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RELATIONSHIP BETWEEN THE TWO MINOR CHLOROPHYLL *a*-PROTEIN COMPLEXES AND THE PHOTOSYSTEM II REACTION CENTRE

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Photosystem II (PS II) particles containing only the two minor chlorophyll *a* complexes CP a-1 and CP a-2 (Green, B.R., Camm, E. and Van Houten, J. (1982) Biochim. 681, 248–55) were prepared from spinach and barley by a simple procedure involving differential solubilization with octylglucoside and sucrose gradient centrifugation. The gradient fractions with highest PS II activity (light-dependent reduction of 2,6-dichlorophenolindophenol by diphenylcarbazide) contained both CP a-1 and CP a-2, but no other chlorophyll-protein complex. Distribution of PS II activity in spinach was matched more closely by CP a-1 distribution than by CP a-2 distribution, suggesting that the former might carry PS II reaction centre chlorophylls, but a similar role for CP a-2 is also possible.

We have recently shown that the non-ionic detergent octylglucoside disrupts chloroplast thylakoid membranes, resulting in an extract rich in the chlorophyll-protein complexes associated with PS II and lacking CP I [1,2]. These complexes include the light-harvesting Chl $a+b$ complex (LHC), and its oligomer, as well as the unrelated Chl $a+b$ complex CP 29, and two minor complexes which contain only Chl *a* [1]. The latter two complexes have apparent molecular masses of about 43000 and 47000 (from spinach) [2], and each complex accounts for about 3% of the total chlorophyll [3]. They are found in dicots, monocots and green algae [2]. They were referred to as CP 47 and 43 in earlier papers [1,2], but we will refer to them here by the more widely used terms CP a-1 and CP a-2 [3]. The absence of these complexes [4,5] or their polypeptides [6,7] from PS II-deficient mutants suggests that they may be part of the PS II reaction centre. In addition, PS II preparations (TSF-2a) have been shown to be enriched in two such complexes [8]. In this paper, we report a simple method for making PS II-active preparations which do not contain any CP complexes other than CP a-1 and CP a-2.

Locally grown spinach was purchased at a local market. Washed, broken chloroplasts were prepared as previously reported [9].

The extractability of CP complexes is inhibited by the presence of cations, although many non-chlorophyllous proteins are not affected and can still be extracted [10]. To extract some extraneous polypeptides, chloroplasts were first suspended at 100 μg Chl/ml in 100 mM sorbitol, 50 mM Tricine, pH 7.6, 10 mM NaCl ('low salt medium'), plus the addition of 0.5 mM $MgCl_2$. After 15 min, the membranes were pelleted and 30 mM octyl glucoside (in 2 mM Tris-maleate buffer, pH 8.0)

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Abbreviations: PS, photosystem; CP, chlorophyll-protein complex; CP I, P-700-chlorophyll *a*-protein complex, the reaction centre core of PS I; LHC, light-harvesting chlorophyll $a+b$ complex; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Chl, chlorophyll; Tricine, *N*-tris(hydroxy methyl)methylglycine.

was added to a final detergent/Chl ratio of 10 : 1. This suspension was centrifuged at 110000 × *g* for 30 min and the pale-green supernatant was discarded. The pellet was re-suspended in 30 mM octyl glycoside (detergent/Chl ratio 10 : 1), and centrifuged again at 110000 × *g* for 30 min to obtain a dark-green supernatant rich in chlorophyll-protein complexes of PS II [2]. Typically, about 20–30% of the total chlorophyll was extracted.

About 3 ml of extract were loaded on a 10–30% sucrose gradient (30 mM octylglucoside, 0.75 mM EDTA, and 2 mM Tris-maleate, pH 8.0). EDTA was included because the presence of divalent cations caused aggregation of complexes. Conditions of centrifugation are shown in the figure legends.

Fractions from the gradient were electrophoresed in the dark at 4°C on 10% polyacrylamide gels containing 0.1% SDS as described previously [10]. Gels were scanned at 680 nm with a Helena R&D densitometer. The amount of chlorophyll in CP a-1 and CP a-2 in each gradient fraction was estimated by expressing the relative area under each peak of the scan, as a proportion of the chlorophyll in the aliquot used for electrophoresis. This was then related back to the gradient fraction and expressed in μg Chl/ml.

The photoreduction of DCIP with diphenylcarbazide was measured at 590 nm to estimate PS II activity. The millimolar extinction coefficient for DCIP was taken as 16. Actinic light was supplied by a 150 W projector bulb filtered through water and a Corning 2-64 red glass filter.

Sucrose gradients of the second octylglucoside extract from fresh spinach yielded a dark-green band peaking at 11% sucrose (fraction 34) and a lighter green shoulder at 15% sucrose (fraction 30) (Fig. 1, upper). Fractions were assayed for PS II activity using diphenylcarbazide as electron donor, since neither the octylglucoside extract nor the fractions derived from it showed any water-splitting activity in the assay system. The peak of PS II activity was clearly associated with the higher density shoulder. In various experiments, specific activity of PS II ranged from 60 to 120 $\mu mol \cdot mg^{-1} \cdot h^{-1}$. Despite the high chlorophyll content of the lower density peak (fraction 34), PS II specific activity and total activity were always very low in this peak.

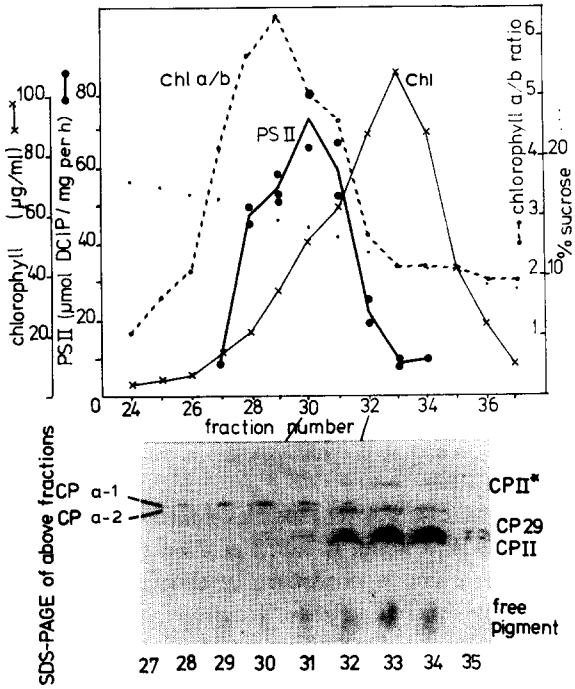

Fig. 1. (Upper) Distribution of chlorophyll and PS II activity along a sucrose density gradient of an octylglucoside extract of spinach. The gradient was centrifuged for 16 h at 81500 × *g* and 4°C in a Beckman SW 27 rotor. (Lower) Eight-drop fractions were collected from the gradient. 50-μl aliquots were electrophoresed on a 10% polyacrylamide gel in the presence of 0.1% SDS. This gel unstained. PAGE, polyacrylamide gel electrophoresis.

When equal aliquots of gradient fractions were electrophoresed in the cold in the presence of 0.1% SDS, the fractions with highest PS II activity were found to contain only CP a-1 and CP a-2 (Fig. 1, lower). The fraction at 11% sucrose, on the other hand, was enriched in CP 29 and LHC and depleted in CP a-1 and CP a-2. At the top of the gradient, fractions contained only CP II and CP 29, with the latter in greater concentration. Similar experiments with Chl *b*-less barley also showed that the fractions with highest PS II activities contained only CP a-1 and CP a-2 [9]. These results suggest that either CP a-1 or CP a-2 may be the reaction centre of PS II.

Staining of gels of the PS II-active fractions revealed an enrichment in the apoproteins of CP a-1 and CP a-2 (of respective molecular mass 47000 and 43000), although other polypeptides were also present. Most preparations from spinach

also showed traces of the α- and β-subunits of CF_1, and a low amount of the CP II apoproteins, although no green complex could be seen. Polypeptides of approximate molecular mass 42000 and 22000 were identified as cytochromes with a haem stain [11], although the cytochromes appeared to sediment above the PS II particles in the gradient. Also present in some preparations were a diffuse band of about 34000 and others of 17000 and 10500.

Both the octylglucoside extract and the PS II-active fractions had decreased sensitivity to the herbicide DCMU, although the degree of sensitivity varied from experiment to experiment. In experiments with a range of concentrations of DCMU, using a chlorophyll concentration of 2–4 μg/ml, washed chloroplasts were 50% inhibited at 0.04 μM while an octylglucoside extract required 2.7 μM for the same degree of inhibition. The gradient fraction richest in the CP a's behaved similarly to the octylglucoside extract. Our work is in agreement with that on peas, in which octylglucoside treatment diminished herbicide sensitivity [12]. On the basis of the present work, it appears likely that detergent extraction has affected the binding protein(s) so as to alter herbicide resistance, rather than removing such a protein or proteins.

In all repetitions of this experiment with spinach, the two CP a's had a slightly different distribution across the gradient. We attempted to correlate the distribution of one or the other of the complexes with PS II activity. In such a case, concentration of the active complex should parallel absolute PS II activity (total PS II activity/ml) rather than specific activity (PS II activity/mg Chl). Fig. 2 shows that absolute PS II activity correlates well with CP a-1 distribution, but not with that of CP a-2. This strongly suggests that CP a-1 plays the major role as the reaction centre of PS II. The results of H. Nakatani (personal communication) also point to the importance of CP 47 (equivalent to CP a-1) in spinach.

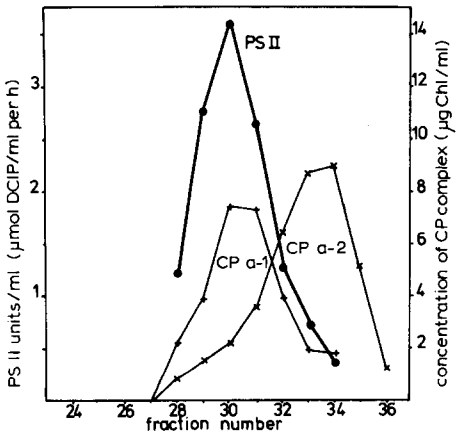

Fig. 2. Distribution of CP a-1, CP a-2 and photosynthetic activity along a sucrose density gradient. Estimates of chlorophyll are from densitometer scans of the gel in Fig. 1.

In summary, this simple procedure has allowed the preparation of a PS II-active preparation, completely free from the LHC holocomplex (as judged by electrophoresis). Our results strongly suggest that CP a-1 is the reaction centre of PS II. However, in view of the observed heterogeneity of the PS II reaction centre in whole thylakoids (reviewed in Ref. 13), the relative roles of the two minor Chl *a* complexes CP a-1 and CP a-2 need careful assessment. Our octylglucoside PS II preparation should facilitate further studies on the roles of these complexes.

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References

1 Camm, E.L. and Green, B.R. (1980) Plant Physiol. 66, 428–432
2 Camm, E.L. and Green, B.R. (1981) Plant Physiol, 67, 1061–1063.
3 Green, B.R., Camm, E. and Van Houten, J., (1982) Biochim. Biophys. Acta 681, 248–255.
4 Krueger, R.W., Metz, J.G. and Miles, D. (1982) Plant Physiol. 69, 74.
5 Metz, J.G. and Miles, D. (1982) Biochim. Biophys. Acta 681, 95–102.
6 Delepelaire, P. and Chua, N.-H. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 111–115.
7 Machold, O., Simpson, D.J. and Møller, B.L. (1979) Carlsberg Res. Commun. 44, 235–254.
8 Laszlo, J.A. and Gross, E.L. (1981) Plant Physiol. 68, 1003–1008.
9 Camm, E.L. and Green, B.R. (1983) J. Cell. Biochem., in the press.
10 Camm, E.L. and Green, B.R. (1982) Arch. Biochem. Biophys. 214, 563–572.
11 Høyer-Hansen, G. (1980) Carlsberg Res. Commun. 45, 167–176.
12 Mullet, J.E. and Arntzen, C.J. (1981) Biochim. Biophys. Acta 635, 236–248.
13 Vermaas, W.F.L. and Govindjee. (1981) Photochem. Photobiol. 34, 775–793.