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Parallel electron donation pathways to cytochrome c_z in the type I homodimeric photosynthetic reaction center complex of *Chlorobium tepidum*

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ABSTRACT

We studied the regulation mechanism of electron donations from menaquinol:cytochrome *c* oxidoreductase and cytochrome *c*-554 to the type I homodimeric photosynthetic reaction center complex of the green sulfur bacterium *Chlorobium tepidum*. We measured flash-induced absorption changes of multiple cytochromes in the membranes prepared from a mutant devoid of cytochrome *c*-554 or in the reconstituted membranes by exogenously adding cytochrome *c*-555 purified from *Chlorobium limicola*. The results indicated that the photo-oxidized cytochrome c_z bound to the reaction center was rereduced rapidly by cytochrome *c*-555 as well as by the menaquinol:cytochrome *c* oxidoreductase and that cytochrome *c*-555 did not function as a shuttle-like electron carrier between the menaquinol:cytochrome *c* oxidoreductase and cytochrome c_z. It was also shown that the rereduction rate of cytochrome c_z by cytochrome *c*-555 was as high as that by the menaquinol:cytochrome *c* oxidoreductase. The two electron-transfer pathways linked to sulfur metabolisms seem to function independently to donate electrons to the reaction center.

1. Introduction

Green sulfur bacteria are strictly anaerobic photoautotrophs that have homodimeric type I reaction center (RC) complex, as do heliobacteria [1,2], and utilize inorganic sulfur compounds (sulfide, thiosulfate, and/or sulfur) as the electron sources for photosynthetic CO_2 fixation [3]. The primary electron donor P840, a special pair of bacteriochlorophyll *a*, in the RC complex initiates the light-driven electron-transfer reaction as the first step in the conversion of light energy into chemical free energy. It is important for the photo-oxidized $P840^+$ to be rereduced rapidly to achieve highly efficient solar energy conversion.

In a thermophilic green sulfur bacterium, *Chlorobium tepidum*, $P840^+$ is rereduced by one of the RC subunits, a PscC subunit, which is also called as cytochrome (cyt) c_z [4,5]. It has been demonstrated that two molecules of cyt c_z are contained in the RC complex [6]. Cyt c_z has three membrane-spanning -helices in its N-terminus and a heme-containing moiety in its C-terminus [4,7]. The C-terminal domain protrudes into the periplasmic space and carry electrons directly from menaquinol:cyt *c* oxidoreductase to P840 [5]. The domain is supposed to be fluctuated as evidenced by the extraordinary dependence of its reaction rates on solvent viscosity [7]. This unique feature of cyt c_z appears to be similar to that of cyt c_y which serves as a shuttle to mediate electron transfer between cytochrome b/c_1 complex and the type II RC in *Rhodobacter* species of purple non-sulfur bacteria [8].

The oxidized cyt c_z^+ then accepts electrons from cyt *c*-554 as well as menaquinol:cyt *c* oxidoreductase [5,9]. Cyt *c*-554 is a soluble monoheme cytochrome with a molecular mass of approximately 10 kDa [10,11], which is named cyt *c*-555 after its α-absorption peak shift in the case of *Chlorobium limicola* [12]. Cyt *c*-554 has been shown to function as an immediate electron donor to cyt c_z^+ by an *in vitro* reconstitution study using purified RC complex from *C. tepidum* [9]. On the other hand, a study using membranes free from soluble cyt *c*-554, as confirmed by heme-staining analysis on SDS-PAGE, demonstrated a direct electron donation from the menaquinol oxidoreductase to cyt c_z [5].

An ascorbate-reduced absorption spectrum of another cyt, which exhibited an α-absorption peak at around 556 nm, was also detected in the membrane preparation [5]. Flash-induced absorption changes indeed revealed the presence of a shoulder at 556 nm in the different spectrum of membranes, which became more prominent by the addition of stigmatellin. Since the activity of the menaquinol:cyt *c* oxidoreductase was inhibited by antimycin A, the menaquinol:cyt *c* oxidoreductase in *C. tepidum* could be classified to the bc_1-type complex. We thus assumed that cyt *c*-556 played a role similar to that of a cyt c_1 subunit in the complex [5], although there has been a strong argument against our conclusion; the shoulder observed at around

Abbreviations: *C*, *Chlorobium*; cyt, cytochrome; RC, reaction center; SQR, sulfide-quinone reductase

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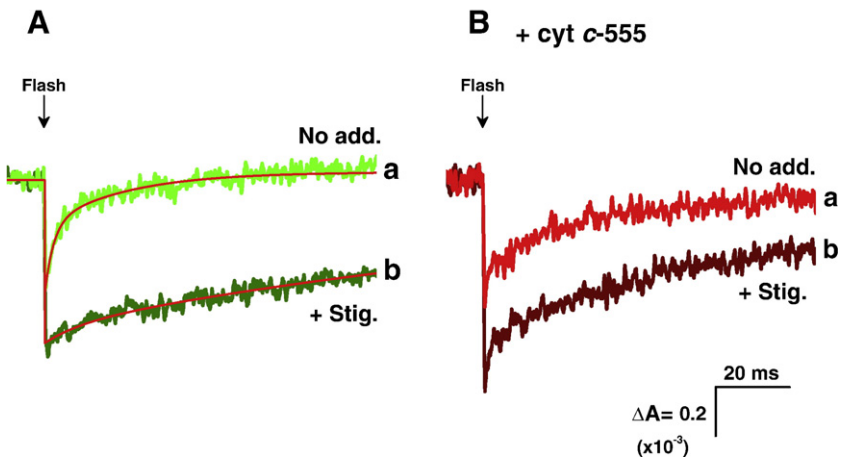

Fig. 1. Flash-induced absorption changes monitored at 552–540 nm (*c*-type cyts) in membranes isolated from Δcyt *c*-554 mutant cells of *C. tepidum* (A) without or (B) with externally added cyt *c*-555 purified from *C. limicola*. Traces a and b represent kinetics in the absence and presence of 20 μM stigmatellin, respectively. Measurements were done at 295 K. The concentrations of membranes and cyt *c*-555 were adjusted to be A_{810}=1.5 (approx. 0.3 μM P840) and 10 μM, respectively. Thin lines indicate the results of kinetic analyses by curve-fitting programs.

556 nm might be derived from the residual cyt *c*-554 that could not be removed completely from the membranes [1,9]. Indeed, the gene encoding probable cyt c_1 has yet been unidentified in the genome of green sulfur bacteria.

We have recently demonstrated that the electron transfer from menaquinol:cyt *c* oxidoreductase to cyt c_z occurred directly in the crude membrane extract prepared from a cyt *c*-554-deleted mutant of *C. tepidum* [13]. However, it still remains uncertain whether soluble cyt *c*-554 can mediate electron-transfer reaction *in vivo* between menaquinol:cyt *c* oxidoreductase and cyt c_z as in the case of purple non-sulfur bacteria, where cyt c_2 shuttles electrons between the bc_1-type ubiquinol oxidoreductase and the type II RC complex [14]. To address this issue, in the present study, we carried out the *in vitro* reconstitution experiments using membranes prepared from the cyt *c*-554-deleted mutant by exogenously adding cytochrome *c*-555 purified from *C. limicola*. The results indicated that cyt c_z accepted electrons from both menaquinol:cyt *c* oxidoreductase and cyt *c*-554/555 independently. The presence of cyt *c*-556 in membranes was also confirmed again as a cyt c_1-like subunit of the menaquinol:cyt *c* oxidoreductase.

2. Materials and methods

2.1. Isolation of photosynthetic membranes from the mutant cells lacking cyt c-554

A deletion mutant of cyt *c*-554 of *C. tepidum* was constructed in our previous study and attained to a full growth in a CL medium containing both thiosulfate and sulfide as electron donors [13]. The photosynthetic membranes of the mutant were prepared according to the procedure described previously [5] in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, USA).

2.2. Purification of CycA

Soluble cyt *c*-554 and *c*-555 were purified from the wild-type strain of *C. tepidum* and *C. limicola* basically according to the previous reports [5,9] with a few modifications described below. Harvested cells were disrupted by three-time passages through a French pressure cell at 20,000 psi (138 MPa). Cell debris was removed by centrifugation at 10,000 *g* for 15 min, and the supernatant was again centrifuged at 110,000 *g* for 1 h. The resultant supernatant was fractionated by ammonium sulfate (40–80% saturation). The precipitated fraction by 80% ammonium sulfate was suspended in a 20 mM Tris–

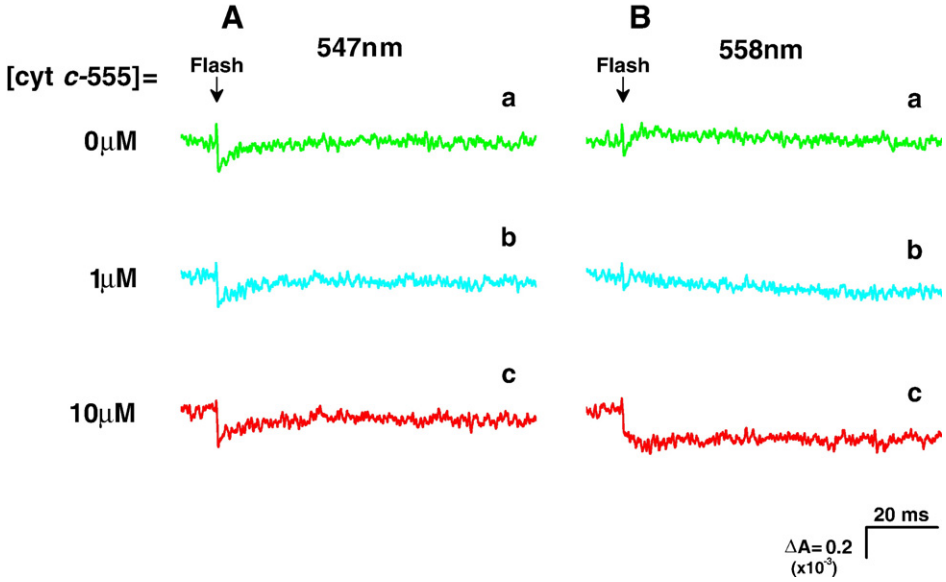

Fig. 2. Flash-induced absorption changes monitored at (A) 547–540 nm (cyt c_z) and (B) 558–540 nm (mainly cyt *c*-555) in membranes. Traces a, b, and c represent no addition and additions of 1 μM and 10 μM cyt *c*-555, respectively. Measurements were done at 295 K. The concentration of membranes was adjusted to be A_{810}=1.5 (approx. 0.3 μM P840).