Reconstitution of light-harvesting chlorophyll–protein complexes with Photosystem II complexes in soybean phosphatidylcholine liposomes

Enhancement of quantum efficiency at sub-saturating light intensities in the reconstituted liposomes

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Received 26 October 1983; revised version received 16 November 1983

Isolated Photosystem II (PS II) complexes and light-harvesting chlorophyll–protein complexes have been reconstituted, both separately and together, into soybean phosphatidylcholine liposomes. PS II activity, as measured by dichlorophenolindophenol reduction, increased by 70% following reconstitution of PS II complexes. Proteoliposomes containing both PS II and light-harvesting chlorophyll–protein complexes also exhibited enhanced quantum efficiencies at sub-saturating light intensities. This indicates that the light-harvesting complexes are interacting with the PS II complexes in proteoliposomes, to increase effectively their antenna size.

Reconstitution *Photosystem II complex* *Light-harvesting chlorophyll–protein complex* *Proteoliposome*

1. INTRODUCTION

The photosynthetic membranes of higher plants contain 3 major membrane-spanning protein complexes that are involved in electron transport: the PS II complex; cytochrome *b/f* complex and PS I complex [1]. Chl *b* is mainly found in the Chl *a/b*-proteins of the light-harvesting complex associated with PS II [2] but is also found in the small amounts of Chl *a/b*-proteins associated with PS I [3,4]. The amount of total chlorophyll associated with the Chl *a/b* light-harvesting complexes relative to PS II core reaction centre complexes has been shown to increase from 3.5 in sun-grown pea plants to 9.0 in blue-light enriched peas grown at very low light intensities [5]. Changes in the ratio of LHCP:PS II core complex have also been found in developing spinach chloroplasts [6]. Finally, the LHCP:PS II core complex ratio increases dramatically in shade-tolerant plant species, such as *Alocasia maccrorhiza* found in tropical rain forests), compared to sun-tolerant species such as spinach or pea [7]. These increases in LHCP: PS II core complex ratio, together with concomitant changes in thylakoid ultrastructure and PS II: PS I stoicheiometry are believed to be adaptive strategies which serve to regulate light capture and energy distribution between PS II and PS I in order to maintain optimal rates of electron transport [1].

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; DPC, diphenylcarbazide; LHCP, light-harvesting chlorophyll–protein; PS, photosystem; Chlorophyll; Chl

Published by Elsevier Science Publishers B.V.
001457593/84/$3.00

Thylakoid membranes also contain two types of PS II complex, which are characterised by their different total chlorophyll:reaction centre (measured as *Q*) ratios. The PS IIα complexes contain 234 Chl molecules per centre, while PS IIβ complexes contain 100 Chl molecules per centre [7]. The ratio of LHCP:PS II core complexes was estimated at 4.75 for PS IIα but only 1.50 for PS IIβ complexes [7]. The ratio of PS IIα:PS IIβ varies in response to different illumination conditions during development [8] and also to short-term changes associated with State 1 to State 2 transitions [9,10]. The latter response is completely reversible and has been proposed to involve a partial dissociation of LHCP subunits from PS IIα complexes to form effective PS IIβ complexes [11].

The association between LHCP and PS II core complexes is thus not in a fixed stoicheiometry. The ratio between these two components of PS II complexes varies between different species occupying different habitats, within members of the same species under different environmental conditions, within an individual plant during development, and even within an individual chloroplast during short-term adaptive responses such as State 1 to State 2 transitions. The manipulation of the LHCP:PS II core complex ratio in vitro and its effect upon PS II activity is therefore of great interest. The most direct method of studying LHCP–PS II core complex interactions involves their reconstitution into liposomes free from other thylakoid membrane components. We describe here the functional reconstitution of purified LHCP complexes with PS II complexes into a PS II–LHCP complex with increased antenna size and greater quantum efficiency at low light intensities.

2. MATERIALS AND METHODS

2.1. *Preparation of chlorophyll–protein complexes*

Dwarf peq plants (*Pisum sativum* L., var. Kelvedon Wonder) were grown in vermiculite under constant environments: 10 h photoperiod; 22°C, for up to 17 days. Batches of 50 g selected leaves were excised for preparation of chlorophyll–protein complexes. PS II complexes were prepared essentially as in [12]. LHCP complexes were prepared as in [13], except that Triton X-100 was excluded from the sucrose density gradient centrifugation. Aliquots of PS II complexes and LHCP complexes were frozen in liquid N_2 and stored at −70°C.

2.2. *Reconstitution of LHCP and PS II into liposomes*

Soybean phosphatidylcholine (Sigma type III-S, over 99% pure as verified using thin-layer chromatography) in chloroform solution was evaporated to dryness under N_2. The lipid was dispersed in 20 mM 4-morpholinepropanesulphonic acid (Mops) (pH 6–8), 5 mM $MgCl_2$, 50 mM KCl by sonication for 20 min in a bath sonicator at 4°C. Suspensions of LHCP and PS II preparations were sonicated for 20 min at 4°C. The chlorophyll–protein complexes were reconstituted, either together or separately, into liposomes by mixing them with the dispersed lipid in a lipid:protein ratio of 20:1. The mixture was immediately frozen in liquid N_2 and incubated at −196°C for 1 min, thawed in water at 20°C, and finally sonicated for 10 min at 4°C. The mixture was layered onto a linear 0.1–2.0 M sucrose gradient and centrifuged at 150000 × *g* for 20 h. Reconstituted chlorophyll–protein containing liposomes were resolved from unreconstituted components by this procedure.

2.3. *PS II assays*

PS II activity was monitored by measuring either 2,5-dimethyl-*p*-benzoquinone (DMQ)-dependent O_2 evolution in a Hansatech oxygen electrode, or DCPIP reduction from DPC in a Bristol dual-wavelength spectrophotometer. In each case the assay medium contained 20 mM Mops (pH 6.8), 5 mM $MgCl_2$, 50 mM KCl. Other concentrations were 2 mM DMQ, 80 μM DCPIP, 0.4 mM DPC (stock solution 100 mM in absolute ethanol). Light intensities were measured using a calibrated photodiode and were varied by means of neutral density filters (Oriel, CT).

2.4. *Spectral analyses*

Absorption spectra were measured from 400–700 nm at 25°C in a Unicam SP8-200 spectrophotometer. Fluorescence emission spectra were measured at 50 μg Chl · ml^{-1} at either room temperature or −196°C using a spectrofluorimeter with a broad-band blue exciting beam. Emission spectra were normally recorded from 625–845 nm.

Chlorophyll concentrations and Chl *a*:Chl *b* ratios were determined in 80% acetone [14]. Protein was determined as in [15].

2.5. *Polyacrylamide gel electrophoresis*

Chlorophyll-proteins were separated under non-denaturing conditions as in [16]. Individual polypeptides were resolved on slab gels in a 10–30% acrylamide gradient as in [17].

3. RESULTS AND DISCUSSION

The purity of chlorophyll-protein complex preparations was monitored by electrophoresis on both denaturing and non-denaturing gels. The LHCP preparations contained little appreciable PS II or PS I contamination and had Chl *a:b* ratios of 1.21. The oxygen-evolving PS II preparations contained virtually no detectable PS I polypeptides but did contain LHCP, as evidenced by their Chl *a:b* ratio of 2.65. However, the Chl *a:b* ratios of the original leaf tissue were in the region of 3.05 and about 40% of this chlorophyll is associated with PS I which has Chl *a:b* ratios of 6–10. Therefore it is likely that the physiological average Chl *a:b* ratio in PS II complexes is less than 2.65, from which it may be concluded that the PS II complexes prepared in this study are relatively depleted in LHCP. It was recently reported that the Chl *a:b* ratios of spinach PS IIα and PS IIβ were 1.73 and 5.6, rspectively [7]. Since the ratio of PS IIα; PS IIβ on a chlorophyll basis is 7.1, the average PS II *a:b* ratio should be 1.96, which would also indicate that our PS II complexes were depleted in LHCP.

It was notable that the O_2-evolving PS II complexes exhibited first-order kinetics in their fluorescence induction curves in the presence of DCMU (not shown). The PS II complexes were therefore behaving as separate packages similar to PS IIβ centres rather than connected PS IIα centres. However, the low Chl *a:b* ratio precludes the possibility that the majority of the PS II complexes purified in this study were in fact PS IIβ centres. It is more likely that most of the PS II complexes are PS IIα centres, but that they are dissociated from neighbouring complexes during detergent solubilisation and rendered incapable of intra-system energy transfer.

PS II activity, as measured by DCPIP reduction from DPC, was altered by incorporation into soybean phosphatidylcholine liposomes (table 1). Simple sonication resulted in a poor yield of incorporated PS II complexes and a reduction in their electron transport capacity. Incorporation of PS II complexes using a freeze-thaw procedure resulted in a good yield of reconstituted complexes and a greater than 70% increase in electron transport capacity. This activity was more than doubled following the purification of the proteoliposomes on a sucrose density gradient. LHCP complexes were also successfully incorporated into soybean phosphatidylcholine liposomes using the freeze-thaw procedure. This is in contrast to a report that relatively little spinach LHCP could be incorporated into such liposomes [18]. This may be due to differences in the grade of soybean phosphatidylcholine employed in the two studies. Incorporation of PS II complexes together with

Table 1

Effect upon PS II activity of the incorporation of PS II preparations into phosphatidylcholine liposomes

Preparation	PS II activity ($\mu mol \cdot h^{-1} \cdot mg^{-1}$ Chl)	
	DCPIP reduction	O_2 evolution ($H_2O \rightarrow DMQ$)
PS II complexes in detergent solution	129	188
PS II proteoliposomes (sonicated only)	63.4	23.6
PS II proteoliposomes (freeze-thawed)	224	242
PS II proteoliposomes (freeze-thawed and purified)	490	trace
LHCP complexes in detergent solution	5	0
LHCP complexes proteoliposomes	trace	0

PS II complexes were prepared and assayed as described in section 2. Reconstitutions were effected by a simple sonication procedure or by a freeze-thaw plus sonication procedure. The reconstituted proteoliposomes were further purified upon sucrose density gradients as described in section 2

LHCP complexes resulted in slightly higher PS II electron transfer rates, as measured on a per mg PS II chlorophyll basis. However, all these measurements were made at saturating light intensities so the effect of any LHCP–PS II interaction would not be apparent.

Although the DPC-dependent DCPIP reduction activity of the PS II complexes was successfully incorporated into soybean phosphatidylcholine liposomes, the oxygen-evolving capacity was not. Some 75% of the original $H_2O \rightarrow DMQ$ activity remained after the freeze-thaw step, but nearly all of this was lost following sucrose density gradient centrifugation. (table 1).

Low-temperature fluorescence emission spectra of the PS II, LHCP and [PS II + LHCP] proteoliposomes showed that purified LHCP proteoliposomes had similar fluorescence emission characteristics to non-incorporated LHPC. There was no long-wavelength emission in the 730–740 nm region. The PS II proteoliposomes showed a small emission peak at 734 nm in addition to the two major peaks around 690 nm. The 734 nm peak did not arise from contamination by PS I as the latter has a major emission peak at 740 nm. The reconstituted [PS II:LHCP] liposomes had a much reduced overall fluorescence yield compared to LHCP proteoliposomes. These data indicate a degree of interaction between the two complexes. However, simple mixing of LHCP proteoliposomes with PS II liposomes also showed some evidence of interaction, albeit not of the same magnitude as in the reconstituted complexes.

Positive confirmation of a putative LHCP–PS II interaction in a reconstituted membrane requires evidence of functional changes in parameters such as quantum efficiency. This is provided by the experiment shown in table 2. Here, the response of PS II activity to sub-saturating light intensities in PS II and [PS II + LHCP] proteoliposomes is described. The reconstituted proteoliposomes had a Chl *a:b* ratio of 2:04, which means that the ratio of PS II complex to LHCP complexes is 2:1. This [PS II + LHCP] ratio does not include the endogenous LHCP already present in the PS II complexes. Thus the PS II complexes have been enriched in LHCP to something approaching the physiological average PS II:LHCP ratio. The latter was shown above to result in a Chl *a:b* ratio of 1.96.

Table 2

Effect of light intensity upon PS II activity in proteoliposomes containing either PS II complexes only or both LHCP and PS II complexes

Light intensity ($W \cdot m^{-2}$)	PS II activity (DPC→DCPIP) ($\mu mol \cdot mg^{-1}$ Chl $\cdot h^{-1}$)	
	PS II proteoliposomes	[PS II + LHCP] proteoliposomes
20	78.7	121.3
38	170.5	257.4
74	302.5	420.2
120	383.6	431.1
175	414.0	442.6
220	449.2	472.1
312	472.8	519.8
437	478.7	485.2

Light intensities were measured as photosynthetically active radiation using a calibrated photodiode. PS II activities were assayed by following DPC-dependent reduction of DCPIP as described in section 2

The reconstituted [PS II + LHCP] proteoliposomes showed a significantly greater capacity for DCPIP reduction at sub-saturating light intensities of up to 175 $W \cdot m^{-2}$ than did PS II proteoliposomes alone. This indicates that the extra LHCP is indeed interacting with the PS II complexes and giving rise to a greater functional antenna size per reaction centre. This is just the first time that such a functional interaction between different chlorophyll–protein complexes in a reconstituted system has been reported from higher plants.

There was no evidence of PS II–PS II interaction from fluorescence induction curves in the presence of DCMU, i.e. the reconstituted PS II proteoliposomes behaved as separate packages whether or not extra LHCP was present. This is possibly due to the lack of a membrane environment favourable for such an interaction or may be due to the particular reconstitution procedure employed here (which also resulted in the loss of O_2 evolving capacity). It is also possible that the ability of PS IIα centres to interact with each other was irreversibly lost during their solubilisation.

The soybean phosphatidylcholine liposomes provided a good vehicle for PS II reconstitution. The environment of a zwitterionic phospholipid

considerably stimulated the capacity of the PS II complexes to reduce DCPIP compared to their DCPIP reduction rate in mixed lipid–Triton X-100 micelles. It has recently been reported that granal thylakoids, which contain most of the PS II of chloroplast membranes, are relatively depleted in galactolipids and that almost half the total granal acyl lipid is made up of the anionic lipids phosphatidylglycerol and sulphoquinovosyldiacylglycerol [19]. The lipid environment of PS II complexes has a dramatic effect on their electron transport capacities and it may be that phosphatidylglycerol and/or sulphoquinovosyldiacylglycerol provide a specific lipid environment in the thylakoid membrane which allows for optimal PS II function and inter-complex interaction. The demonstration that LHCP can be added to PS II complexes in vitro considerably strengthens the case that reversible dissociation of LHCP from PS IIα centres is responsible for the changes observed during State 1 to 2 transitions [11].

ACKNOWLEDGEMENTS

We are grateful to other members of the ARC Research Group on Photosynthesis and in particular to Dr P. Horton, for their assistance and useful discussions. This work was funded by the Agricultural Research Council, UK, and I.E.W. is a recipient of a Queen Elizabeth II Postdoctoral Fellowship.

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