Replacement of Galactolipids in Chloroplasts by Lecithin in the Presence of Lysolecithin

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Replacement of Galactolipids in Chloroplasts by Lecithin in the Presence of Lysolecithin

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Abstract The binding of lysolecithin (lysophosphatidyl choline) to chloroplast membranes and resulting release of membrane lipids were investigated. At low ratios of lysolecithin to chlorophyll, lysolecithin molecules were incorporated into the chloroplast membranes without loss of membrane components. High ratios of lysolecithin to chlorophyll extracted 50–60 % of monogalactosyl and digalactosyl diglycerides, and lecithin (phosphatidyl choline), and 10–15 % of phosphatidyl glycerol, sulfoquinovosyl diglyceride and chlorophyll from chloroplasts. When soybean lecithin liposomes were added to lysolecithin-treated chloroplasts, lysolecithin was stoichiometrically replaced by lecithin. The distribution of lipid molecules in the lateral plane of thylakoid membranes is discussed.

Introduction

Hoshina (1979a), and Hoshina and Nishida (1975) reported that when lysolecithin was added to spinach chloroplasts, the activity of electron transport was stimulated as a result of the uncoupling of photophosphorylation at low ratios of lysolecithin to chlorophyll (0.2–1.0 LPC/chl*), and inhibition of electron flow also was observed at 1.0–5.0 LPC/chl. Hoshina (1979b) also reported that the Hill reaction inhibited by lysolecithin was reactivated by an addition of lecithin containing unsaturated fatty acids. Lysolecithin, which is well-known for its lytic properties (Hoshina and Nishida, 1975; Neumann and Habermann, 1957; Tanford and Reynolds, 1976), belongs to a group of soluble amphiphiles with lyotropic mesomorphism described by Helenius and Simons (1975). Lysolecithin is known to bind strongly to membranes, such as myeline (Gent *et al.*, 1964) or sarcoplasmic reticulum (Deamer, 1973; Leibovitz-BenGershon *et al.*, 1972) and to cause a dispersion or solubilization of the membrane components, such as lipids and proteins.

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* Abbreviations used are: LPC, lysolecithin (lysophosphatidyl choline); LPC/chl, ratio of μg lysolecithin per μg chlorophyll as a amount of lysolecithin added to chloroplast suspension.

There have been many papers in the literature (Helenius and Simons, 1975 ; Tanford and Reynolds, 1976) on the use of detergents in the research of membrane structure and functions. A type of these studies with detergents is an attempt to explain the exact mode of association of membrane proteins with lipid molecules, and the specificity of interaction with different chemically defined portions of lipid molecules on the influence of bound lipid in the structure of the protein. There are a few reports about the interaction of detergent and thylakoid membranes, although many detergents have been used for preparation of active subchloroplast particles, reaction centers or light-harvesting chlorophyll protein complexes (Boardman, 1970 ; Thornber, 1975). Okamoto and Katoh (1977) and Okamoto *et al.* (1977) recently reported the instructive results of studies on the interaction of linolenic acid and chloroplast membranes. They showed that binding of linolenic acid to spinach chloroplasts occurred through partition of fatty acid between the membrane and aqueous medium (Okamoto and Katoh, 1977). They also reported that linolenic acid-induced changes in chloroplast structure were the main cause of the changes in capacity of chloroplasts to bind free fatty acid (Okamoto *et al.*, 1977).

The purpose of the present study is to determine the amount of lysolecithin incorporated into chloroplast membranes and the classes of lipids released from the membranes, and to elucidate the interaction between lysolecithin and chloroplasts. In addition, the present paper describes the interaction between lysolecithin and lecithin in chloroplast membranes as the lysolecithin-induced inhibition of the Hill reaction was reactivated by the addition of lecithin.

Materials and Methods

Preparation of chloroplasts

Type C chloroplasts (Hall, 1972) were prepared from spinach leaves (purchased at the local market) in a chilled medium containing 0.3 M NaCl and 50 mM Tris-HCl (pH 7.5) as previously described (Hoshina, 1979a). The isolated chloroplasts were resuspended in the same medium to obtain 0.2-0.4 mg chlorophyll per ml, and kept at 0-4°C before use. Chlorophyll concentrations were measured by the method of Arnon (1949).

Treatment with reagents

Chloroplasts were incubated with lysolecithin at about 25°C (room temperature) for 8-10 min as the incubation time longer than 4 min was required to complete the effect of lysolecithin on the Hill reaction (Hoshina and Nishida, 1975). When soybean lecithin liposomes were added to the lysolecithin-incubated chloroplasts, the incubation was continued for anotehr 8-10 min at about 25°C, because the effect of soybean lecithin on restoration of the Hill reaction in lysolecithin-treated chloroplasts was complete within 6 min (Hoshina, 1979b). The basal medium contained 0.3 M NaCl, 50 mM Tris-HCl (pH

7.5) and 0.6 % ethanol. After the incubation of the chloroplasts with lysolecithin or with lysolecithin and lecithin, the suspensions were rapidly cooled by placing on ice and centrifuged at 144,000 x g for 1 hr at 4°C.

Lipid analysis

Lipids were extracted from the supernatant or the chloroplast suspension using chloroform-methanol-water mixture according to the method of Bligh and Dyer (1959). Lipids were purified with DEAE-cellulose column chromatography followed by silica gel G thin-layer chromatography as described previously (Hoshina *et al.,* 1975). For lipid analyses, the locations of the compound were revealed by iodine vapor and each lipid was carefully removed by scraping off the silica gel with a razor blade after the iodine was evaporated. Glycolipids were determined colorimetrically in the presence of the silica gel by the phenol-sulfuric acid procedure of Roughan and Batt (1968). Phospholipids were measured in the presence of the silica gel by the phosphomolybdate colorimetric method of Parker and Peterson (1965).

Reagents

Solvents of analytical grade were used without redistillation. Lysolecithin (type I, from egg yolk) and soybean lecithin (commercial grade, type II-S) were purchased from Sigma. Lysolecithin was used without purification, although it was always contaminated by small amounts of impurities. Soybean lecithin was purified by silicic acid column chromatography followed by DEAE-cellulose column chromatography. Soybean lecithin purified in this way showed a single spot by silica gel G thin-layer chromatography. Soybean lecithin liposomes were prepared as previously described (Hoshina, 1979b).

Results

Hoshina and Nishida (1975) previously described that effect of lysolecithin on the activity of ferricyanide photoreduction depended on the ratio of lysolecithin to chlorophyll rather than on its concentration alone. Thus, the amounts of lysolecithin bound to chloroplasts were determined as a function of the ratio of μg lysolecithin per μg chlorophyll (Fig. 1). At low ratios of lysolecithin to chlorophyll (lower than 1 LPC/chl), free lysolecithin was barely detected, indicating most of lysolecithin added were bound to chloroplasts. At ratios lower than 1.0-1.5 LPC/chl, the amount of lysolecithin incorporated into chloroplasts was found to be strongly dependent on the amount of lysolecithin added, but at ratios higher than that, the dependency was much less. At 1.0-1.5 LPC/chl, the concentration of free lysolecithin was 10-15 nmoles/ml. This is very close to the critical micelle concentration of lysolecithin (Leibovitz-BenGershon *et al.,* 1972). A plot of 1/[bound LPC] versus 1/[free LPC] (Klotz, 1946) did not show a straight line. This indicates that the increase in binding at higher ratios of lysolecithin

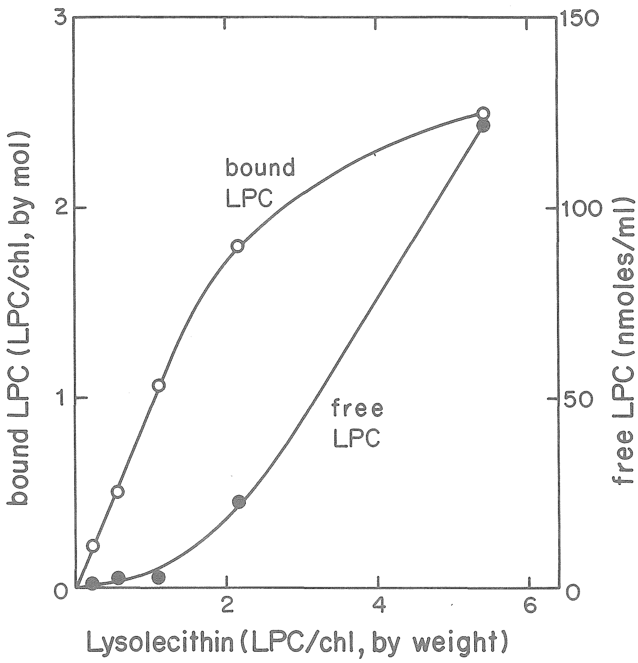

Fig. 1. Binding of lysolecithin with chloroplast membranes as a function of lysolecithin added. Chloroplasts (30.2 μg chl/ml) were incubated with various concentrations of lysolecithin. The suspensions were centrifuged and the supernatant was assayed to determine the concentration of lysolecithin. The amounts of bound lysolecithin which was given as the ratio of mole lysolecithin per mole chlorophyll, were calculated from the difference of the concentrations of free and added lysolecithin. In the absence of chloroplasts, lysolecithin added was totally recovered in the supernatant.

to chlorophyll is cooperative, although the binding site could not accurately be determined.

Fig. 2 shows that chlorophyll and galactolipids were released from chloroplast membranes by adding various amounts of lysolecithin. At 1.1 LPC/chl, the release of galactolipids was observed to be significant, but no chlorophyll was detected. The amounts of monogalactosyl and digalactosyl diglycerides released, increased with increasing ratio of lysolecithin to chlorophyll. At 5.4 LPC/chl, 50–60 % of monogalactosyl and digalactosyl diglycerides in original chloroplasts were detected in the supernatant. The extraction of chlorophyll began at 2.2 LPC/chl, and about 10 % of chlorophyll was released at 5.4 LPC/chl. Protein was slightly detected in the supernatant obtained from chloroplasts treated with 1.1 LPC/chl, and the release of protein increased with increasing ratio of lysolecithin to chlorophyll (data were not shown).

Table 1 shows that lysolecithin preferentially extracted glycerolipids from chloroplast membranes. The extraction of lecithin as well as monogalactosyl and

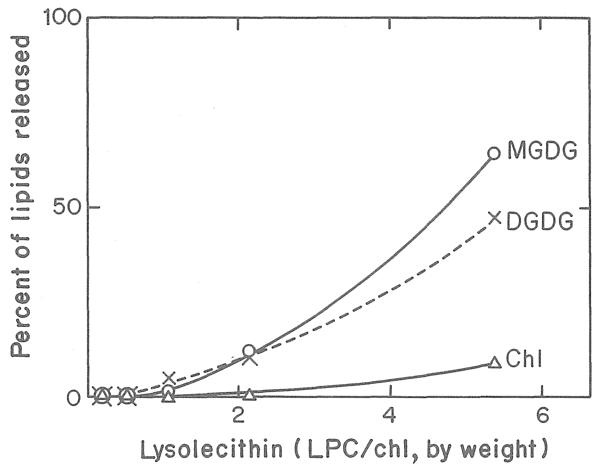

Fig. 2. Release of chlorophyll, monogalactosyl and digalactosyl diglycerides by the addition of lysolecithin. Experimental conditions are described in Fig. 1. The suspension of control chloroplasts and the supernatant of lysolecithin-treated chloroplasts were assayed to determine the amounts of chlorophyll and galactolipids. In the suspension of control chloroplasts, contents of monogalactosyl and digalactosyl diglycerides, and chlorophyll were 82.6, 39.4 nmoles/ml and 30.2 μg/ml, respectively. MGDG, monogalactosyl diglyceride; DGDG, digalactosyl diglyceride.

digalactosyl diglycerides was 50–60 % of the value in the untreated membranes, while the release of sulfoquinovosyl diglyceride, phosphatidyl glycerol and chlorophyll was only 10–15 % of the original value. In addition, the release of phosphatidyl inositol was 25 %. Thus, lysolecithin caused the selective extraction of chloroplast lipids. Total amounts of glycerolipids in the supernatant were 63.1 nmoles/ml and the amount of bound lysolecithin corresponded to 76.6 nmoles/ml, as was calculated from Table 1. It was obtained from three separate experiment that total amounts of glycerolipids released were 82–98 % of the value of bound lysolecithin.

The data shown in Table 1 was obtained from the chloroplasts prepared in the summer. It may be noticed that the amounts of lecithin and phosphatidyl glycerol in this experiment are lower compared with data reported in the literature (Allen *et al.*, 1966; Hoshina *et al.*, 1975; Lichtenthaler and Park, 1963; Wintermans, 1960). The results obtained from chloroplasts with normal lipid composition in winter and spring materials also showed a similar trend to the selective extraction of glycerolipids as illustrated in Table 1.

As the reactivation of the Hill reaction in lysolecithin-incubated chloroplasts was observed on the addition of lecithin containing unsaturated fatty acids (Hoshina, 1979b), it was suggested that the chloroplasts delipidated lysolecithin were reconstituted with lecithin. Table 2 shows that when soybean lecithin liposomes were added to the lysolecithin-incubated chloroplasts, the amounts of lysolecithin in the supernatant were increased, indicating replacement of lysolecithin by soybean lecithin added. At the concentration of lecithin, 107 nmoles/ml, which induced the slight reactivation (Hoshina,

Table 1 Lysolecithin-induced release of glycerolipids from chloroplast membranes

	chl	LPC	MGDG	DGDG	SQDG	PC	PG	PI
	μg/ml	nmoles/ml						
suspension of control chloroplasts	27.5	Trace	77.9	32.6	12.2	2.3	1.8	2.0
supernatant of lysolecithin-treated chloroplasts	4.0	160.9	40.4	18.7	1.9	1.3	0.3	0.5

Experimental procedures are described in Fig. 2 except that chloroplasts (27.5 μg chl/ml) were incubated with lysolecithin (5.9 LPC/chl). The concentration of lysolecithin was 237.5 nmoles/ml. MGDG, monogalactosyl diglyceride; DGDG, digalactosyl diglyceride; SQDG, sulfoquinovosyl diglyceride; PC, lecithin (phosphatidyl choline); PG, phosphatidyl glycerol; PI, phosphatidyl inositol.

1979b), the amount of bound lysolecithin was reduced to about half of the value without lecithin, and the chloroplasts took up lecithin instead of lysolecithin. When 417 nmoles/ml of lecithin was added, the amount of bound lysolecithin further decreased, and the amount of lecithin taken up by chloroplasts almost corresponded to the amount of lysolecithin released. Table 2 also shows that chlorophyll released by lysolecithin is again taken up by the reconstituted chloroplast membranes. However, the amounts of galactolipids in the supernatant were not changed by the addition of lecithin (data were not shown).

Table 2 Replacement of lysolecithin bound to chloroplast membranes by soybean lecithin

No	Incubation with		Lipids in supernatant			Calculation		
	LPC	PC	chl	LPC	PC	binding of LPC	binding of PC	release of LPC by PC
	nmoles/ml		μg/ml	nmoles/ml		moles/mole chl		
1	157	—	1.6	15	Trace	5.54	—	—
2	157	107	1.1	75	82	3.20	0.98	2.34
3	157	426	0.4	112	323	1.76	3.95	3.78

No 1: Chloroplasts (23.0 μg chl/ml) were incubated with lysolecithin. No 2 and 3: Chloroplasts (23.0 μg chl/ml) were incubated with lysolecithin, followed by incubation with soybean lecithin. After the incubation, the suspensions were centrifuged and the contents of lipids in the supernatant were determined. The amounts of lysolecithin released by lecithin were calculated from the difference in the amounts of bound lysolecithin in the presence and in the absence of lecithin. The amount of lysolecithin added was 4.7 LPC/chl. When lecithin was incubated with lysolecithin in the absence of chloroplasts, both molecules were totally recovered in the supernatant. PC, lecithin.

Discussion

The present study suggests the following sequence of events as increasing amounts of lysolecithin are added to chloroplast membranes.

Stage 1 : At ratios lower than 1.0 LPC/chl, lysolecithin molecule was incorporated into chloroplasts without loss of membrane components (Figs. 1 and 2). However, the decrease of chloroplast volume was observed (Hoshina and Nishida, 1975). The binding of lysolecithin may cause changes in physical properties of thylakoid membranes, resulting in uncoupling of photophosphorylation (Hoshina, 1979a ; Hoshina and Nishida, 1975).

Stage 2 : At 1.0–1.5 LPC/chl (probably critical micelle concentration of lysolecithin), lysolecithin caused the extraction of monogalactosyl and digalactosyl diglycerides and proteins from chloroplast membranes without release of chlorophyll. Lysolecithin also caused the inhibition of electron transport activities of both photosystems I and II (Hoshina, 1979a ; Hoshina and Nishida, 1975).

Stage 3 : At ratios higher than 1.5 LPC/chl, lysolecithin incorporated into chloroplast membranes further increased with loss of chlorophyll, glycerolipids and protein (Figs. 1 and 2, Table 1), resulting in disintegration of membranes into vesicles, spherical particles or strands (Hoshina and Nishida, 1975).

It was clearly shown that lysolecithin caused the selective extraction of chloroplast lipids (Fig. 2, Table 1). The release of phosphatidyl inositol was intermediate between that of galactolipid and sulfoquinovosyl diglyceride (Table 1), but the release of phosphatidyl inositol greater than 25 % was often observed in the other experiments. This indicates that phosphatidyl inositol may belong to the group of lipids which are more easily extracted. The selectivity of lipid solubilization may have many different explanations according to those suggested by Helenius and Simons (1975). 1. Detergents may have different solubilizing powers for different lipids. 2. The selectivity of lipid solubilization may reflect the difference of lipid distribution in different regions of the membranes. 3. The selectivity may result from specific interactions between lipids and other membrane components (mainly proteins) which would influence the ability of extraction.

It may be impossible that explanation 1 can be applied to the selectivity because the chemical and physical properties of lipids easily extracted by lysolecithin, such as charged groups and fatty acid composition (Allen *et al.,* 1966 ; Lichtenthaler and Park, 1963) or phase behavior in aqueous solution (Shipley *et al.,* 1973), are not similar.

It has been suggested that monogalactosyl diglyceride is located chiefly in the external half of the membrane bilayer and digalactosyl diglyceride is located predominantly in the inner half (Anderson, 1975 ; Heise and Jacobi, 1973 ; Radunz, 1972 ; Radunz, 1976). Allen *et al.* (1972) reported that the molar amounts of glycerolipids were similar in grana and stroma lamellae, as they were expressed either on a protein or a total lipid basis. It may be, therefore, also impossible to understand the selectivity by explanation 2.

It may be reasonable that lipids, specifically interacted with membrane proteins, are more difficult to extract by the action of lysolecithin. In some experiments using detergents or spin labelled reagents, membrane lipids are shown to be classified into two groups. One of these is called fluid matrix lipids (or bilayer lipids), able to diffuse rapidly in the plane of the bilayer. The other group is immobile lipids, bound tightly to membrane proteins (Jost *et al.,* 1973 ; Träuble and Overath, 1973 ; Warren *et al.,* 1975). The latter are so-called boundary lipids (Jost *et al.,* 1973) or annular phospholipid (Warren *et al.,* 1975). The selective solubilization of chloroplast lipids suggests that thylakoid membranes may be composed of a lipid bilayer consisting of lipids, such as monogalactosyl and digalactosyl diglycerides, lecithin, and probably phosphatidyl inositol which are more easily extracted. Sulfoquinovosyl diglyceride, phosphatidyl glycerol, and chlorophyll may be boundary lipids attached to chlorophyll-protein complexes or other intrinsic proteins in thylakoid membranes. Anderson (1975) and Leech and Murphy (1976) assumed that galactolipids containing polyunsaturated fatty acids might be the candidates for the fluid bilayer, because the greater the degree of unsaturation of the acyl groups, the greater the degree of membrane fluidity, and that boundary lipids in thylakoid membranes might be sulfoquinovosyl diglyceride and phospholipids (especially phosphatidyl glycerol) from a view point of their fatty acid composition. Recently, Hirayama and Nomotobori (1978) reported that by hydrolyzing chloroplast phospholipids with phospholipase A_2, 82.6 % of the original phospholipids were lost and the residual phospholipids were 24.0 % of total phosphatidyl glycerol and 7.9 % of total lecithin. They suggested that phospholipids resistant to the enzyme action were assigned to the tightly bound phospholipids. In addition, Menke *et al.* (1976) reported that the circular dichroism of a polypeptide fraction (24,000 dalton) from thylakoids was increased by the addition of sulfoquinovosyl diglyceride but not of phosphatidyl glycerol, monogalactosyl and digalactosyl diglycerides. This special interaction of thylakoid membrane components observed in vitro indicates probably the special distribution of membrane components in native chloroplasts.

It is interesting that the amounts of lysolecithin incorporated into chloroplast membranes are approximately equal to those of glycerolipids released (Table 1), and about 50 % of total glycerolipids was extracted by the action of lysolecithin (Table 1). In addition, the amounts of lecithin incorporated were almost equal to that of lysolecithin released when soybean lecithin liposomes were added to the lysolecithin-incubated chloroplasts (Table 2, No 3). This shows that about half of the lipids in the original chloroplast membrane can be replaced by other lipids. Investigation of the properties of these reconstituted membranes may be useful in understanding the function of thylakoid membranes.

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