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Evidence for the coupling reaction between *bc* complex and the P798 RC in *Heliobacterium gestii*

H Oh-oka[1], M Iwaki[2], S Itoh[3]

[1]*Department of Biology, Graduate School of Science, Osaka University, Osaka 560-0043, Japan. Fax: +81-6-6850-5425; E-mail: ohoka@bio.sci.osaka-u.ac.jp*

[2]*National Institute for Basic Biology, Okazaki 444-8585, Japan.*

[3]*Division of Material Science, Graduate School of Science, Nagoya University, Nagoya, 464-8602, Japan. Fax: +81-52-789-2883; E-mail: itoh@bio.phys.nagoya-u.ac.jp*

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Introduction

The reaction center (RC) complexes can be divided into two major groups based on the difference in the terminal acceptor molecules and their structures, that is, quinone type (type 2) and iron-sulfur type (type 1), respectively (Blankenship 1992). The RCs in purple and green filamentous bacteria belong to type 2, and those in green sulfur bacteria and heliobacteria belong to type 1. Plants and cyanobacteria have both type-1 (photosytem I (PS I)) and -2 (PS II) RCs. In the type-1 RC of a green sulfur bacterium *Chlorobium tepidum*, we showed the tight coupling between the cytochrome *bc* complex and the P840 RC complex via the membrane-bound cytochrome c_z (Oh-oka et al. 1998). However, in heliobacteria, which also has the homodimeric type-1 RC, the coupling mechanism has not yet been clarified well (Kramer et al. 1997). Heliobacteria are strictly anaerobic gram-positive bacteria according to the 16S rRNA analysis and use the bacteriochlorophyll (BChl) *g* molecule, as the major pigment within its RC (Amesz 1995). Since heliobacteria have no outer membranes, there is no soluble cytochrome *c* which functions on the donor side of the P798 RC. In this study, we analyzed the reaction mechanism on the donor side using the whole cells and membranes of *Heliobacterium gestii*.

Materials and methods

Cultures of *H. gestii* were kindly provided by Dr. M. T. Madigan (Southern Illinois University, Carbondale). Cells were grown anaerobically in PYE medium in a 1-L bottle under continuous illumination by tungsten lamps (Madigan 1992). In order to avoid accumulation of appreciable amounts of lysed cells in the late logarithmic growth phase, cultivation was performed at 39 ^{o}C for 20-22 h using 1% inocula. The preparation of membranes as well as the kinetic measurements were carried out under anaerobic conditions. The cells were harvested by centrifugation at 12,000 x g for 10 min, suspended in 7-8 ml buffer A [20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 2 mM DTT] and disrupted by passage through a French pressure cell three times at 20,000 psi. After removal of the cell debris by centrifugation at 12,000 x g for 10 min, the membranes were collected by the following ultracentrifugation at 110,000 x g for 1 h. The pelleted membranes were suspended again in buffer A. Flash-induced absorption changes at selected wavelengths in the μs-ms time range

were measured with a home-built split-beam spectrometer equipped with a combination of two monochrometers, a mechanical shutter and a 100 W tungsten-iodine lamp as previously described (Iwaki et al. 1999). Samples for the SDS-PAGE followed by heme staining were treated as previously described (Oh-oka et al. 1998).

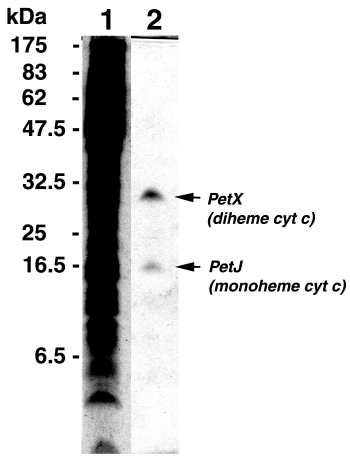

Fig.1. SDS-PAGE analysis of membrane-bound *c*-type cytochromes stained with CBB (lane 1) and TMBZ (lane 2)

Results

Two TMBZ-stained bands with apparent molecular masses of 17 and 30 kDa were detected after the SDS-PAGE analysis followed by the heme staining (Fig. 1). They seem to correspond to the *c*-type cytochromes encoded by the *petJ* and *petX* genes, respectively, based on their masses. The PetJ protein is reported to be a monoheme-type cytochrome whose amino-terminal cysteine residue is modified with fatty acid chains and would serve as the direct electron donor to P798 (Albert et al. 1998). The PetX protein is a diheme-type cytochrome which is considered to be functional as a subunit within the cytochrome *bc* complex (Xiong et al. 1998).

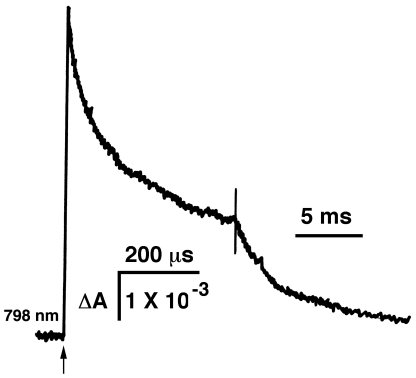

Fig.2. Flash-induced absorption change of P798 in whole cells

In the whole cells, the flash-oxidized $P798^+$ was rereduced with half times ($t_{1/2}$s) of 10 μs, 300 μs and 4ms in relative amplitudes 40, 35, 25%, respectively (Fig. 2). The fast two phases were almost parallel with the absorption changes of cytochrome *c* (data not shown) and attributable to the electron transfer from cytochrome *c* to $P798^+$. The 5-ms phase seems to be ascribable to the back reaction from the terminal acceptor (FeS center) to $P798^+$. However, the fast electron transfer rates from cytochrome *c* to P798 decreased dramatically upon disruption of cells. This has made the kinetic studies in heliobacteria difficult for more than a decade (Nitschke et al. 1995) despite of the first report in 1985 (Fuller et al.) that showed the photooxidation of a membrane-bond *c*-type cytochrome.

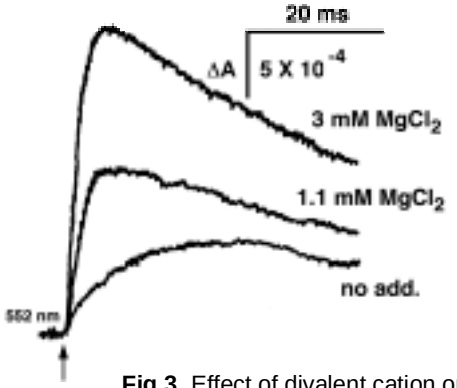

Fig.3. Effect of divalent cation on the kinetics of cytochrome *c* oxidation in membranes

We found that the addition of a divalent salt, $MgCl_2$, to the suspension of isolated

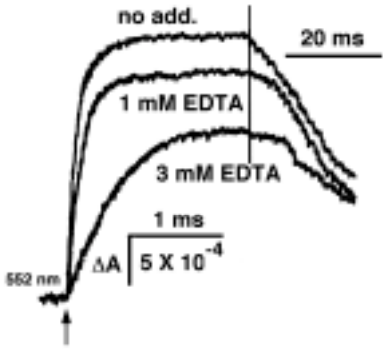

Fig.4. Effect of EDTA on the kinetics of cytochrome *c* oxidation in whole cells

membranes recovered its slowed rate of cytochrome oxidation ($t_{1/2}$ = 3 ms) as shown in Fig. 3. The amplitude of cytochrome oxidation gradually increased with the addition of $MgCl_2$ and reached the maximum at a final concentration of 3 mM. Its kinetic behavior did not changed afterwards. A monovalent salt, NaCl, had almost no effect on the acceleration of the electron donation rate from cytochrome *c* to P798 (data not shown).

Consistent with the observed requirement of Mg^{2+} in the isolated membranes, the oxidation rate of cytochrome *c* decreased with the addition of a chelator reagent, EDTA, for a divalent cations to the reaction mixture of whole cells (Fig. 4). These results indicate the involvement of an electrostatic interaction between the membrane-bound cytochrome *c* and the P798 RC. The rate also showed high temperature dependency exhibiting the apparent activation energy of 88.2 kJ/mol in whole cells and 58.9 kJ/mol in isolated membranes, respectively (data not shown).

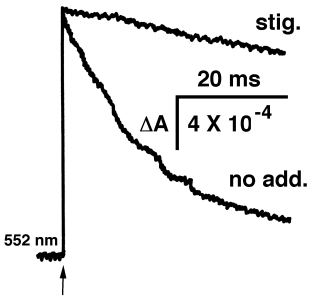

Fig.5. Effect of stigmatellin on the kinetics behaviors of cytochrome *c* oxidation in whole cells

Upon the addition of stigmatellin, the rereduction of the flash-oxidized cytochrome *c* was almost completely suppressed in the whole cells (Fig. 5). A similar result was obtained using isolated membranes (data not shown). Since stigmatellin inhibits quinol oxidation at the Q_o-site in the cytochrome *bc* complex, this clearly indicates an electron transfer from the cytochrome *bc* complex to the P798 RC with no participation of soluble electron carrier(s).

Discussion

We here demonstrated that the membrane-bound cytochrome *c* (PetJ) mediates the electron transfer from the cytochrome *bc* complex to the P798 RC with no involvement of water-soluble electron carrier proteins. This is similar to the case in the green sulfur bacteria, *Chlorobium*, as previously reported (Oh-oka et al. 1998). The membrane-bound cytochrome c_y in *R. capsulatus* has also been proved to mediate the electron transfer between cytochrome bc_1 and the RC complexes (Jenney et al. 1994). Although there are no meaningful similarities among the amino acid sequences of these three cytochromes, all of them are anchored to membranes through their N-terminal hydrophobic moieties (see Figure 6) and carry out analogous functions. The membrane-anchored cytochromes, which can move only in the laterally limited area, seem to be effective on shuttling electrons between closely adjacent complexes or within the so-called supercomplex. Such machinery might be a prototype for the development of more flexible soluble cytochromes which function by searching for high affinity sites between long-distantly spaced complexes.

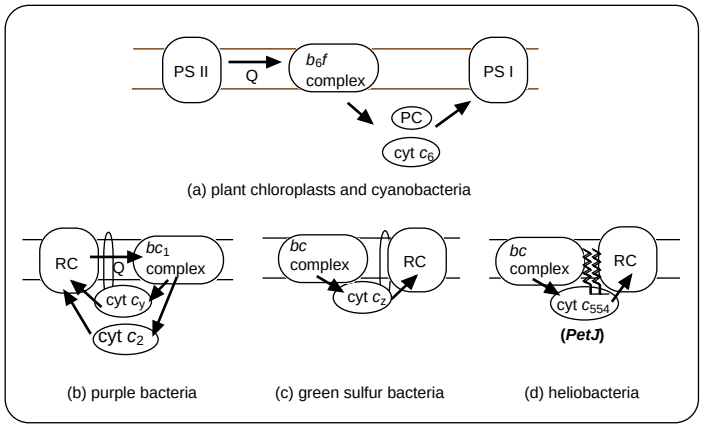

Fig. 6 Schematic representation of the coupling reactions Between cytochrome complexes and RCs

References

Albert I, Rutherford AW, Grav H, Kellermann J and Michel H (1998) *Biochemistry* **37**, 9001-9008

Amesz (1995) *In*: *Anoxygenic Photosynthetic Bacteria*, pp 687-697. Kluwer Academic Publishers, Dordrecht, The Netherlands

Blankenship (1992) Photosynthesis Research **33**, 91-111

Fuller RC, Sprague SG, Gest H and Blankenship RE (1985) *FEBS Letters* **182**, 345-349

Iwaki M, Itoh S, Kamei S, Matsubara H and Oh-oka H (1999) Plant and Cell Physiology **40**, 1021-1028

Jenney FE, Prince RC and Daldal F (1994) *Biochemistry* **33**, 2496-2502

Madigan MT (1992) *In*: *The Prokaryotes*, second edition pp 1981-1992. Springer-Verlag, New York

Kramer DM, Schoepp B, Liebl U and Nitschke W (1997) *Biochemistry* **36**, 4203-4211

Nitschke W, Liebl U, Matsuura K and Kramer DM (1995) *Biochemistry* **34**, 11831-11839

Oh-oka H, Iwaki M and Itoh S (1998) *Biochemistry* **37**, 12293-12300

Xiong J, Inoue K and Bauer CE (1998) *Proceedings of the National Academy of Sciences of the United States of America* **95**, 14851-14856