The effect of thylakoid lipids on an oxygen-evolving Photosystem II preparation

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The lipid content of a Photosystem II preparation, derived by Triton X-100 fractionation of spinach thylakoid membranes, which retained high rates of oxygen evolution was examined. It was found that the detergent treatment resulted in a preferential loss of digalactosyldiacylglycerol. The effect on this preparation of exogenously added lipids, or lipid mixtures, purified from thylakoids was investigated. Addition of total polar lipid extract stimulated the rates of oxygen evolution, the same effect being achieved with isolated digalactosyldiacylglycerol or phosphatidylcholine alone. The stimulation of oxygen evolution was dependent on the degree of unsaturation of the lipids used and on the relative amounts of acidic lipids present in the mixtures. Addition of isolated acidic lipids alone resulted in a complete inhibition of oxygen evolution. The results are discussed in terms of the possible involvement of thylakoid lipids in the molecular organisation of the Photosystem II oxygen-evolving system.

Thylakoid lipid *Photosystem II* *Oxygen evolution* *Triton X-100 fragmentation* *Lipid content* *Photosynthesis*

1. INTRODUCTION

Several methods have been employed to isolate active oxygen-evolving PS II-enriched preparations [1–5], most of which [2–5] rely on the use of mild detergents. The availability of these methods has enabled a better characterisation of PS II and several components have been identified with specific polypeptides of the complex [6]. Little, however, is known about the amount and composition of thylakoid polar lipids associated with these preparations, although a recent study has been carried out with PS II-enriched appressed membrane vesicles [7].

Abbreviations: PS II, Photosystem II; TLE, total lipid extract; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; SQDG, sulphoquinovosyldiacylglycerol; PC, phosphatidylcholine; OPL, other phospholipids; LHCP, light-harvesting chlorophyll *a/b* protein; TLC, thin-layer chromatography; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; SDS, sodium dodecyl sulphate

Here we give an analysis of the lipid content and composition of an active, oxygen-evolving PS II preparation obtained by Triton X-100 fractionation of spinach thylakoid membranes and show the effect of exogenous thylakoid lipids on the activity of this preparation. We have found that the capacity of such preparations for oxygen evolution is enhanced by the addition of thylakoid lipids and that the observed stimulation depends on the type of lipid added and on the degree of unsaturation of its fatty acid chains. We postulate that thylakoid polar lipids are required for the optimal organisation of the protein complexes involved in O_2 evolution and that the fluidity and surface charge of the membrane are crucial parameters.

2. EXPERIMENTAL

2.1. *Isolation of the Photosystem II preparation*

Chloroplasts were isolated from deveined spinach leaves (*Spinacia oleracea* L.) as in [8] ex-

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cept that the washing medium was 330 mM sorbitol, 1 mM EDTA, 5 mM $MgCl_2$ and 50 mM Hepes-KOH (pH 7.5). The PS II preparation, capable of oxygen evolution, was obtained by treating the final chloroplast pellet as in [3] except that the pH of the solutions was 7.3. Small aliquots of the preparation were stored in liquid nitrogen. Rates of O_2 evolution were assayed at pH 6 in 100 mM sorbitol, 20 mM Mes-KOH, 15 mM KCl; conditions which we found gave optimal rates.

2.2. *Isolation of chloroplast lipids*

Chloroplast lipids were extracted from spinach thylakoid membranes as in [9]. Pigments and neutral lipids were removed from the extract on silicic acid columns as in [10]. Separation of the total polar lipid extract thus obtained into lipid classes was carried out on acid-washed florisil columns in combination with preparative TLC. The methods used for the purification of the lipid classes have been given in detail elsewhere [10]. Saturation of lipids was achieved by catalytic hydrogenation in the presence of Adams' catalyst as in [11]. Lipids were quantitated as in [12].

2.3. *Lipid class and fatty acid analysis of thylakoids and PS II preparations*

Analyses of the amounts of different lipid classes and fatty acids present in the PS II preparations and in the spinach thylakoids were carried out as in [7] and [12]. The presence of Triton X-100 in the PS II preparation resulted in a slight interference with the TLC procedures employed. In order to ensure unequivocal identification of the lipid classes, TLC pure standards were always chromatographed together with the PS II lipid extract.

2.4. *Addition of exogenous lipids*

Lipids or lipid mixtures were dried under N_2 in test tubes and appropriate volumes of buffer (100 mM sorbitol, 15 mM KCl, 20 mM Mes-KOH, pH 6) were added. The solution was sonicated under N_2 for approximately 60 s in a Kerry water-bath sonicator. This procedure did not lead to fatty acid oxidation as judged by gas-liquid chromatographic analysis. Aliquots of the PS II preparation were added to the lipid dispersions and the mixture was either incubated at 25°C for 10 min or frozen in liquid N_2 (60 s) and thawed back to 25°C. Both procedures yielded identical results and the incubation method was employed. The chlorophyll to lipid ratio in these experiments was routinely adjusted to 1:14 (w/w).

2.5. *Other methods*

Oxygen evolution was measured in the presence of ferricyanide (5 mM) and dimethylbenzoquinone (0.5 mM) using a Clark type electrode. Chlorophylls were estimated as in [13].

3. RESULTS

Using SDS-polyacrylamide gel electrophoresis, it was found that the O_2-evolving PS II preparation contained at least ten clearly separated Coumassie blue-stained bands similar to that reported in [14]. The purpose of our work was to investigate and fully characterise the lipid properties of the PS II preparation and table 1a and b gives their fatty acid and lipid class compositions, respectively. When compared with the values obtained by analysing spinach thylakoid membranes it can be seen that the fatty acids of the O_2-evolving fragments are more saturated. This is mainly due to a reduction in linolenic acid (C18:3) and an increase in the palmitic (C16:0) and stearic (C18:0) acids as compared with the intact thylakoids. This finding could indicate either the presence of more saturated polar lipid species in the PS II preparation compared to the intact membrane or that there is a reduction in the levels of specific lipids which normally contain high levels of linolenic acid. The latter possibility seems to be the case since as table 1b shows, the PS II preparations are significantly depleted of DGDG as well as having a lower concentration of MGDG compared with the levels of the two galactolipids found in untreated thylakoids. Another important feature of the lipid class analyses is that the two acidic lipids, sulphoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) are present in higher proportions in the PS II preparations compared with the normal content of thylakoids. Coupled to these findings it can also be seen in table 1b that the total lipid content of the PS II preparation was low as indicated by the lipid to chlorophyll ratios. This observation is not surprising since Triton X-100 was used at levels well above its micellar

Table 1

(a) Fatty acid composition of spinach thylakoids and PS II preparation obtained by Triton X-100 fragmentation of the membranes

Sample	Fatty acid composition (mole %)							% saturated fatty acids	Average no. of double bonds per lipid molecule
	16:0	16:1	16:3	18:0	18:1	18:2	18:3		
Thylakoids	9.9	3.4	10.8	0.4	2.2	6.01	67.3	10.3	5.03
PS II preparation	10.0	4.2	13.1	2.4	3.6	5.30	53.4	20.4	4.34

(b) Lipid class composition of the Triton X-100-derived PS II preparation compared with that of the untreated thylakoids

Sample	Lipid class composition (mole %)						moles of lipid per mole total chlorophyll
	MGDG	DGDG	PG	SQDG	OPL	PC	
Thylakoids	48.2	24.5	13.7	8.2	3.4	2.0	2.45
PS II preparation	30.9	5.9	24.1	14.5	11.4	13.2	0.53

concentration in conditions which would be expected to displace lipid from the membranes.

The PS II preparations used for lipid analyses showed reasonably high rates of O_2 evolution which were inhibited by DCMU. Although the analyses were remarkably constant from preparation to preparation (for example, the data in tables 1a and b are the mean for five different preparations with a maximum variation within any analysis of less than 1.5%), the actual rates of O_2 evolution varied considerably. This variation is therefore most likely due to changes in the level of Triton X-100 associated with different PS II preparations.

In order to investigate whether addition of exogenous lipid would affect the rates of O_2 evolution a number of lipids and lipid mixtures were added to the PS II preparations. Fig.1 shows that incubation with total lipid extract gave a 80% stimulation above the control rate. In an attempt to identify the requirement of a specific lipid for this stimulatory effect, different lipid classes were used. As can be seen in fig.1, omission of MGDG, which represents nearly 50% of the total lipid content in thylakoids, had no apparent effect in the observed stimulation. In fact addition of MGDG

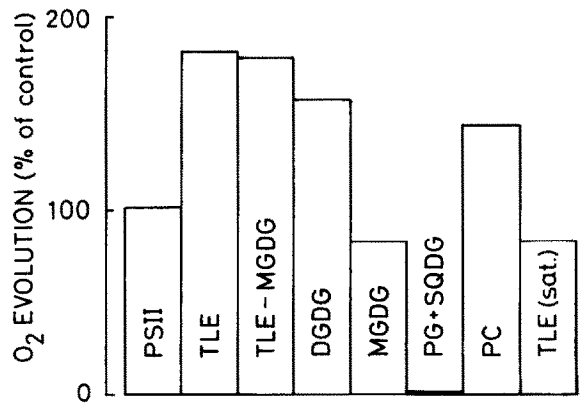

Fig.1. The effect of polar thylakoid lipids on the rates of O_2 evolution of Triton X-100-derived PS II preparation. 100% is 83 μmol O_2/h per mg chlorophyll. (This is a typical rate but variations from about 40–190 μmol O_2/h per mg chlorophyll were also observed without significant changes in protein or lipid composition.) The values given represent the average of 9 independent measurements on the same PS II preparation. The saturated TLE had an average number of double bonds per lipid molecule of 0.717 and contained 86.0% saturated fatty acids (16.7% palmitate and 69.3% stearate). Variations were within 5% with the same PS II preparation.

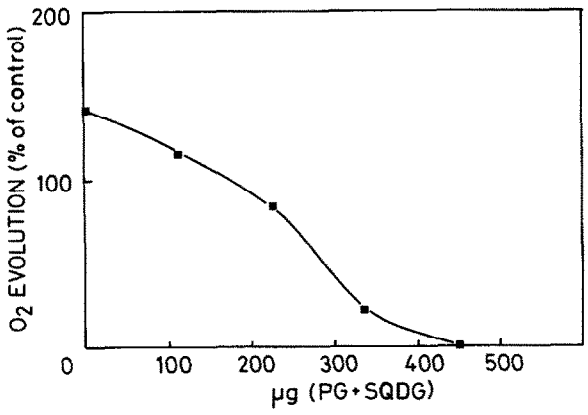

Fig.2. The dependent of oxygen evolution on the relative amounts of acidic lipids present. 100% represents the rate of oxygen evolution of the PS II preparation (83 μmol O_2/h per mg chlorophyll). Values were obtained by varying the amounts of (SQDG + PG) and PC, so as to maintain a constant lipid amount corresponding to a ratio of chlorophyll to added lipid of 1:14.

alone resulted in a slight inhibition. In contrast to the monogalactosyl lipid, DGDG and phosphatidylcholine (PC) stimulated O_2 evolution although not to the same extent observed with total polar lipid extract. As fig.1 also shows, the stimulation by the total lipid extract did not occur if the lipid was subjected to hydrogenation prior to addition to the sample, indicating the importance of unsaturated fatty acids for the enhancement of O_2 evolution.

A most striking observation was the inhibition of the capacity of the PS II preparation to evolve oxygen by the addition of the two acidic lipids. Complete loss of oxygen evolution was invariably observed under the conditions used. To examine whether this was a concentration effect, possibly related to the amount of net electrical charge present, samples were prepared in which the levels of charged lipids were varied by the addition of the zwitterionic lipid PC. As shown in fig.2, the anionic lipids inhibited O_2 evolution when present in amounts above 25–30% in the lipid mixture. This indicates that the inhibition observed is related to the net electrical charge present in the added lipid.

4. DISCUSSION

It is very likely that the PS II preparations used in this study are derived from the appressed region of the grana. However, it seems that these fragments are not portions of normal granal appressed lamellae as assumed in [15], since their lipid content is lower and different from that of untreated thylakoids and from granal appressed membranes obtained by mechanical disruption and phase separation [7]. A possible explanation is that Triton X-100 selectively solubilises the galactolipids, particularly DGDG. Even so the Triton preparations do maintain an O_2-evolving capacity which is DCMU sensitive. Thus, it must be concluded that the protein complexes involved in the water oxidation process are maintained in a functional conformation after isolation. Nevertheless, the stimulation by exogenous lipids indicates that the tertiary protein relationships are not the same as in vivo and that the addition of lipids allows optimisation of protein configurations to occur which enhances the rate of O_2 evolution. It is well known that Triton X-100 binds to hydrophobic parts of proteins [16] which could in our preparations partially modify the normal protein–protein and protein–lipid interactions necessary for maximum rates of O_2 evolution. It seems likely that the variation in O_2-evolution rates which we obtained from preparation to preparation (see legend to fig.1) reflects different extents of Triton interaction with the fragments.

The character of the acyl chains of the lipids appears to be important in that the saturated, unlike the unsaturated, total lipid extract caused no stimulation of O_2 evolution. An explanation for this may be that the more rigid hydrocarbon chains cannot satisfy the requirements for optimal protein–lipid interactions. The lack of effect of the major lipid component MGDG could be due to the inability of this lipid to adopt bilayer configurations in aqueous environments [11]. It should be noted that MGDG has recently been shown to facilitate close association of LHCP to the PS II core complex [17]. Our results do not contradict this conclusion since our preparations contain residual amounts of this lipid. The precise mechanism by which DGDG, PC and total lipid extract stimulate O_2 evolution in contrast to the action of MGDG is not yet clear but may reflect their

ability to form stable bilayers.

The inhibition of O_2 evolution by the acidic lipids, and the dependence of this inhibition on their relative amounts, suggests that besides the hydrophobic regions of the lipid, the polar head group characteristics are also of importance. Bearing in mind the already high levels of acidic lipids present in the preparation our results indicate that the introduction of increased amounts of negative charge into the system destabilizes the protein–protein and lipid–protein interactions necessary for O_2 evolution.

Clearly, our results indicate that certain types of lipids in certain proportions are required for optimising the rate of O_2 evolution and electron transfer on the oxidising side of PS II. Whether a complete delipidation of the PS II preparation would result in total inhibition of O_2 evolution, and if a specific lipid is required to restore activity, is as yet unknown and gives the basis for further experiments.

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