\text{-}551^+$ or directly to $P840^+$, we analyzed ΔA_{1150} (oxidation of P840 gives rise to an increase in ΔA) obtained by multiple flash experiments in which the intervals between the first and the second flash was varied (Fig. 2B). From the redox equilibrium between P840 and bound cyt *c*-551, this RC preparation was found to contain about 1.8 cyt *c*-551/RC on the average (Kusumoto et al. 1999). At 1 ms after the first flash, + charge resides less than 0.1/RC in P840 and more than 0.9/RC in cyt *c*-551. Increasing the interval between the first and the second flash induced measurable effects on the reduction kinetics of $P840^+$ after the third flash, which indicates an efficient electron donation from cyt *c*-554 during the above intervals. At 1 ms after the third flash, calculations show that + charges reside about 0.8/RC in P840 and 1.7/RC in cyt *c*-551 (curve **a**), but the rate of the reduction of $P840^+$ (decrease of absorbance) was much slower than that predicted from direct reduction of $P840^+$ by cyt *c*-554.

The second-order rate constant for the electron donation from cyt *c*-554 to cyt *c*-551 was estimated to be $1.7 \times 10^7\ M^{-1}s^{-1}$. A fast reduction of the oxidized donors of PS-C by the cyt bc_1 complex with a $t_{1/2}$ = 150 μs ($K_{dl} = 4.6 \times 10^3\ s^{-1}$) is reported with a membrane preparation from *C. tepidum*. One can calculate the concentration of cyt *c*-554 that would give a similar rate assuming a second-order rate constant k_2 of $1.7 \times 10^7\ M^{-1}s^{-1}$ and with $k_{d1} = 0.94 \times k_2 \times$ [cyt *c*-554], which leads to [cyt *c*-554] = 0.29 mM. This is a high concentration, which may suggest that reduction of PS-C by cyt *c*-554 occurs only when electron transfer from cyt bc_1 complex to PS-C is limited. However, as cyt *c*-554 is assumed to be localized in the periplasmic space, it is conceivable that the *in vivo* concentration of cyt *c*-554 is high and that cyt *c*-554 plays an

important role in electron transfer to PS-C. The reaction center of *Rp. viridis* binds tetraheme cyt *c*, and periplasmic soluble cyt c_2 is generally admitted to be a physiological electron donor to the tetraheme cyt *c*, although Fe-S proteins (HiPIP) may function as an alternative donor. The rate constant between the tetraheme cyt *c* and cyt c_2 is reported to be 1.1×10^7 $M^{-1}s^{-1}$ (Ortega et al. 1999), a value comparable to that estimated above for the reaction between cyt *c*-551 and cyt *c*-554 in *C. tepidum*. Our results thus raise the question of whether cyt bc_1 complex or soluble cyt *c*-554 is the physiological donor to cyt *c*-551 bound to PS-C in *C. tepidum* (Fig.1). (For full discussions, see Itoh M. et al. (Photosynthesis Reaearch, submitted)).

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