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REQUIREMENT OF GALACTOLIPIDS FOR PHOTOSYSTEM I ACTIVITY IN LYOPHILIZED SPINACH CHLOROPLASTS

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SUMMARY

1. The effect of monogalactosyl diacylglycerol and digalactosyl diacylglycerol on reconstitution of Photosystem I activity in heptane-extracted and galactolipase-treated spinach chloroplasts was investigated.

2. Both galactolipids, in a molar ratio with chlorophyll of 2.5, partially restored Photosystem I activity in heptane-extracted chloroplasts. An addition o saturating amounts of plastocyanin caused complete reactivation of Photosystem I.

3. Similarly, with galactolipase-treated chloroplasts, both galactolipids partially restored Photosystem I activity and additional amounts of plastocyanin were required for complete reactivation.

4. The action of galactolipids on partial reconstitution of Photosystem I supports the suggestion of their structural role in the restoration of thylakoid membranes.

INTRODUCTION

Lyophilized chloroplasts extracted with non-polar organic solvents lose the ability for photosynthetic electron transport (see ref. 1). Restoration of Photosystem II and I activity is possible on addition of lipid extract or some endogenous compounds [2–6].

In 1971, Brand et al. [7], measuring Photosystem I activity by transfer of electrons from TMPD through the chloroplast photosystem to methylviologen dye, found that exogenous triacylglycerols partially restored the activity of Photosystem I. Also, a crude heptane extract restored Photosystem I activity of extracted chloroplasts, although some components of this extract such as galacto-, sulpho- and phospholipids were not involved in the restoration.

Recently, it was shown [8] that α-tocopherol completely restored Photosystem I activity in heptane-extracted spinach chloroplasts. The concentration of α-tocopherol used for reconstitution of Photosystem I in these experiments suggested that α-tocopherol plays a structural role in restoration of the thylakoid membranes.

Abbreviations: DCMU, (3,4-dichlorophenyl)-1,1-dimethylurea; TMPD, *N,N,N,N*-tetramethyl-*p*-phenylene diamine; Tricine, V-tris(hydroxymethyl)methylglycine.

The heptane extract of spinach chloroplasts contain easily detectable concentrations of galactolipids. They are the main lipid components of the chloroplast membranes of higher plants and comprise about 80 % of their nonpigmented lipids. It was important, therefore, to test these compounds for ability to restore the activity of extracted chloroplasts and to define the catalytic or structural role of these lipids.

To obtain additional evidence for the effect of lipids on chloroplast structure and function, galactolipid lipase was used to deplete partly chloroplasts of their galactolipids. Recently it has been shown that endogenous and exogenous lipases cause disruption of membrane structure and affect chloroplast photochemical activities [9–12, 25].

The data reported in this paper concern the effect of monogalactosyldiacylglycerol and digalactosyldiacylglycerol on reconstitution of Photosystem I in heptane-extracted or galactolipase-treated chloroplast membranes. Preliminary experimental results support the examination of the role of galactolipids in Photosystem I [13].

MATERIALS AND METHODS

Spinach chloroplasts were isolated from market spinach leaves according to the method of Sane et al. [14].

The procedure for lyophilization of pelleted chloroplasts and for heptane extraction has been described earlier [8].

Galactolipids (mono- and digalactosyldiacylglycerol) necessary for reactivation of Photosystem I were extracted from freeze-dried chloroplasts with a mixture of chloroform and methanol (v/v 2 : 1). The lipid extract containing plastid pigments, galactolipids and phospholipids was separated on a DEAE cellulose column according to Allen et al. [15]. Purification of the galactolipids was achieved by rechromatography of the lipids on DE 11 cellulose columns. Thin-layer chromatography on silica gel G after Pohl et al. [16] was used for the purity test of the galactolipids. Galactolipids were assayed by determination of galactose according to Roughan and Batt [17], and factors of 4.3 and 2.6 were used to convert galactose to mono- and digalactosyldiacylglycerol, respectively [18].

Reconstitution was achieved by resuspending the extracted chloroplasts in heptane solution containing the quoted amounts of galactolipids. After evaporation of the solvent, dry preparations, resuspended in 0.05 M phosphate buffer (pH 7.4) plus 0.15 M KCl, were used for activity measurements.

Electron transfer from ascorbate/TMPD to methylviologen was measured by oxygen uptake in the reaction vessel with a Clark-type electrode at 24 °C. The reaction vessel was illuminated with red light at an incident intensity 2.5×10^5 ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. NADP photoreduction was measured by following the change in absorbance at 340 nm caused by illumination with red light of an intensity equal to 1.5×10^5 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. Experimental details for both assays are described in the figure and table legends.

Chlorophyll was assayed by the method of Arnon [19].

Ferredoxin and plastocyanin were prepared from spinach according to the procedure of Böger et al. [20]. Molar concentration of plastocyanin was calculated after Katoh [21].

A galactolipid lipase (ammonium sulphate fraction) was isolated and partially

purified from the chloroplast fraction of the primary leaves of bean according to Anderson et al. [22].

Protein content was estimated by the method of Schacterle and Pollack [23].

RESULTS

The relationship between the time of heptane extraction of lyophilized chloroplasts and Photosystem I activity is presented in Fig. 1. After a 10-hour extraction the activity decreased to about 20–30 % of its original rate and it slightly decreased as extraction was continued. An addition of extracted amounts of mono- and digalactosyldiacylglycerol to heptane-extracted chloroplasts stimulated electron flow in Photosystem I to about 50–60 % of the original Photosystem I activity. The saturating amounts of plastocyanin added to the reaction mixture completely restored Photosystem I activity independent of the time of extraction.

Fig. 2 shows galactolipid contents in chloroplast membranes after different times of heptane extraction. The decrease in the amount of both galactolipids removed during heptane extraction paralleled the decline in Photosystem I activity (see Fig. 1). However, large quantitative differences were observed, i.e. lipids were removed by about 20 % in relation to their content in the membrane, whereas Photosystem I activity decreased by about 80 %. In the course of extraction, the molar ratio of monogalactosyldiacylglycerol to digalactosyldiacylglycerol in the heptane extract was maintained in the range 1.5–1.7, thus approximating the molar ratio of galactolipids in

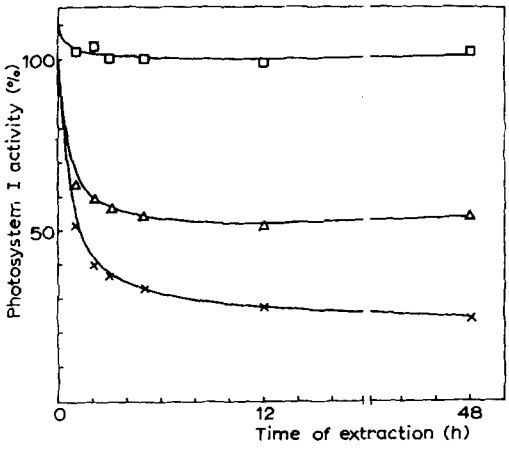

Fig. 1. Photosystem I activity in heptane-extracted spinach chloroplasts and in chloroplasts reconstituted with galactolipids and plastocyanin after different times of extraction. For reconstitution of chloroplast membrane, galactolipids extracted at different times and saturating amounts of plastocyanin were used. Addition of lipids to extracted chloroplasts were performed as described in the methods. The reaction mixture for measuring O_2 uptake contained the following components, in μmol: Tricine/NaOH buffer (pH 8.0), 150; DCMU, 0.03; sodium ascorbate, 50; TMPD, 0.2; methylviologen, 0.4; chloroplast equivalent to 15 μg of chlorophyll in a final volume of 3 ml. Rate values are expressed as percentage of the rate of lyophilized chloroplast activity which was 653 μmol O_2 uptake/mg chlorophyll per h. $\times - \times$, extracted chloroplasts; $\triangle - \triangle$, reconstituted chloroplasts with heptane-extracted galactolipids; $\square - \square$, galactolipid-reconstituted chloroplasts plus saturating amounts of plastocyanin.

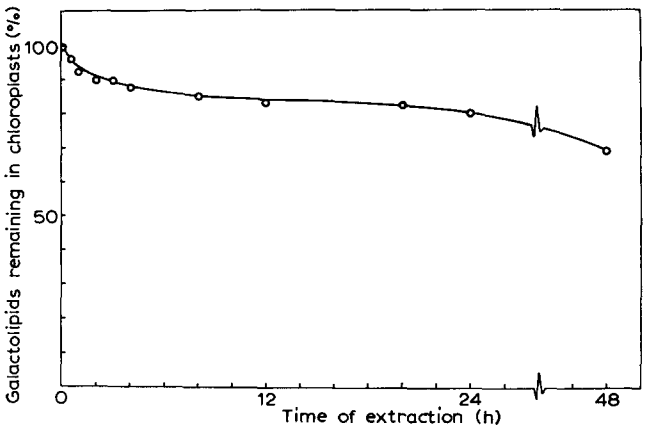

Fig. 2. Residual galactolipids in lyophilized spinach chloroplasts after different times of extraction with heptane. Estimation of galactolipids as described in the methods.

lyophilized chloroplasts which is 1.66.

Fig. 3 shows the reconstitution of Photosystem I of extracted chloroplasts in relation to increasing amounts of galactolipids. Both galactolipids partially reactivate this photosystem. The maximum rate of methylviologen in reconstituted chloroplasts was obtained at a galactolipid to chlorophyll molar ratio of 2.5. Under such conditions, monogalactosyldiacylglycerol caused an increase in Photosystem I activity to about 65 %, whereas digalactosyldiacylglycerol caused an increase to about 55 % of original rate before extraction. Simultaneous addition of both galactolipids in optimal amounts to extracted chloroplasts did not result in a significant increase in Photosystem I activity in comparison to monogalactosyldiacylglycerol and digalactosyldiacylglycerol used separately.

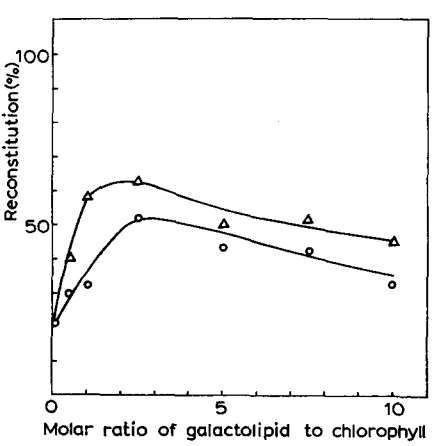

Fig. 3. The effect of mono- (△—△) and digalactosyldiacylglycerol (○—○) on reconstitution of Photosystem I in heptane-extracted spinach chloroplasts. The Photosystem I activity was assayed as described in Fig. 1.

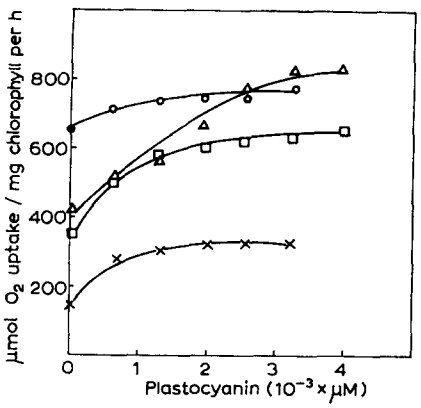

Fig. 4. The effect of plastocyanin on Photosystem I activity of heptane-extracted and reconstituted with galactolipids spinach chloroplasts. See Fig. 1 for measuring O_2 uptake. ○—○, lyophilized chloroplasts; × — ×, extracted chloroplasts; △ — △, extracted chloroplasts reconstituted with monogalactosyldiacylglycerol; □ —□, extracted chloroplasts reconstituted with digalactosyldiacylglycerol.

Assuming, after Elstner et al. [24], that heptane extraction partially removes plastocynin, the influence of plastocyanin on Photosystem I activity was tested. It appears from Fig. 4 that plastocyanin has little effect on the activity of lyophilized chloroplasts. Plastocyanin, however, increases the oxygen uptake in heptane-extracted chloroplasts and in those reconstituted with optimal amounts of galactolipids. In chloroplasts reconstituted with monogalactosyldiacylglycerol the effect of plastocyanin seems to be more distinct.

TABLE I

RECONSTITUTION OF PHOTOSYSTEM I ACTIVITY OF HEPTANE-EXTRACTED SPINACH CHLOROPLASTS

Experimental details for measuring O_2 uptake are given in Fig. 1. The reaction for $NADP^+$ photoreduction measurements contained the following components, in µmol: Tricine/NaOH buffer (pH 8.0), 150; DCMU, 0.03; sodium ascorbate, 50; TMPD, 0.2; $NADP^+$, 2.5; saturating amounts of ferredoxin, chloroplasts containing 30 µg chlorophyll. In both assays, where indicated, saturating amounts of plastocyanin were added. Addition of galactolipids to 24-h-extracted chloroplasts were performed as described in the methods. MGDG, DGDG, mono- and digalactosyldiacylglycerol.

Chloroplasts	Additions (mol/mol chlorophyll)	TMPD → methylviologen (µmol O_2 uptake/mg chlorophyll per h)		TMPD → $NADP^+$ (µmol NADP reduced/mg chlorophyll per h)	
		Plastocyanin		Plastocyanin	
Lyophilized	—	653	766	44	50
	2.5, MGDG	660	702		
	2.5, DGDG	643	730		
Heptane-extracted	—	145	309	5	22
	2.5, MGDG	418	815	9	38
	2.5, DGDG	360	634	12	33

The results summarized in Table I show reconstitution of Photosystem I activity of lyophilized and heptane-extracted chloroplasts measured by oxygen uptake in reaction with methylviologen as electron acceptor and by NADP photoreduction. Electron transport flow through Photosystem I measured by photoreduction of methylviologen is considerably restored by galactolipids and it reaches its original rate on addition to the reaction mixture of saturating amounts of plastocyanin.

The photoreduction of NADP reaction with ascorbate-TMPD is considerably lower and it depends more on plastocyanin being added than on galactolipids.

Further experiments were carried out to partially deplete chloroplast membranes of their galactolipids only by endogenous galactolipase isolated from the chloroplast fraction of the primary leaves of bean.

The dependence of Photosystem I activity on galactolipase concentration and incubation time of the reaction mixture is shown in Fig. 5. Using optimal enzyme concentration, the effect of incubation time of lyophilized chloroplasts with galactolipase on photosynthetic activity was determined. The character of the curve as well as the rate of Photosystem I inhibition are similar to the curve of heptane extraction. Galactolipase decreases the activity of chloroplasts to about 25 % of original activity just after 30 min of incubation.

As can be seen in Table II, 2-h-galactolipase treatment removed about 40 % of galactolipids from chloroplast membrane, however, a larger amount of monogalactosyldiacylglycerol than of digalactosyldiacylglycerol was removed. The molar ratio of monogalactosyldiacylglycerol/digalactosyldiacylglycerol in galactolipase-treated chloroplasts was significantly lower than in lyophilized ones. This is in agreement with the results of Anderson et al. [22], which suggest that digalactosyldiacylglycerol is hydrolized by the lipase at a slower rate than monogalactosyldiacylglycerol. Optimal concentration of mono- and digalactosyldiacylglycerol and of both these lipids used together in a molar ratio of 1.6 reactivated Photosystem I reduced by the action of galactolipase to about 40 % of original activity. The addition

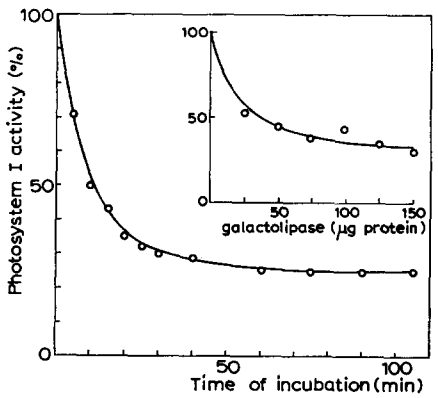

Fig. 5. The effect of galactolipase on Photosystem I activity in lyophilized spinach chloroplasts (upper curve, in relation to galactolipase content; lower curve, in relation to incubation time). Rate values are expressed as percentage of the rate of lyophilized chloroplast activity which was 510 μmol O_2 uptake/mg chlorophyll per h. The reaction conditions were identical to those described in Fig. 1. The galactolipase content in the lower curve was 200 μg protein per ml.

TABLE II

RECONSTITUTION OF PHOTOSYSTEM I ACTIVITY WITH GALACTOLIPIDS IN GALACTOLIPASE-TREATED SPINACH CHLOROPLASTS

Experimental details for measuring O_2 uptake are given in Fig. 1. In the experiment where both galactolipids were used, molar ratio of monogalactosyldiacylglycerol (MGDG) to digalactosyldiacylglycerol (DGDG) = 1.6. Where indicated, saturating amounts of plastocyanin were used. Addition of lipids to galactolipase-treated chloroplasts was performed as in heptane-extracted ones, see Methods for details. The galactolipase was added in an amount of 200 μg protein per ml. Incubation time was 2 h at 25 °C.

Chloroplasts	Additions (μmol/μmol chlorophyll)	TMPD → methylviologen (μmol O_2 uptake/mg chlorophyll per h)				% Galactolipids remaining in chloroplasts			MGDG/DGDG molar ratio
			%	Plastocyanin	%	MGDG	DGDG	Total	
Lyophilized	–	483	100			100	100	100	1.54
Galactolipase-treated	–	124	26	269	56	44	85	60	0.84
	2.5, MGDG	199	41	379	78				
	2.5, DGDG	199	41	380	79				
	2..5 MGDG + DGDG	212	44	396	82				

of saturating amounts of plastocyanin increased Photosystem I activity to about 80 % in relation to the activity of lyophilized chloroplasts. Thus, reconstitution of Photosystem I of galactolipase-treated chloroplasts seems to be similar to analogous processes in heptane-extracted chloroplasts.

The total inhibition of Photosystem I activity under the influence of lipase observed by Ostrovskaya et al. [11, 12] has not been confirmed in our experiments. It is possible that NADP photoreduction in Photosystem I is more sensitive to the action of lipase than photoreduction of methylviologen.

DISCUSSION

As appeared from the studies carried out, heptane extraction as well as hydrolysis by galactolipase of small amounts of galactolipids cause a considerable decrease in Photosystem I activity. The readiness of lipids to restore Photosystem I activity accounts for both heptane extraction and galactolipase action causing some small damage to the membrane. Such opinion with respect to heptane extraction was represented by Magree et al. [5] who found that extraction does not cause any major disruption of membrane structure. It should be stressed here that Anderson et al. [22], when studying the effect of lipase treatment of subchloroplast particles on electron transport have found that at least half of the galactolipids could be removed by lipase without strong effect on electron flow.

It is possible that heptane extraction and galactolipase treatment remove only this part of the galactolipids which are localized at the membrane surface and are responsible for Photosystem I activity.

It is known from the studies of Radunz [26] on localization of galactolipids with serological methods that some of the chloroplast monogalactosyldiacylglycerol is located at the surface of the thylakoids. Digalactosyldiacylglycerol did not react with the antiserum.

Bamberger and Park [9] concluded from their studies that galactolipids are not associated with chlorophyll but do constitute a continuous matrix, which is responsible for the properties of the thylakoid membranes.

Photosystem I activity decrease during lipase treatment is attributed, as is known, by some authors to inhibitory action of unsaturated fatty acids on electron flow. It is known that fatty acids are potent inhibitors of Photosystem II activity [27–29]. Brody et al. [30], however, showed that with a concentration ratio of linolenic acid to chlorophyll of $\leqslant 4$, inhibition of Photosystem I does not occur. This fact is supported by the results of Ostrovskaya et al. [11] which indicate that light subchloroplast fragments incubated with galactolipase for 4 h released fatty acids in amounts which did not cause Photosystem I inhibition.

Partial reconstitution of Photosystem I activity of lipase-treated chloroplasts with galactolipids occurs irrespective of a possible presence of fatty acids released during enzyme treatment.

Restoration of activity in heptane-extracted and galactolipase-treated chloroplasts was achieved by mono- and digalactosyldiacylglycerol to about the some extent with no synergistic effect on addition of both lipids together. This suggests that the restoration effect is non-specific.

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