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Photosystem I Reaction Centers from *Chlamydomonas* and Higher Plant Chloroplasts

Rachel Nechushtai[1] and Nathan Nelson[1]

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Abstract

A photosystem I reaction center has been isolated from *Chlamydomonas* chloroplasts and compared with the photosystem I reaction center from higher plants. While the higher plant reaction center is active in cytochrome 552 photooxidation, the *Chlamydomonas* preparation was not active unless salts were included in the assay medium or the pH was lowered to 5. Subunit III-depleted photosystem I reaction center from higher plants is also inactive in cytochrome 552 photooxidation in the absence of salts. As with the *Chlamydomonas* reaction center, salts induced its activity. Subunit I of the photosystem I reaction center has tentatively been identified as the binding site of cytochrome 552.

Key Words: Photosystem I; reaction center; chloroplasts; cytochrome 552; light induced oxidation; effect of salts.

Introduction

The functional asymmetry of the chloroplast membrane is maintained by tight protein complexes that have specific biochemical functions in energy transduction. Photosystem I reaction center is one of these complexes that must retain its orientation in the membrane for its function (Junge, 1977, Nelson, 1981). This complex has been isolated from higher plant chloroplasts, and it was demonstrated that the purified complex retains all of the known photobiochemical activities of photosystem I (Bengis and Nelson, 1975; Orlich and Hauska, 1980; Hauska *et al.* 1980). The subunit structure of the photosystem I reaction center has been preserved in various higher plant species (Bengis and Nelson, 1977; Nelson and Notsani, 1977; Orlich and Hauska, 1980; Nechushtai *et al.*, 1981; Okamura *et al.* 1981). Six different subunits have been resolved in SDS gels run according to Weber and Osborn (1969). The subunits were designated by Roman numerals I to VI in the

[1]Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel.

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order of decreasing molecular weights from 70,000 to 8000 (Bengis and Nelson, 1975, 1977). In slab gels run according to Laemmli (1970), subunit VI has been resolved into two bands (Orlich and Hauska, 1980; Nechushtai *et al.*, 1981). Recently a photosystem I reaction center consisting of four different polypeptides was isolated from *Chlamydomonas* chloroplasts (Nechushtai and Nelson, 1981). This reaction center was active in the reconstitution of photophosphorylation but not active in NADP photoreduction.

It is the purpose of this communication to indicate some properties of the purified reaction center and to compare it with the photosystem I reaction center from higher plants.

Experimental

Published procedures were used for chlorophyll (Arnon, 1949) and protein (Lowry *et al.*, 1951) determinations and reconstitution of photophosphorylation (Hauska *et al.*, 1980). Gel electrophoresis in slabs containing an exponential gradient of 10 to 15% acrylamide was performed according to Douglas and Butow (1976). The amounts of P_{700} and the assay of light-induced cytochrome 552 oxidation were performed as previously described (Bengis and Nelson, 1977). Swiss chard photosystem I reaction center and subunit III-depleted reaction center were prepared as before (Bengis and Nelson, 1975, 1977). *Chlamydomonas* photosystem I reaction center was prepared as described by Nechushtai and Nelson (1981). P_{700} reaction center (purified subunit I) was prepared from *Chlamydomonas* photosystem I reaction center as described by Bengis and Nelson (1975, 1977). Cytochrome 552 was purified from Euglena cells according to Perini *et al.* (1964).

Results

The subunit structures of *Chlamydomonas* and Swiss chard photosystem I reaction centers are shown in Fig. 1. Subunits I of both preparations are similar in their position on the gel (M.W. 70,000) and even in their appearance as diffused bands. Subunit II of the *Chlamydomonas* reaction center has a molecular weight of about 19,000 and its position in the gel is near subunit IV of the reaction center from Swiss chard or spinach chloroplasts. However, subunits II of both reaction centers are products of cytoplasmic ribosomes and they might be analogous to each other (Nechushtai and Nelson, 1981). Subunit IV of the *Chlamydomonas* reaction center is parallel in its position in the gel to subunit VI_b of the higher plant reaction center and

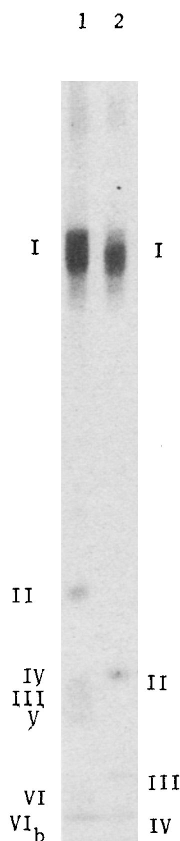

Fig. 1. Sodium dodecyl sulfate gels of photosystem I reaction centers from Swiss chard and *Chlamydomonas* chloroplasts. The reaction centers were purified as described in the Experimental section. The preparations were dissociated for about 2 hr at room temperature in the presence of 2% β-mercaptoethanol and 2% SDS. Samples containing about 20 μg protein were electrophoresed on exponential 10 to 15% acrylamide gel. (1) Photosystem I reaction center from Swiss chard chloroplasts; (2) photosystem I reaction center from *Chlamydomonas*.

it is a good candidate to function as a bound ferredoxin. Subunit III of the *Chlamydomonas* reaction center is close to subunit VI of the preparation from higher plants.

Photosystem I reaction center from *Chlamydomonas* chloroplasts is active in photooxidation of P_{700}, but photoreduction of NADP could not be detected in this preparation. Figure 2 shows that the purified complex was not active in cytochrome 552 photooxidation. However, addition of high concentrations of Na^+ or K^+ or quite low concentrations of divalent cations rendered it active in cytochrome 552 photooxidation. Figure 2 also demonstrates that the re-reduction of $P_{700}{}^+$ in the dark was markedly stimulated by salts or high proton concentrations. Therefore, it seems as if salts facilitate the formation of a complex between the reaction center and cytochrome 552. Figure 3 depicts the effect of various cations on the extent of cytochrome 552 photooxidation. It is apparent that the divalent cations are more effective

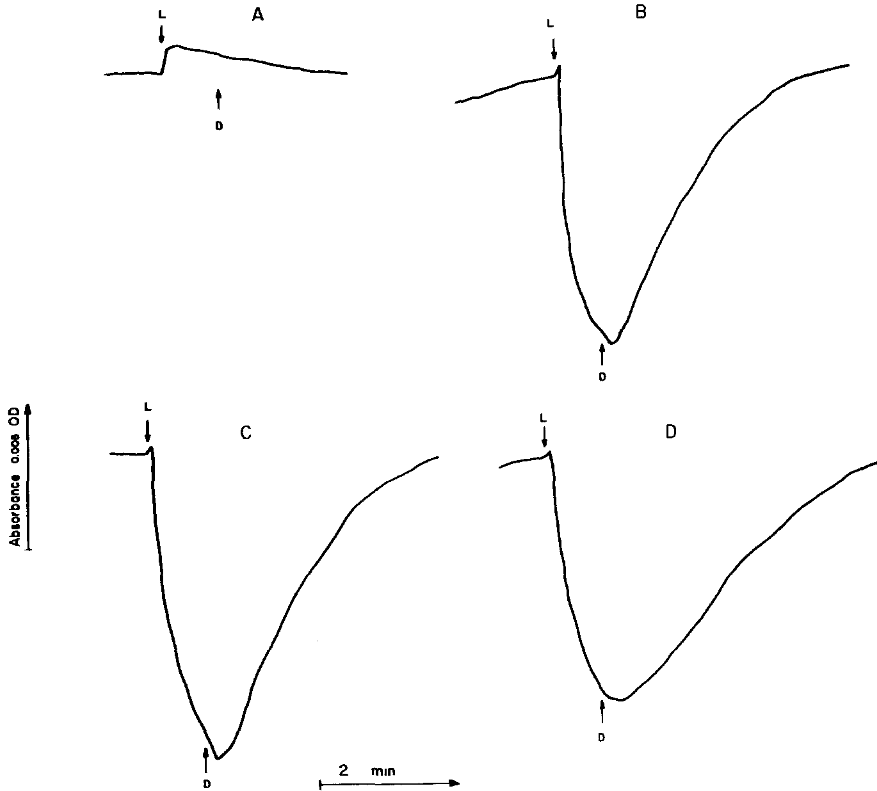

Fig. 2. Photooxidation of cytochrome 552 by *Chlamydomonas* photosystem I reaction center and the effect of salts on the reduction of $P_{700}{}^{+}$ in the dark. The reaction mixture contained, in a final volume of 1 ml, 20 μmol of MES Tricine (pH 7.0), 0.8 nmol of cytochrome 552, 50 nmol of sodium ascorbate, and photosystem I reaction center equivalent to 13 μg chlorophyll. Light-induced absorbance changes were recorded by a Cary 118C at 552 nm for the cytochrome photooxidation (left) and at 430 nm for P_{700} (right) as previously described (Bengis and Nelson, 1977). A to D, light induced absorbance changes at 552 nm; A′ to D′, light-induced absorbance changes at 430 nm; A and A′, control; B and B′ in the presence of 0.2 M NaCl; C and C′, in the presence of 8 mM $MgCl_2$; D and D′, in the presence of 20 mM MES-Tricine (pH 5).

then monovalent cations at the same ionic strength. Figure 4 shows the effect of pH on photooxidation of cytochrome 552 by photosystem I reaction center from *Chlamydomonas* chloroplasts. In the absence of salts the preparation was not active in cytochrome 552 photooxidation at all pH values above 6. At pH 5 it was fully active and the addition of salts had no effect.

In light of these findings we reinvestigated the Swiss chard photosystem I reaction center depleted of subunit III (Bengis and Nelson, 1977). It was shown that depletion of subunit III prevented NADP photoreduction and cytochrome 552 photooxidation. Figure 5 shows that the addition of salts markedly enhanced the cytochrome 552 photooxidation activity of the sub-

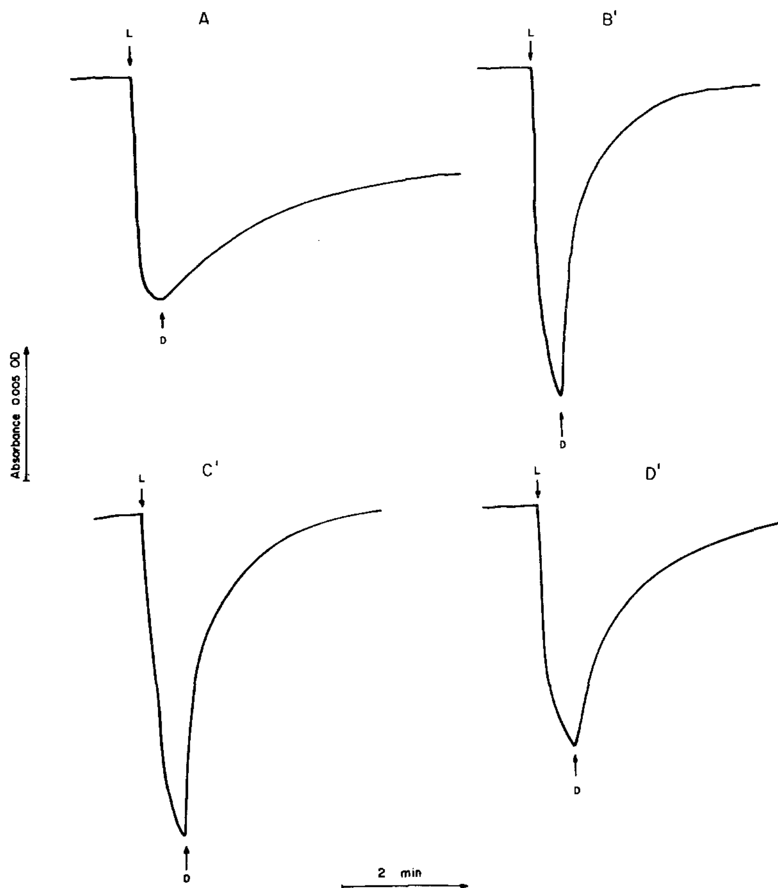

Fig. 2. Continued.

unit-III depleted photosystem I reaction center from Swiss chard chloroplasts. Similarly lowering the pH rendered the depleted reaction center active in cytochrome 552 photooxidation. On the other hand, the reaction center that contained subunit III was active in cytochrome 552 photooxidation in the absence of salts.

Figure 6 shows that a similar treatment that yielded P_{700} reaction center from higher plants produced also purified P_{700} reaction center from *Chlamydomonas* membranes. The preparation consisted of only subunit I and it was active in P_{700} photooxidation.

Figure 7 demonstrates that even the purified subunit I (P_{700} reaction center) is active in cytochrome 552 photooxidation in the presence of salts. This suggests that subunit I might provide the binding site for cytochrome 552.

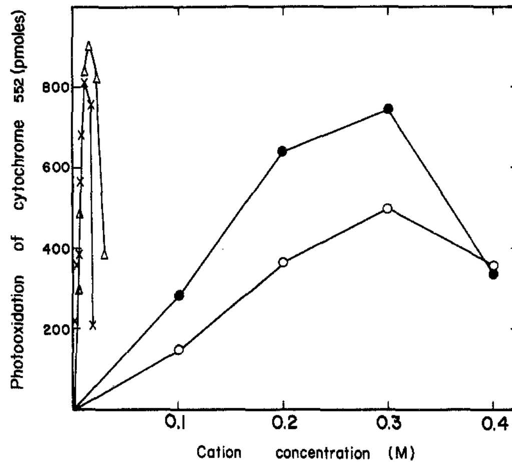

Fig. 3. Effect of cations on photooxidation of cytochrome 552 by *Chlamydomonas* photosystem I reaction center. The experimental conditions were as described in Fig. 2 except that the MES-Tricine buffer was at pH 8.0. (●) in the presence of NaCl; (○) in the presence of KCl; (△) in the presence of $CaCl_2$; (x) in the presence of $MgCl_2$.

Discussion

A photosystem I reaction center active in NADP photoreduction was originally isolated from Swiss chard chloroplasts (Bengis and Nelson, 1975; Nelson and Bengis, 1975). This reaction center was reported to contain six different subunits with molecular weights of about 70,000 (subunit I), 25,000 (II), 20,000 (III), 18,000 (IV), 16,000 (V), and 8000 (VI). The subunit composition and the molecular weights were obtained from calibrated SDS gels performed according to Weber and Osborn (1969). In the same gel system photosystem I reaction center isolated from spinach has a similar subunit composition except that subunit II appeared in a position corresponding to a molecular weight of 22,000. Photosystem I reaction centers resembling the one from Swiss chard have been isolated from lettuce, pea, and Spirodela chloroplasts. In Tris-glycine-SDS gels subunit III switches places with subunit IV and subunit VI is resolved into two bands (Fig. 1 and Hauska *et al.*, 1980; Nechushtai *et al.*, 1981). The subunit composition of photosystem I reaction center has been confirmed in other laboratories (Mullet *et al.*, 1980; Orlich and Hauska, 1980), and it appears as a typical protein complex like cytochrome oxidase in mitochondria (Nelson, 1981). A minimal reaction center (P_{700} reaction center) has been isolated from the photosystem I reaction center by a mild SDS treatment followed by a sucrose gradient

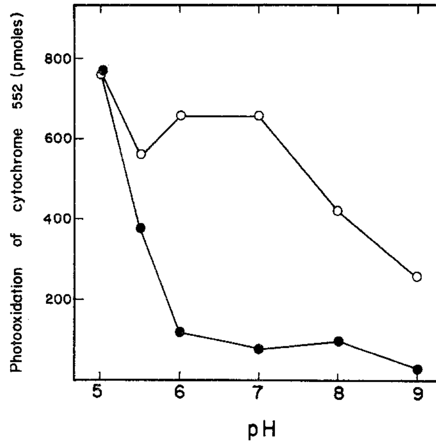

Fig. 4. Effect of pH on the photooxidation of cytochrome 552 by *Chlamydomonas* photosystem I reaction center. The experimental conditions were as described in Fig. 2 except that 50 mM MES-Tricine at the given pH values was used. (●) control; (○) in the presence of 0.1 M NaCl.

centrifugation (Bengis and Nelson, 1975, 1977; Nelson and Notsani, 1977). It contains two copies of subunit I, about 40 chlorophyll *a* molecules that are mutually oriented to one P_{700} pigment (Junge *et al.*, 1977), one carotene molecule, and the primary electron acceptor A_1 (Nelson and Notsani, 1977; Sauer *et al.*, 1978; Shuvalov *et al.*, 1979). It is not clear as yet what stabilizes the charge separation in this preparation. An oxygen molecule might serve as a secondary electron acceptor from A_1 (Nelson and Notsani, 1977; Okamura *et al.*, 1981). In this work we reported on the isolation of a similar P_{700} reaction center from *Chlamydomonas* chloroplasts (Fig. 6). Therefore it seems as if the structure and function of P_{700} reaction center (subunit I of photosystem I reaction center) have been strictly maintained in green algae and higher plants. On the other hand, the structure of the other subunits has not been preserved so strictly and even in the same family a small change in the molecular weight of subunit II has been detected (Swiss chard versus spinach).

The photosystem I reaction center isolated from *Chlamydomonas* chloroplasts contains four different subunits (Fig. 1). Its subunits were designated by Roman numerals I to IV in the order of decreasing molecular weights from 70,000 to 8000. Subunit I is similar to that of higher plants while subunit II (M.W. 19,000) is lighter than the corresponding subunit from higher plants. Subunits II of both systems are products of cytoplasmic ribosomes and therefore they might fulfill a similar function in those reaction centers

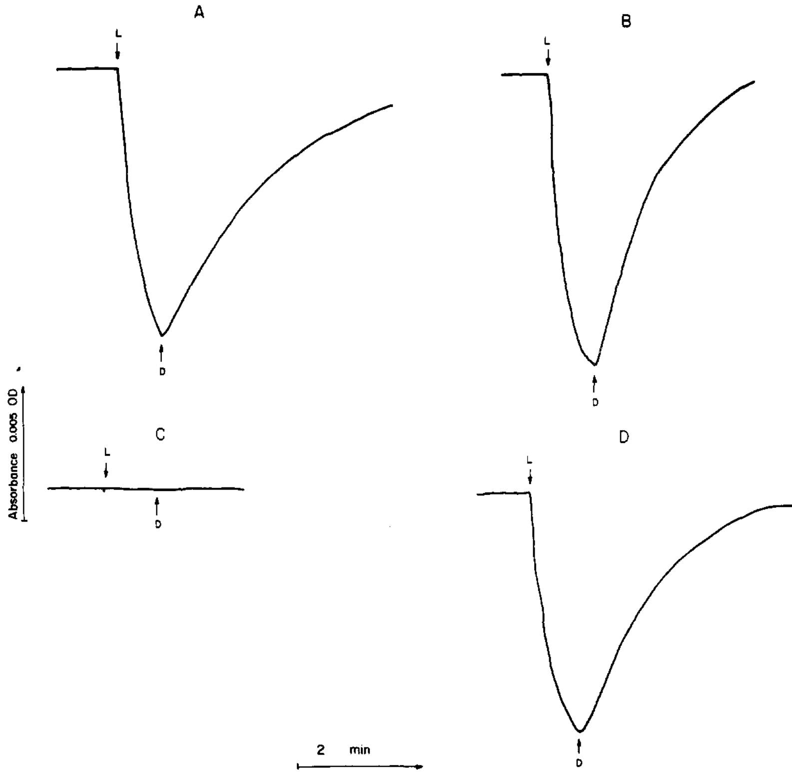

Fig. 5. Photooxidation of cytochrome 552 by subunit III-depleted photosystem I reaction center from Swiss chard chloroplasts. Photosystem I reaction center and subunit III-depleted reaction center were prepared as previously described (Bengis and Nelson, 1977). The preparations were dialyzed against 500 ml of 10 mM Tris-Cl (pH 8) for 2 hr with buffer changing every 30 min. Left—photooxidation of cytochrome 552 at 552 nm. Photooxidation of cytochrome 552 was assayed as described in Fig. 3 except that Swiss chard photosystem I reaction center equivalent to 1.1 μg chlorophyll and subunit III-depleted reaction center equivalent to 1.3 μg chlorophyll replaced the *Chlamydomonas* reaction center. (A) Photosystem I reaction center, control; (B) photosystem I reaction center in the presence of 8 mM $MgCl_2$; (C) subunit III-depleted photosystem I reaction center; (D) subunit III-depleted photosystem I reaction center in the presence of 8 mM $MgCl_2$. Right—SDS gels of the photosystem I reaction center used in this experiment (track 1) and subunit III-depleted reaction center (track 2).

(Nechushtai *et al.*, 1981; Nechushtai and Nelson, 1981). Subunit IV (M.W. 8000) is a good candidate for a "bound ferredoxin" while subunit III (M.W. 10,000) has no cysteine and therefore cannot contain a non-heme iron cluster. The reaction center was inactive in NADP photoreduction and we classified it as photosystem I reaction center because it was isolated as a tight protein complex with a fixed subunit stoichiometry of 2:1:1:1 for subunits I, II, III, and IV respectively (Nechushtai and Nelson, 1981). The *Chlamydomonas*

Fig. 5. Continued.

reaction center was inactive in cytochrome 552 photooxidation, but inclusion of salts induced this activity (Fig 2). Similar phenomenon was observed in photosystem I preparations from *Chlamydomonas* and higher plants (Lien and San Pietro, 1979, Davis *et al.*, 1980). These observations prompted us to investigate the subunit III-depleted photosystem I reaction center from higher plants. It was reported that upon depletion of subunit III the higher plant preparation lost NADP-photoreduction and cytochrome 552 photooxi-

Fig. 6. Sodium dodecyl sulfate gel of *Chlamydomonas* photosystem I reaction center and P_{700} reaction center. The *Chlamydomonas* photosystem I reaction center was purified as previously described (Nechushtai and Nelson, 1981). Aliquots of the purified reaction center containing about 80 μg chlorophyll/ml were treated with 0.6% SDS diluted by equal volume of 10 mM Tricine pH-8.0 and applied on sucrose gradients identical to those used for the preparation of the reaction center. After centrifugation at 40,000 rpm for 15 hr in SW 50 rotor the lower green band was collected as P_{700} reaction center. The upper green band contained the three low-molecular-weight subunits (II, III, and IV). (1) Control of about 25 μg of photosystem I reaction center; (2) the lower green band formed after treatment with 0.6% SDS and sucrose-gradient-centrifugation P_{700} reaction center containing about 20 μg protein; (3) the upper green band containing about 3 μg protein.

dation activities (Bengis and Nelson, 1977). In the present work it is shown that inclusion of salts renders the subunit III-depleted photosystem I reaction center active in cytochrome 552 photooxidation (Fig 5). Therefore, the *Chlamydomonas* photosystem I reaction center is shown to be analogous to subunit III-depleted photosystem I reaction center from higher plants. Two possible mechanisms were proposed for the function of subunit III in the activity of photosystem I reaction center (Bengis and Nelson, 1977; Nelson and Notsani, 1977). The first was that subunit III provides the binding site

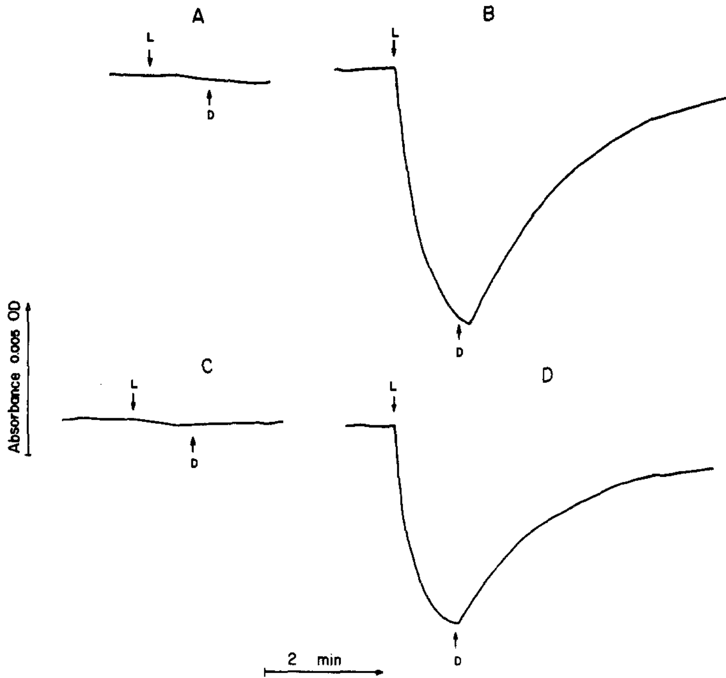

Fig. 7. Cytochrome 552 is photooxidized by purified P_{700} reaction center. The conditions of cytochrome 552 photooxidation are as described in Fig. 2 (A) *Chlamydomonas* photosystem I reaction center shown in Fig. 6, Track 1, containing 8.5 μg chlorophyll; (B) as in A, but in the presense of 8 mM $MgCl_2$; (C) *Chlamydomonas* P_{700} reaction center shown in Fig. 6, track 2, containing 5.4 μg chlorophyll; (D) as in C, but in the presence of 8 mM $MgCl_2$.

for plastocyanin (or cytochrome 552), and the second was that this subunit induces proper conformation for the binding of plastocyanin on other subunits. Haenel *et al.* (1980) showed that the presence of subunit III accelerated by an order of magnitude the photooxidation reaction of plastocyanin. The results of this study clearly support Haenel's conclusion that subunit III induces proper conformation for the binding of plastocyanin elsewhere. Moreover, the results depicted in Fig. 7 tentatively suggested that subunit I provides the binding site for cytochrome 552 and most probably for plastocyanin as well.

What is the biological significance of subnit III in photosystem I reaction center? Even though salts markedly stimulated the rate of cytochrome 552 photooxidation in subunit III-depleted reaction center or the *Chlamydomonas* preparations, the maximum rates were always slower than those which were obtained in reaction centers containing subunit III. A polypeptide analogous to subunit III in higher plants might exist in *Chlamy-*

domonas chloroplasts. On the other hand, it might be an invention of higher plants for better response and faster electron transport. It is noteworthy that in the dark the thylakoids contain sufficient Mg^{+2} to induce electron transport to photosystem I and in the light the internal pH drops to about 5 to allow continuation of the electron transport even in the absence of subunit III.

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