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Electron Transfer between Plastocyanin and P700 in Various Types of Photosystem I Complex [1]

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Three types of PS I Chl-protein complex, PS I 180, PS I 65, and PS I 30, have been prepared and the kinetic properties of the transfer of electrons from plastocyanin to P700 in the PS I complexes with different sized antennae were examined. The PS I 180 complex, which consists of 180 Chl per P700, showed the almost same rate constant and effects of cations for the transfer of electrons from plastocyanin to P700 as those obtained with PS I-enriched membrane fragments. The rate constant increased with the addition of low concentrations of monovalent and divalent cations, but decreased with high concentrations of cations. However, the rate was severely reduced in the case of the PS I 65 and PS I 30 complexes, and quite different effects of cations were observed. Given the presence of additional 25- to 28-kDa polypeptides in the PS I 180 complex as compared to the PS I 65 and PS I 30 complexes, we discuss a possible function for these polypeptides in the regulation of the reaction between plastocyanin and P700.

Key words: Antenna chlorophyll — Chlorophyll protein — Electron transfer — Photosystem I — Plastocyanin.

The PS I Chl-protein complex is the photochemical reaction complex that catalyzes the transport of electrons from plastocyanin to ferredoxin (Malkin 1982). The PS I complex is known to contain the P700-apoprotein of 60–65 kDa and several smaller polypeptides with molecular weights lower than 30 kDa. Although recent studies have shown that cations regulate the rate of electron transfer from plastocyanin to P700, different effects of cations on this reaction have been reported which depended on whether or not P700 was in the PS I particles with large antennae size (Tamura et al. 1981, Olsen and Cox 1982) or in the highly purified PS I complex (Lien and San Pietro 1979, Burkey and Gross 1981, Takabe et al. 1983). In thylakoid membranes, low concentrations of monovalent (<20 mM) or divalent (<3 mM) cations stimulated the rate of reduction of P700 by plastocyanin at neutral pH, whereas inhibition was observed at high concentrations of cations.

However, when the highly purified PS I complex was used, increasing concentrations of divalent cations gradually stimulated the rate of reduction of P700 by plastocyanin. The polypeptide compositions of the PS I particles with large antennae have not been reported. The reason for the different effects of cations in these two systems is not clear. The reconstitution of the PS I 30 complex, which has 30 Chl per P700, into lipid vesicles proved ineffective for the retention of the electrostatic interactions that can be observed in preparations of thylakoid membrane fragments (Ishikawa et al. 1984). Therefore, it has been suggested that some factors other than lipid vesicles may regulate the transfer of electrons from plastocyanin to P700.

Mullet et al. (1980) have prepared a functional PS I 110 protein complex, which contains 110 Chl per P700. The existence of PS I-specific light-harvesting Chl *a/b* protein complexes (LHC I) was predicted from the reduction in the size of antennae of a PS I core preparation (PS I 65).

Purification of LHC I has been reported by several

Abbreviations: P700, photosystem I reaction center chlorophyll; LHC I, light-harvesting chlorophyll *a/b* protein associated with photosystem I; LHC II, light-harvesting chlorophyll *a/b* protein associated with photosystem II; PAGE, polyacrylamide gel electrophoresis.

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groups (Haworth et al. 1983, Lam et al. 1984, Dunahay and Staehelin 1985, Nechushtai et al. 1987, Bassi and Simpson 1987), but the number of LHC I per P700 and its polypeptide composition are the subject of controversy. A preparation of the PS I complex that contains both LHC I and LHC II has also been reported (Bassi and Simpson 1987). Therefore, it seems useful to compare the kinetic properties of the transfer of electrons between plastocyanin and P700 using different types of PS I complex that vary with respect to their polypeptide compositions. Here we describe the preparation of three kinds of PS I complex (PS I 180, PS I 65, and PS I 30) and their kinetic properties. A possible function of peripheral polypeptides in the PS I 180 complex in the regulation of the transfer of electrons from plastocyanin to P700 is discussed.

Materials and Methods

The PS I 180 complex was prepared from fresh spinach leaves, obtained from a local market, by the procedure of Mullet et al. (1980). The concentrations of Chl and Triton X-100 in the extraction mixtures were 0.8 mg/ml and 0.75% (v/v), respectively. Although Mullet et al. reported a Chl/P700 ratio in their preparation from pea of 110, we obtained a Chl/P700 ratio of 180 with our preparation, which we have denoted as PS I 180. The PS I 65 complex was prepared from the PS I 180 complex essentially by the method of Mullet et al. (1980). The PS I 180 complex was subfractionated by incubation with 0.45% (v/v) Triton X-100 for 30 min at 20°C. The final concentration of Chl in this mixture was 0.2 mg/ml. The solubilization mixture was loaded onto a linear sucrose gradient (0.1–1.0 M sucrose) which contained 10 mM Tris-HCl, pH 8.0, and 0.35% (v/v) Triton X-100, and was centrifuged at 4°C for 16 h, in a Hitachi RPS 27-2 rotor at 27,000 rpm. The lower green band (PS I 65) was collected and stored at −20°C. The PS I 30 complex was extracted from spinach chloroplasts with 2% (v/v) Triton X-100 and purified by column chromatography (on DEAE-Sephacel and Sephacryl S-300) and sucrose density centrifugation (Takabe et al. 1983). The P700 content was calculated using an extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$, as determined by Hiyama and Ke (1972). Plastocyanin was measured using an extinction coefficient of 4.9 $mM^{-1} \cdot cm^{-1}$ at 597 nm after oxidation with ferricyanide (Takabe et al. 1980). The concentrations of Chl were measured by the method of Arnon (1949).

Flash-induced photooxidation and the subsequent dark reduction of P700 were measured at 697 nm using a Union Giken single-beam spectrophotometer, as previously described (Ishikawa et al. 1984). The reaction mixture contained PS I complexes equivalent to 0.25 μM P700, 2.5 mM ascorbate, 2 μM plastocyanin, 100 μM methylviologen, 10 mM $MgCl_2$, and 10 mM Tris-HCl, pH 8.0, unless otherwise mentioned. Fluorescence emission spectra at 77°K were measured using a Jasco FP-550 spectrometer, as previously described (Takabe et al. 1980).

The polypeptide compositions of PS I complexes were determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at room temperature in a slab-gel apparatus using a 6% (w/v) polyacrylamide stacking gel and a 13% polyacrylamide running gel, as previously described (Takabe et al. 1983). The Chl complexes were analyzed by SDS-PAGE at 4°C, by the procedure of Anderson et al. (1978).

The cross-reactivities of polypeptides were measured by the Western blotting method, as previously described (Takabe et al. 1986).

Results and Discussion

Preparation of PS I 180, PS I 65, and PS I 30—The polypeptide composition of our PS I 180 complex is shown in Fig. 1. The PS I 180 complex shows many polypeptides and includes the apo-P700 peptide at 60–65 kDa, and several bands at 25–28 kDa, 23 kDa, 20 kDa, 19 kDa, 18 kDa, 16 kDa, 15 kDa, and 12 kDa. No contamination by photosystem II is apparent in the PS I 180 complex as evidenced by the absence of the polypeptides CP 43 and CP 49. However, the presence of peptides in the range of 25–28 kDa shows the presence of the light-harvesting Chl *a/b* protein complex (LHC II), as also indicated by the Western blotting technique with antibody raised against apo-LHC II (data not shown). Alteration of the concentrations of Triton X-100 and/or the ratio of Chl/Triton X-100 failed to generate a preparation of PS I 110 with a ratio of Chl/P700 as low as 110 but without LHC II. Bassi and Simpson (1987) have reported the preparation of a functional PS I complex with a Chl/P700 ratio of 300. It contained no PS II but did contain a significant amount of LHC I and LHC II, which were both functionally connected to P700. Therefore, our PS I 180 complex appears to be similar to the PS I 300 complex prepared by Bassi and Simpson.

The PS I 65 complex was prepared from the PS I 180 complex by the method of Mullet et al. (1980). Its polypeptide composition was relatively simple, as shown in Fig. 1. The complex consisted of the P700-apoprotein at 60–65 kDa, and bands at 23 kDa, 20 kDa, 19 kDa, 18 kDa, 15 kDa, and 12 kDa. The PS I 65 complex was found to be depleted with respect to the 25- to 28-kDa polypeptides when compared to the PS I 180 complex. Other polypeptides appeared at relatively constant levels in comparisons of PS I 180 and PS I 65, although the relative intensities of each stained band in two complexes were different. The ratio of Chl/P700 and the polypeptide composition of our preparation of PS I 65 are similar to those of the corresponding particle reported by Mullet et al.

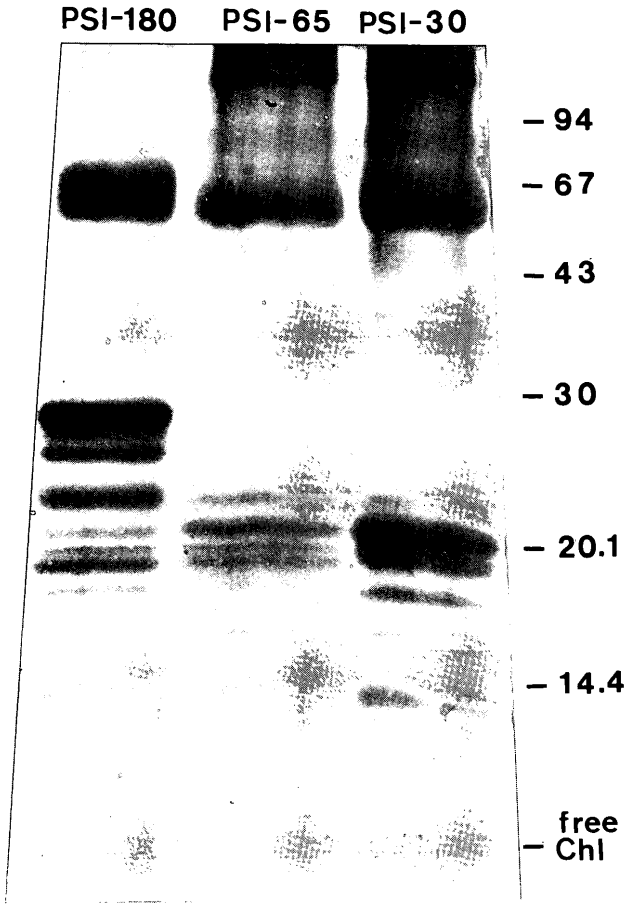

Fig. 1 Fractionation by electrophoresis on a SDS-polyacrylamide gel of PS I 180, PS I 65, and PS I 30 complexes. The electrophoresis of purified PS I complexes (40 μg) was carried out as described in Materials and Methods. The molecular weights (in kDa) of standard protein markers are indicated on the right side of the figure.

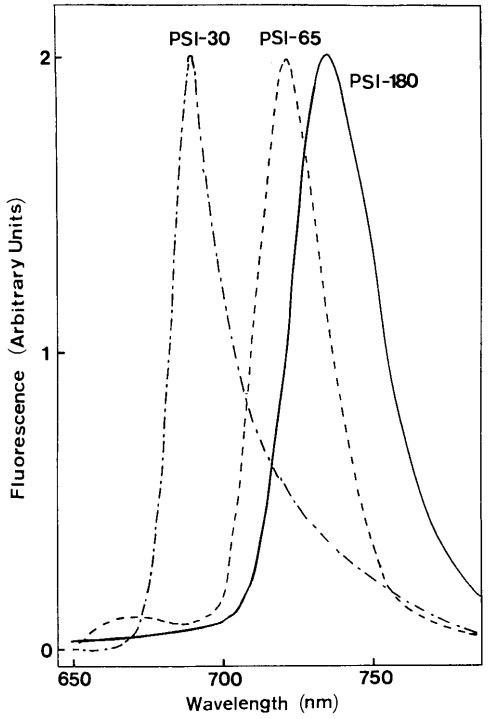

Fig. 2 Low temperature (77°K) fluorescence emission spectra of the PS I 180, PS I 65, and PS I 30 complexes. Spectra were obtained using 435 nm excitation with a slit width of 5 nm, and an emission slit width of 10 nm.

(1980). As shown in Fig. 1, the PS I 30 complex, which was prepared by column chromatography (Takabe et al. 1983), showed a similar polypeptide composition to that of the PS I 65 complex. Again, in this case, the relative intensities of staining of each band in the two complexes were different.

Fig. 2 shows the fluorescence emission spectra at 77°K of three PS I protein complexes. The spectrum of PS I 180 exhibited a fluorescence maximum at 735 nm which arises from the LHC I component (Mullet et al. 1980). The fluorescence emission maximum of PS I 65 at 77°K was shifted towards lower wavelengthes than that of PS I 180, and had a peak at 725 nm. The blue shift in emission exhibited by PS I 65 reflects the absence of LHC I (Mullet et al. 1980). The peak of emission maximum of PS I 30 was further shifted to lower wavelengthes and had a maximum value at 695 nm, which may be due to the absence of long-wavelength-absorbing forms of Chl *a* from this particles.

Chl-proteins in the PS I complexes were analyzed by SDS-PAGE under mildly dissociating conditions. The unstained pattern of migration of Chl-protein complexes is shown in Fig. 3. The most intense band, designated as CPI, was the P700-containing reaction center protein of PS I which contained the 60- to 65-kDa polypeptide. Three bands, which were absent from the PS I 65 and PS I 30 complexes, were generated from Chl *a*/*b* protein complexes. The center band of the three Chl-protein complexes, designated as LHC I, contained Chl *a* and *b* with a ratio of Chl *a*/*b* of 2.4. The fluorescence spectrum at 77°K of this band exhibited long-wavelength fluorescence at 735 nm as well as 685 nm, characteristic of the LHC I complex (Mullet et al. 1980), as shown in Fig. 3B. It has only one protein of about 23 kDa (data not shown), and assumed to be LHC I. The other two bands fluoresced at 680 nm and were probably a mixture of LHC I and LHC II. All the above data are consistent with the presence of both LHC I and LHC II in the PS I 180 complex and the absence of LHC from the PS I 65 and 30 complexes.

Kinetic Properties of PS I 180, PS I 65, and PS I 30 Complexes—Since it has been shown that surface charges on proteins are important in the transfer of electrons from plastocyanin to P700 (Lien and San Pietro 1979, Tamura et al. 1981, Burkey and Gross 1981, Olsen and Cox 1982, Takabe et al. 1983), we have examined the effects of cations

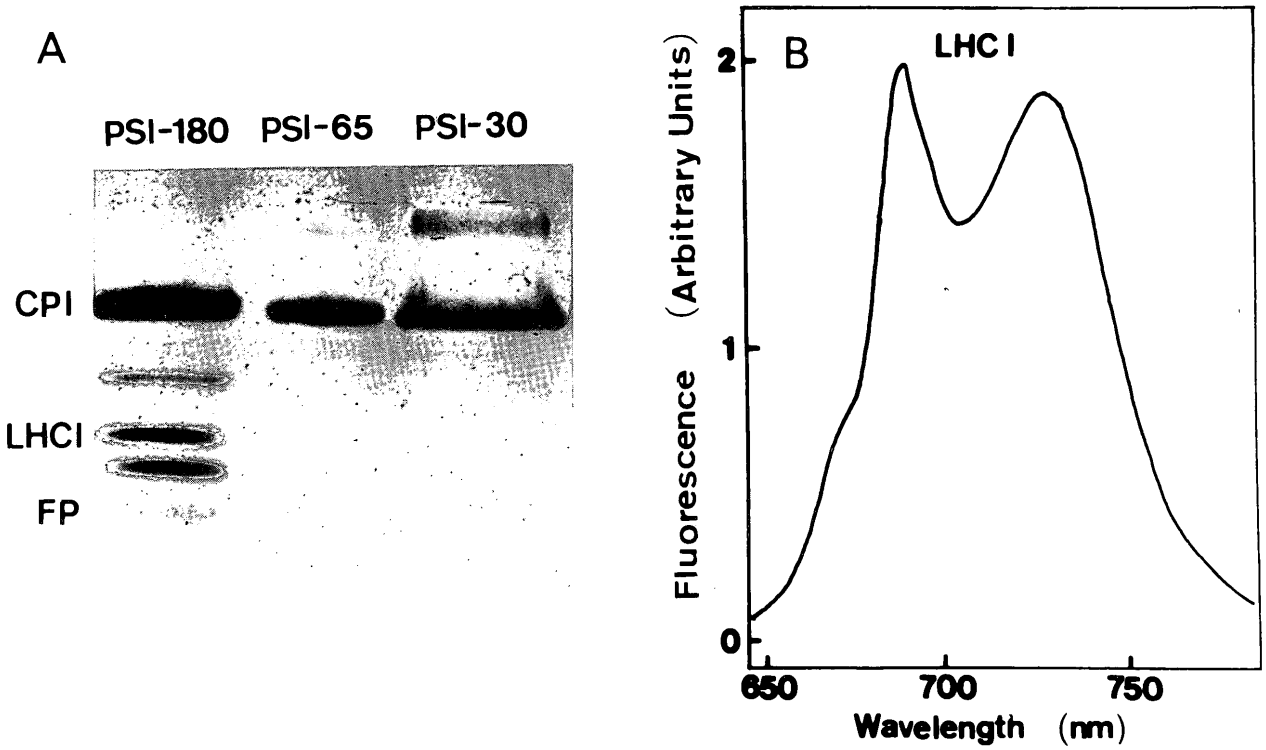

Fig. 3 Unstained pattern of migration of pigment-protein complexes of PS I 180, PS I 65, and PS I 30 after mild SDS-polyacrylamide gel electrophoresis (A), and low temperature (77°K) fluorescence emission spectrum of the LHC I complex (B). The electrophoresis was carried out as described in Materials and Methods. CP I, P700-containing reaction center protein; FP, free pigment zone. Conditions for the fluorescence emission spectrum were the same as in the legend to Fig. 2.

on this reaction using three types of PS I complex. Plots of pseudo-first-order rate constants vs. concentrations of plastocyanin gave straight lines in the case of PS I 30 and PS I 65 complexes, but showed saturation kinetics in the case of the PS I 180 complex (data not shown). Therefore, the data are consistent with a second-order rate constant in the former cases and with a first-order rate constant in the latter case. The rate of electron transfer from plastocyanin to P700 in the PS I 30 complex was very low at neutral pH in the absence of cations, as shown in Fig. 4. The rate was accelerated by the addition of divalent cations such as Mg^{2+} and Ca^{2+}, but not of monovalent cations. These results confirm the data in a previous paper (Takabe et al. 1983). The use of the PS I 65 complex in place of the PS I 30 complex did not change the effect of cations on this reaction, although the absolute rate constant was about 1.6 times larger, as shown in Fig. 4. These data indicate the presence of similar surface charges around the binding sites of both the PS I 65 and PS I 30 complexes.

In contrast to the cases of the PS I 65 and PS I 30 complexes, the rate of transfer of electrons from plastocyanin to P700 in the PS I 180 complex changed dramatically upon the addition of monovalent cations, as shown in Fig. 5. The rate constant increased with increasing concentrations of monovalent cations up to 20 mM, and reached to a plateau value of about 300 s^{-1}. Above 50 mM NaCl, the rate constant decreased. Divalent cations, such as Mg^{2+} and Ca^{2+}, also changed the rate constant dramatically. The addition of low concentrations of $MgCl_2$ (<5 mM) increased the rate constant dramatically, but higher concentrations of $MgCl_2$ (>5 mM) gradually decreased the rate constant. These data are essentially the same as those obtained with the thylakoid membrane fragments (Tamura et al. 1981, Olsen and Cox 1982). Compared to the PS I 65 and PS I 30 complexes, the PS I 180 complex contains the additional 25- to 28-kDa polypeptides, some of which are LHC I and LHC II protein complexes. Therefore, the above data suggest a possible function for the peripheral antenna complex in the regulation of transfer of electrons from plastocyanin to P700.

The numbers of species and of copies of LHC complexes are controversial (Haworth et al. 1983, Lam et al. 1984, Dunahay and Staehelin 1985, Nechushtai et al. 1987, Bassi and Simpson 1987). Therefore, it is not clear at present whether or not all the 25- to 28-kDa polypeptides are associated with Chl and are essential for the efficient electron transfer reaction. The conformational change or the loss of lipid(s) in the PS I core complex upon the deple-

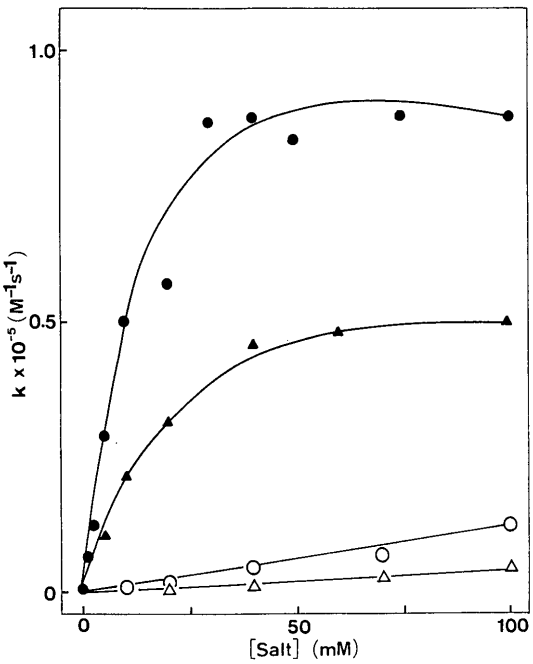

Fig. 4 Effects of salts on the transfer of electrons from plastocyanin to P700 in the PS I 65 and PS I 30 complexes. The standard reaction mixture for the electron transfer contained 0.25 μM P700, 2 μM plastocyanin, 2.5 mM ascorbate, 0.05% Triton X-100, 10 mM Tris-HCl (pH 8.0), and the indicated concentrations of salts. ●, PS I 65 complex plus $MgCl_2$; ○, PS I 65 complex plus NaCl; ▲, PS I 30 complex plus $MgCl_2$; △, PS I 30 complex plus NaCl.

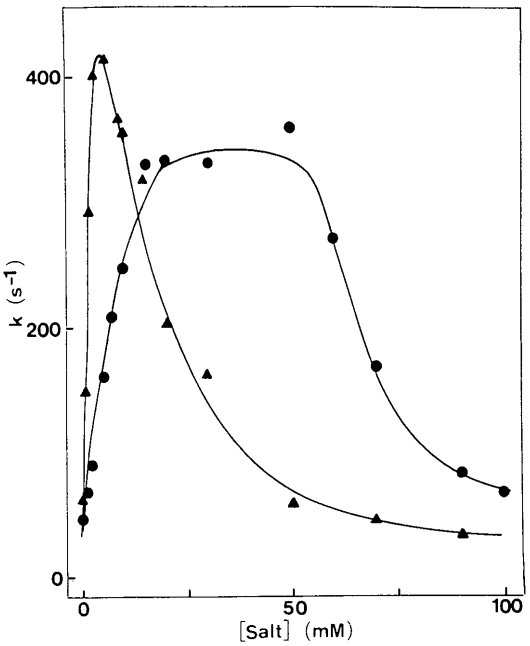

Fig. 5 Effects of salts on the transfer of electrons from plastocyanin to P700 in the PS I 180 complex. The reaction mixture was the same as in the legend to Fig. 4. ▲, $MgCl_2$; ●, NaCl.

tion of the peripheral antenna complex might also change the reactivity and electrostatic interactions between P700 and plastocyanin. Further study is required to clarify these points. We have tried to reconstitute the PS I 180 complex from the PS I 65, LHC I, and LHC II complexes but have been unsuccessful.

The molecular mechanisms of the effects of cations on the transfer of electrons from plastocyanin to P700 in the PS I complex are not clear at present. One possible explanation for the acceleration of electron transfer from plastocyanin to P700 at low concentrations of cations may be an increased concentration of plastocyanin molecules on the surface of the PS I complex when repulsive negative charges on both plastocyanin and the PS I complex are neutralized by the presence of cations such as Mg^{2+}. The linear dependence of the rate of reduction of P700 in the PS I 65 and 30 complexes on concentrations of plastocyanin indicates the low affinity of P700 for plastocyanin in the PS I 65 and 30 complexes, as compared to the PS I 180 complex. The reduced rate constants of electron transfer between plastocyanin and P700 in the PS I 65 and 30 complexes are also consistent with the above conclusion. These results indicate the presence of some factor(s) with high affinity for plastocyanin in the PS I 180 complex. The sites of interaction on plastocyanin with P700 may be the negative patch of carboxyl residues around residues 42–45 (Burkey and Gross 1981, Tamura et al. 1981, Takabe et al. 1983, Takano et al. 1985). Decreases in the rate of electron transfer at high concentrations of cations may be the result of the same factor(s) in the PS I 180 complex having a high affinity for plastocyanin. The decreases in rate may be due to the aggregation of the PS I 180 complex by excess cations, and consequently the inaccessibility of plastocyanin to P700. In this regard, a recent paper by Williams et al. (1987) is of interest. They prepared a PS I protein complex that contained both LHC I and LHC II, and they observed the control of excitation energy transfer from LHC II to LHC I in the complex by Mg^{2+}. They suggested that the aggregation of PS I complexes was due to the screening of negative charges of the N-terminal domain on the LHC II component by Mg^{2+}. Further work is clearly need to clarify whether or not these mechanisms operate in the transfer of electrons between plastocyanin and P700.

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