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Plastocyanin participation in chloroplast Photosystem I

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SUMMARY

French pressure cell disruption of spinach chloroplasts releases much of the plastocyanin from chloroplast membranes. Heavy particles obtained from French pressure cell disrupted chloroplasts lose most of their plastocyanin while light particles retain a high plastocyanin to chlorophyll ratio. Photosystem I activity is dependent on the presence of plastocyanin in our preparations.

In 1968, Michel and Michel-Wolwertz[1] reported that two distinct photosystems could be separated by disrupting chloroplasts with a French pressure cell and then subjecting the chloroplast fragments to sucrose density gradient centrifugation. In 1970, Sane *et al.*[2] extended these results and concluded that the French pressure cell treatment broke the grana stacks from the stroma lamellae. The stroma lamellae fraction showed only Photosystem I activity while the grana lamellae fraction retained both Photosystem I and II activities. Goodchild and Park[3] concluded that Photosystem I particles showed the same activities when prepared either by digitonin or French pressure cell disruption of the chloroplast.

Arnon *et al.*[4] and Tsujimoto *et al.*[5] have reported that plastocyanin is not essential for $NADP^+$ reduction when electrons are supplied from an artificial donor to Photosystem I. The observation that plastocyanin has only a slight effect on activity of the Photosystem I particle derived by French pressure cell treatment argues against the participation of plastocyanin in this photosystem[6,7].

The role of plastocyanin in Photosystem I might be clarified by measurement of plastocyanin concentration in Photosystem I particles. Recently Plesničar and Bendall[8] have published a catalytic method for measuring plastocyanin concentration with great

Abbreviations: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochlorohydride; DCIP, 2,6-dichlorophenolindophenol.

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sensitivity. Application of this method to Photosystem I particles prepared by French pressure cell disruption of chloroplasts reveals substantial amounts of plastocyanin. Removal of the plastocyanin from the Photosystem I particles results in a partial diminution of photochemical activity and addition of plastocyanin to these depleted particles causes large increases in Photosystem I activity.

In the experiments to be reported here plastocyanin was solubilized by digitonin treatment of chloroplasts or chloroplast subfractions. Plastocyanin concentration was estimated by measuring the extent of restoration of plastocyanin-dependent cytochrome *c* photooxidation by digitonin-treated chloroplasts[8]. Chlorophyll concentrations were estimated by the method of Arnon[9]. Chloroplasts were isolated from market spinach and the French pressure cell fractions were prepared according to Sane *et al.*[2]. The light (P_{160}) and heavy (P_{10}) particles were resuspended in 0.05 M phosphate buffer, pH 7.4, containing 0.15 M KCl to a chlorophyll concentration of 0.4 mg/ml. These particles were incubated in 0.5% digitonin for 40 min, then subjected to differential centrifugation. Large debris was removed by centrifugation for 1 h at 40 000 × *g*. The small particles from both P_{160} and P_{10} were then pelleted by centrifugation at 160 000 × *g* for 60 min. These digitonin-derived particles (P_{160}/D_{160} and P_{10}/D_{160}) were resuspended in phosphate buffer and assayed for Photosystem I activity. Photosystem I activity was measured with sodium ascorbate and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochlorohydride (TMPD) as the electron donor and methyl viologen as the electron acceptor[10] or with sodium ascorbate and 2,6-dichlorophenolindophenol (DCIP) as the electron donor and $NADP^+$ as the electron acceptor.

Table I demonstrates that French pressure cell treatment releases a significant amount of plastocyanin. However, plastocyanin is not released uniformly from particles of different sizes. P_{10} particles contain less than half the amount of plastocyanin seen in control chloroplasts. In contrast to previous observations[6], P_{160} particles contain on a

TABLE I

PLASTOCYANIN CONTENT IN SUBCHLOROPLAST PARTICLES

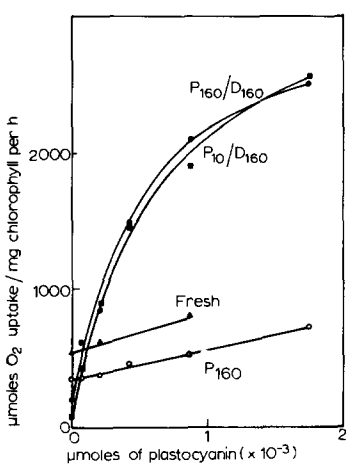
Chloroplast fractions were prepared as described in the text. Control, P_{10} and P_{160} were treated with 0.5% digitonin to extract plastocyanin. All fractions (including French pressure cell-treated chloroplasts) were then centrifuged at 160 000 × *g* for 1 h. Supernatant fractions were dialysed for 8 h against 0.02 M phosphate buffer, pH 7.5, and plastocyanin was concentrated on a DEAE-cellulose column. Cytochrome *c* photooxidation assays were run in an Eppendorf 1100 photometer, illuminated with a 750-W tungsten projection lamp and equippped with a Baird Atomic narrow band pass filter (λ_{max} 685 nm) situated between the lamp and reaction vessel, and at right angles to the measuring beam. A 546-nm narrow band pass filter was placed between the reaction vessel and the photocell. The course of the reaction was followed continuously with a strip chart recorder. The reaction cell contained in 3 ml volume: Tricine buffer, pH 8.0, 150 μmoles; reduced cytochrome *c*, 0.3 μmole; sodium azide, 10 μmoles; methyl viologen, 0.4 μmole; DCMU, $5 \cdot 10^{-3}$ μmole; digitonin, 5 mg; fresh chloroplasts containing 20–30 μg chlorophyll; and standard or unknown aliquots of plastocyanin.

Fraction	*μmoles plastocyanin per mmole chlorophyll*
Control (fresh) chloroplasts	4.9
French pressure cell-treated chloroplasts	1.5
P_{10} particles	2.1
P_{160} particles	3.6

chlorophyll basis about 75% of the plastocyanin present in control chloroplasts. As shown in Fig. 1, plastocyanin gives only a slight stimulation to P_{160} particles in Photosystem I activity. Treatment of P_{160} particles with digitonin, which releases plastocyanin, induces a dramatic influence of plastocyanin on Photosystem I activity. Treatment of P_{10} with digitonin to obtain light particles produces a chloroplast fragment with Photosystem I activity identical with that obtained from P_{160}. Fig. 2 demonstrates that the same influence of plastocyanin in Photosystem I activity is observed when using TMPD as electron donor and methyl viologen as accepter.

The observations reported here indicate that a requirement for plastocyanin exists in Photosystem I activity of light particles obtained from French pressure cell and detergent-treated chloroplasts. The apparent lack of requirement of plastocyanin in P_{160} is due to retention of plastocyanin by the French pressure cell-derived particles. The requirement is only demonstrated when plastocyanin is completely removed by digitonin treatment. Similar results were obtained when heptane extraction[10] or sonication[11] was used to remove the plastocyanin from Photosystem I particles prepared by French pressure cell disruption of chloroplasts. The role of plastocyanin in Photosystem I is supported by Avron and Shneyour[12] who have recently reported that plastocyanin-depleted chloroplasts require plastocyanin for electron transport from ascorbate/DCIP to $NADP^+$ and for photoinduced oxidation of cytochrome *f*.

The hypothesis that $NADP^+$ reduction from an artificial donor is independent of added plastocyanin[4] may only hold in cases where plastocyanin is still present in the particles. Tighter binding of plastocyanin to the stroma lamella fragments would account



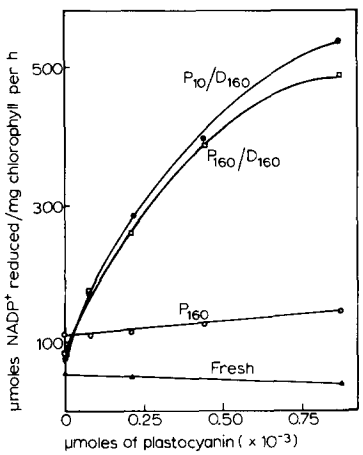


Fig. 1. $NADP^+$ reduction in subchloroplast particles. P_{160}, P_{10}/D_{160} and P_{160}/D_{160} particles were prepared from fresh chloroplasts as described in the text. $NADP^+$ photoreduction was measured by following the change in absorbance at 340 nm caused by illumination with red light of an intensity equal to 10^5 ergs/cm^2 per sec. The reaction cell contained in 3 ml: Tricine buffer, pH 8.0, 150 μmoles; $NADP^+$, 1.4 μmoles; DCMU, $5 \cdot 10^{-3}$ μmole; sodium ascorbate, 50 μmoles; DCIP, 0.2 μmole; saturating amounts of ferredoxin and NADPH–ferredoxin oxidoreductase (EC 1.6.99.4); chloroplast particles containing approx. 50 μg chlorophyll; and an appropriate amount of plastocyanin.

Fig. 2. Methyl viologen reduction in subchloroplast particles. Particle preparations were as in Fig. 1. Oxygen uptake was measured with a Y.S.I. oxygen monitor. Assay conditions and reagent concentrations were as described previously[10].

for the apparent lack of a plastocyanin requirement in these particles.

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