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Electron transport from cytochrome b_6-f complexes to Photosystem I reaction center complexes in *Synechococcus* sp. Is cytochrome *c*-553 a mobile electron carrier?

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Numbers of the Photosystem I reaction center complexes and the cytochrome b_6-f complexes with which a cytochrome *c*-553 molecule can interact within the limiting time of photosynthetic electron transport were examined by measuring flash-induced absorption changes of P-700, cytochrome *c*-553 and cytochrome *f* in the thermophilic cyanobacterium *Synechococcus* sp. The addition of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) did not affect the common 2 ms half-time of P-700, cytochrome *c*-553 and cytochrome *f* reduction, which is ascribed to electron transfer from the plastoquinone pool. The inhibitor decreased, however, amounts of the three electron carriers which underwent the 2 ms reduction in the order of cytochrome *f*, cytochrome *c*-553 and P-700. On excitation with weak flashes which oxidized only a small fraction of cytochrome *c*-553 molecules present in cells, P-700 remained in the oxidized state after the flashes was reduced with electrons from the Rieske center or plastoquinone but not from cytochrome *c*-553. The ratios of cytochrome *c*-553 to cytochrome *f* oxidized at various flash intensities were constant and similar to the ratio of the two cytochromes present in cells. It is concluded that cytochrome *c*-553 cannot exchange electrons with large numbers of the Photosystem I reaction center complexes and the cytochrome b_6-f complexes in the limiting time, but has a mobility sufficient to mediate electron transfer between the two complexes, which are present at an unbalanced ratio in *Synechococcus* cells.

Introduction

Photosynthetic electron transport in higher plants, algae and cyanobacteria contains three integral membrane protein complexes, the reaction center complexes of PS I and PS II and the cytochrome b_6-f complex [1–3]. Plastocyanin and plastoquinone are considered to serve as mobile electron carriers in chloroplasts of higher plants, where the reaction center complexes of PS I and PS II are located in different membrane regions [4,5]. The in situ reaction kinetics of plastocyanin which connects the PS I complexes and the cytochrome b_6-f complexes is, however, rather difficult to determine spectrophotometrically due to its weak and broad absorption bands [6]. The mobile character of the copper protein has been deduced mainly from reduction kinetics of P-700 [7–13].

A soluble cytochrome *c* with the α-band maximum at 552–553 nm (cytochrome *c*-553) widely

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (dibromothymoquinone); DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem.

distributes among algae and cyanobacteria [14,15] and is considered to serve, instead of plastocyanin, as electron carrier between the PS I and the b_6-f complexes [16–18]. Cytochrome c-553 has an advantage over plastocyanin that it has sharp and intense absorption bands, and hence is more amenable to spectrophotometric investigation in vivo [6,19]. Recently, we have resolved photoresponses of P-700, cytochrome c-553 and cytochrome f in cells of the thermophilic cyanobacterium *Synechococcus* sp. [20]. The kinetics of the three electron carriers are consistent with the functioning of cytochrome c-553 between P-700 and cytochrome f [20].

In the present work, we focussed on the mobility of cytochrome c-553 as electron carrier in photosynthetic electron transport of *Synechococcus*. There is no evidence for the lateral heterogeneity in the distribution of the two photosystems in the cyanobacterial thylakoids which lack the grana structure. A notably unbalanced stoichiometry exists, however, among electron carriers and the reaction centers in photosynthetic electron transport of *Synechococcus* [21]. As illustrated schematically in Fig. 1, P-700 is present nearly twice in excess of cytochrome c-553, while the cytochrome f content is less than the cytochrome c-553 content. Thus cytochrome c-553

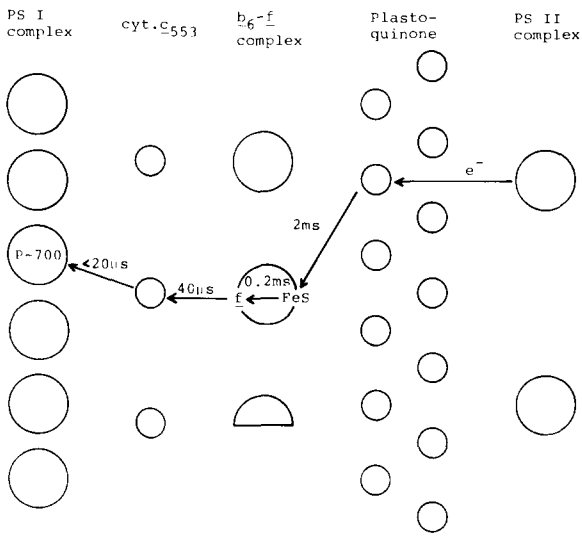

Fig. 1. Relative abundance of the PS I and PS II reaction center complexes, the cytochrome b_6-f complexes, cytochrome c-553 and plastoquinone in *Synechococcus*.

should have some mobility to distribute electrons from the cytochrome b_6-f complexes to all the PS I reaction center complexes present in the cells. To determine numbers of the two complexes with which a cytochrome c-553 molecule can interact within the limiting time of electron transport, flash-induced absorption changes of P-700, cytochrome c-553 and cytochrome f were analyzed under conditions where electron transport was limited by the addition of an electron transport inhibitor DBMIB, or by the use of low-intensity flashes.

Materials and Methods

The thermophilic cyanobacterium *Synechococcus* sp. was grown for 24 h at 55°C as described previously [22,23]. For spectrophotometric measurement, the cells were suspended in the fresh culture medium containing 25 mM Hepes-NaOH (pH 7.5) and 20 mM fructose to give a final chlorophyll a concentration of about 10 µg/ml. The suspension was kept under illumination with white light of 1000 lx at 22–24°C prior to measurement [20,24]. To the suspension, 5 µM gramicidin D, 50 mM KCl and 2 mM sodium ascorbate were added just before measurement. In the absence of ascorbate, DBMIB which is highly autooxidizable at 55°C, caused a slow oxidation of the plastoquinone pool [25,26].

Absorption changes were measured with a single beam spectrophotometer as described previously [20,26]. A xenon lamp was a source of the actinic flash with a half-peak height of 5 µs but a rather long tailing (about 80 µs). The flash was passed through a Toshiba VR-65 and a VR-66 filters. The photomultiplier was protected against the actinic light with two Corning 4-96 filters. Photoresponses of P-700, cytochrome c-553 and cytochrome f in the Soret band region were resolved by the computer subtraction as described previously [20]. The flashes were fired at 0.5–1 Hz and 200–250 signals were averaged. All measurements were carried out at 55°C. DBMIB was a gift from Dr. A. Trebst, University of Bochum, and Dr. M. Nishimura, Kyushu University.

Chlorophyll was determined as described by Mackinney [27].

Results

Effects of DBMIB

Fig. 2 shows effects of DBMIB on the reduction kinetics of flash-oxidized P-700 in *Synechococcus* cells. In the absence of the inhibitor, P-700 was reduced first rapidly and then slowly (A). When a line drawn in the center of noises was plotted on the semilogarithmic scale, three kinetical components were distinguished; approx. 40, 40 and 20% of P-700 oxidized were reduced with half times of less than 0.2 ms, 2.1 ms and 17 ms, respectively (C).

The reduction kinetics of P-700 were studied in detail previously [20]. P-700 is reduced showing three first-order phases with half times of about 40 μs, 0.2 ms and 2 ms, which, for the simplicity, are called the 40 μs, 0.2 ms and 2 ms phases, in spite of variations in the actual half times with cultures. The appearance of the three kinetic phases reflects the unique organization of the *Synechococcus* electron transport as illustrated in Fig. 1. During a single excitation with a flash having a rather long tailing (about 80 μs), P-700 is oxidized, on average, about 1.5 times because P-700 is present twice in excess of cytochrome *c*-553 and a part of oxidized P-700 is very rapidly rereduced by the cytochrome and undergoes second oxidation [20]. Since cytochrome *c*-553 is totally oxidized during the flash, P-700 remained in the oxidized state after the flash accepts electrons successively from cytochrome *f*, the Rieske iron-sulfur center and plastoquinone. The 40 μs reduction phase, which is ascribed to electrons provided from cytochrome *f*, is not seen in Fig. 2 because of the slow recording time used. We showed recently that the 0.2 ms phase represents P-700 reduction with electrons from the Rieske iron-sulfur center [26]. The 2 ms phase is considered to be due to electrons originated from plastoquinone. The 17 ms phase, which would reflect an incompletely reduced state of the plastoquinone pool, was significant in the particular sample used here but not in most of the cultures.

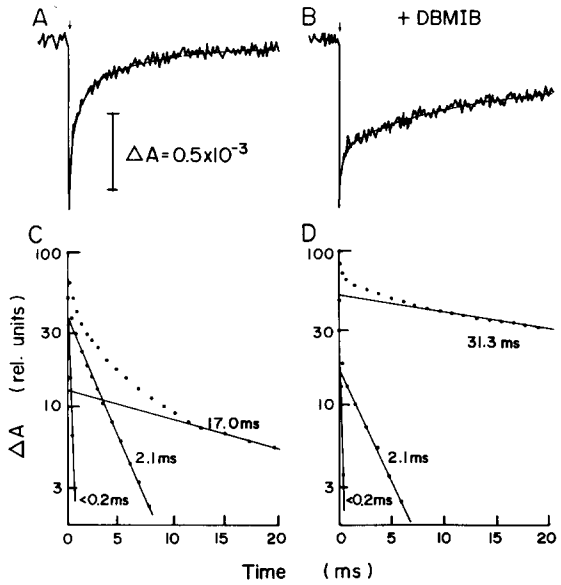

Fig. 2. Effects of DBMIB on the reduction kinetics of P-700. Cells were suspended in fresh growth medium containing 25 mM Hepes-NaOH (pH 7.5), 5 μM gramicidin D, 50 mM KCl and 2 mM sodium ascorbate. Flashes were fired at 0.5 Hz, and 200 signals were averaged. Downward arrows mark flash illuminations. (A) No addition; (B) 10 μM DBMIB was added; (C) and (D) are semilogarithmic plots of (A) and (B), respectively. Numbers indicate the decay half times.

As shown previously [20], DBMIB slowed down the P-700 reduction without affecting the 0.2 ms phase (B). Fig. 2D reveals, however, that DBMIB decreased only the magnitude of the 2 ms phase without affecting its half-time. A slow phase with a half-time of 31 ms appeared at the cost of the 2 ms phase so that the total signal size remained unchanged. DBMIB is considered to inhibit electron transport by interacting at a specific binding site of the b_6-*f* complexes [26,28–30]. The results are explained by assuming that electron transport from the Rieske center to P-700 occurs in separate and independent chains; a part of P-700 present in chains containing DBMIB-blocked b_6-*f* complexes is reduced slowly, while the other in the uninhibited chains accepts electrons from plastoquinone with the unaltered rate. Thus, cytochrome *c*-553 seems to be unable to visit large numbers of the PS I complexes and the b_6-*f* complexes to exchange electrons within the limiting time.

The effects of DBMIB observed here are just opposite to the effects of the inhibitor on the P-700 reduction kinetics in spinach chloroplasts [7]. DBMIB increases the half-time of P-700 reduction with electrons from plastoquinone without causing any biphasic kinetics, leading to the con-

clusion that there is a rapid electron exchange among a considerable number of chains between the inhibition site and P-700.

The effects of DBMIB on reduction kinetics of cytochrome *c*-553 is shown in Fig. 3. Cytochrome *c*-553 was reduced monophasically with a half-time of 1.4 ms in unpoisoned cells. The addition of DBMIB did not alter the half-time, but reduced the amount of the cytochrome which underwent the 1.4 ms reduction. The result again suggests that the cytochrome cannot accept electrons from a large number of the b_6-*f* complexes within the limiting time.

The actual half-time (2.1 ms) of the 2 ms phase of P-700 reduction was significantly longer than the half time (1.4 ms) for the cytochrome reduction determined in the same batch of cells. We found that this is due to a slow absorption decrease centering around 430 nm, which occurs overlapping on the P-700 response several-ms after the flash excitation (data not shown). The absorption decrease may be ascribed to oxidation of cytochrome b_6 which has hitherto been undetected in *Synechococcus*.

The effects of DBMIB on the magnitude of the 2 ms phase of P-700, cytochrome *c*-553 and cytochrome *f* are compared in Fig. 4. If electron transport occurs in completely independent chains, DBMIB should diminish the 2 ms phase of the three electron carriers equally. On the contrary, if a cytochrome *c*-553 molecule intercommunicates between, for instance, more than ten each of the PS I reaction center complexes and the cytochrome b_6-*f* complexes, one expects that the inhibition of a small fraction of the b_6-*f* complexes will affect only the 2 ms reduction phase of cytochrome *f*, but not that of cytochrome *c*-553 or P-700. The inhibitory effect of DBMIB on the 2 ms phase of cytochrome *c*-553 and P-700 reduction should become apparent only after most of the b_6-*f* complexes are blocked by the inhibitor. The result presented in Fig. 4 is an intermediate between the two extreme cases considered. Cytochrome *c*-553 and P-700 reduction were appreciably inhibited at the lowest concentration of DBMIB used, which reduced the 2 ms phase of cytochrome *f* only by 30%. However, DBMIB affected the reduction kinetics of the three electron carriers differently: the amount of cytochrome *f* that undergoes the 2 ms reduction was most strongly diminished, while the 2 ms phase of P-700 was least sensitive to the inhibitor. The total signal

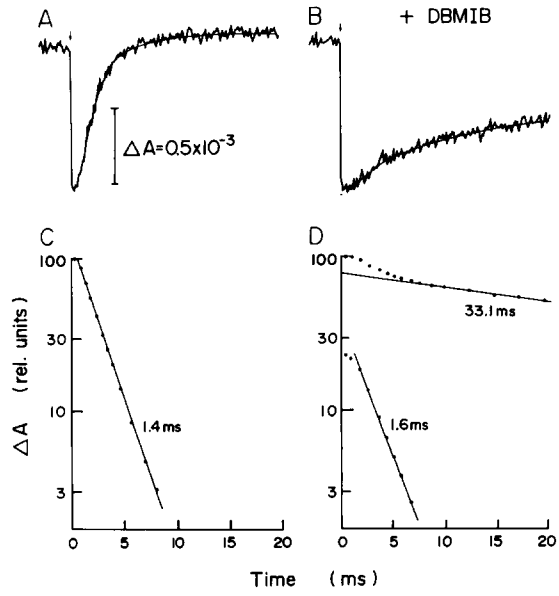

Fig. 3. Effects of DBMIB on the reduction kinetics of cytochrome *c*-553. Experimental conditions were as described in Fig. 2. (A) No addition; (B) 10 μM DBMIB was added. (C) and (D) are semilogarithmic plots of (A) and (B), respectively.

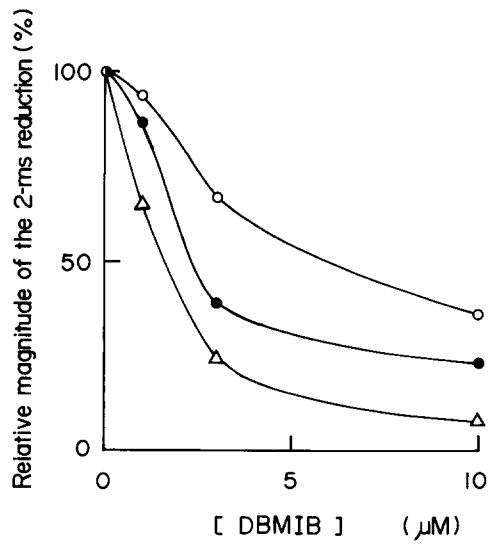

Fig. 4. Effects of DBMIB on magnitudes of the 2 ms reduction phase of P-700 (○), cytochrome *c*-553 (●) and cytochrome *f* (△). The magnitudes of the 2 ms phase in the absence of DBMIB were taken as 100%.

sizes of the three electron carriers remained constant in the range of DBMIB concentrations used. The results strongly suggest that cytochrome *c*-553 is mobile in an extent that one cytochrome b_6-*f* complex gives electrons to more than one cytochrome *c*-553 molecules, and in turn one cytochrome *c*-553 molecule transfers them to more than one PS I complexes.

Effects of flash intensity

Information on the electron exchange in the cytochrome *c*-553 region was also obtained by limiting electron transport on the oxidizing side of the cytochrome. When the flash intensity was reduced by 90%, the magnitude of cytochrome *c*-553 oxidation decreased to 26% of that obtained at the 100% flash intensity (Fig. 5). Since the unattenuated flash oxidized about 90% of cytochrome *c*-553 present in cells [26], nearly 80% of the cytochrome remained unoxidized. If cytochrome *c*-553 can donate its electrons to, for instance, more than ten PS I complexes, P-700 oxidized by the weak flashes should be very rapidly rereduced by cytochrome *c*-553. Fig. 5 shows, however, that P-700 oxidized at the 10% flash intensity was reduced with slower kinetics indicating electron donation from the Rieske center or plastoquinone to P-700. Evidently, a fraction of P-700 which remained in the oxidized state after the flash cannot accept electrons from reduced cytochrome *c*-553 molecules abundantly present in cells. The results are compatible with the view that cytochrome *c*-553 is not a highly mobile electron carrier.

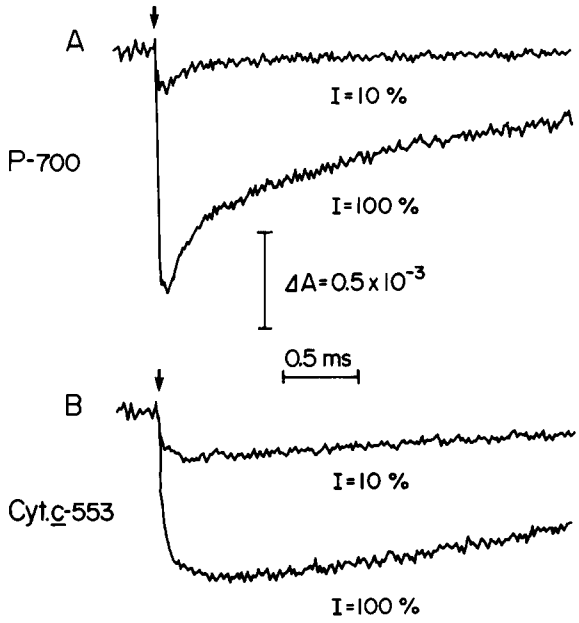

Fig. 5. Redox changes of P-700 (A) and cytochrome *c*-553 (B) at 100% and 10% flash intensities. Flashes were fired at 2 Hz, and 250 and 500 signals were averaged at 100 and 10% flash intensities, respectively. Other experimental conditions were as described in Fig. 2, except that no ascorbate was added. Flash intensity was varied with neutral density filters.

Fig. 6 shows amounts of P-700, cytochrome *c*-553 and cytochrome *f* oxidized as a function of the flash intensity. The magnitude of P-700 oxidation was more strongly diminished than that of cytochrome *c*-553 or cytochrome *f* oxidation as the flash intensity was lowered. This is consistent with the interpretation that the amount of P-700 remained in the oxidized state after the flashes largely depends upon the double hit. Note that the magnitude of cytochrome *c*-553 oxidation decreased in parallel with that of cytochrome *f* oxidation with decreasing intensities of flashes. The ratios of cytochrome *c*-553 oxidized to cytochrome *f* oxidized were about 0.7 at all the flash intensities examined. A similar molar ratio was obtained in the previous work [26] in which the two cytochromes present in cells were determined

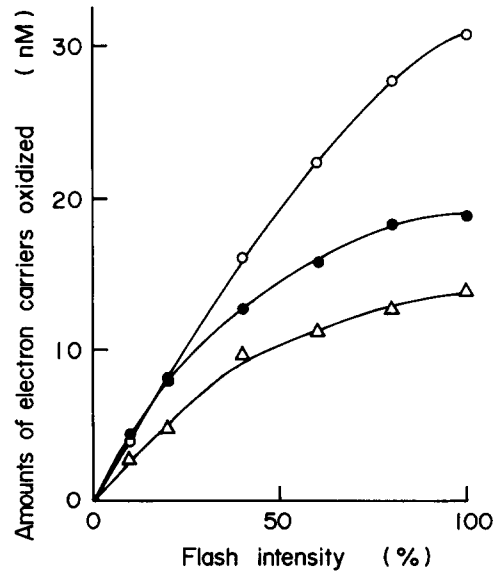

Fig. 6. P-700 (○), cytochrome *c*-553 (●) and cytochrome *f* (△) oxidation as a function of the flash intensity.

photochemically. The results support the view that a cytochrome *c*-553 molecule cannot withdraw electrons from a large number of the cytochrome b_6-*f* complexes.

Discussion

The present work demonstrates that there is no extensive electron exchange among photosynthetic electron-transport chains in the cytochrome *c*-553 region in *Synechococcus*. This indicates that cytochrome *c*-553 is not a highly mobile electron carrier, i.e., a cytochrome molecule cannot migrate a long distance to interact with large numbers of the PS I reaction center complexes and the cytochrome b_6-*f* complexes within the limiting time of electron transporet. Cytochrome *c*-553 is, however, mobile in an extent that one cytochrome b_6-*f* complex donates electrons to more than one cytochrome *c*-553 molecules and in turn one cytochrome *c*-553 molecule reduces more than one PS I complexes. Thus, cytochrome *c*-553 has a mobility sufficient to mediate electron transport from cytochrome *f*, which is present less abundantly than cytochrome *c*-553, to P-700, which occurs twice in excess of cytochrome *c*-553 in *Synechococcus* cells [21,26].

The mobility of plastocyanin in chloroplasts is still in dispute [7–13]. Recently, Haehnel, who studied flash-induced oxidation kinetics of cytochrome *f* in KCN-treated spinach chloroplasts, showed that the mobility of plastocyanin varies depending upon the length of the flash duration [31]. Sensitivity of linear electron transport to $HgCl_2$ is considerably higher in tightly packed than in swollen thylakoids [31]. He proposed that the luminal space of the thylakoid is sufficiently large so that plastocyanin can migrate a considerable distance in the dark or after short flashes, whereas the distance between the membranes becomes too short to allow free-diffusion of the copper protein in the light or after long flashes. It is to be stressed in this respect that the mobility of cytochrome *c*-553 was determined with short flashes of not only high but also low intensities and in the presence of a strong uncoupler gramicidin D in the present work. Clearly, the mobility of cytochrome *c*-553 is low irrespective of light-induced shrinkage of the thylakoids.

The electron exchange among different chains is important for efficient operation of photosynthesis, especially under the light-limiting conditions. Plastoquinone has been shown to serve as a mobile electron carrier through the fluid inner phase of the thylakoids [32]. This, together with the results obtained here, indicates the electron exchange occurs predominantly in the plastoquinone region in *Synechococcus*.

It has long been puzzling to us that any photoresponse attributable to cytochrome b_6 could not be resolved from the flash-induced absorption changes in *Synechococcus* cells. A small absorption change at 430 nm detected in the present work may be ascribed to cytochrome b_6. At present, however, it is difficult to analyze its spectral and kinetical properties in detail due to its small signal size and large overlapping absorption changes.

Acknowledgements

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