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Lipid Composition of Plasma Membranes and Tonoplasts Isolated from Etiolated Seedlings of Mung Bean (*Vigna radiata* L.)[1,2]

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ABSTRACT

The lipid composition of plasma membranes and tonoplasts from etiolated mung bean hypocotyls was examined in detail. Phospholipids, sterols, and ceramide monohexoside(s) were the major lipid classes in both membranes. The content of phospholipids on a protein basis was higher in the tonoplast, but the content of total sterols was similar in both membranes. Accordingly, the sterol to phospholipid molar ratio in the plasma membrane was higher than that of the tonoplast. Phosphatidylethanolamine and phosphatidylcholine comprised the major phospholipids in both membranes. Phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol were identified as minor phospholipid components. The content of phosphatidylinositol and phosphatidylglycerol was relatively high in the tonoplast, comprising 11 and 5% of the total phospholipids, respectively. Although special care was taken against the degradative action of phospholipase D and phosphatidic acid phosphatase during the isolation of these membranes, by adding EDTA, EGTA, KF, choline, and ethanolamine to the homogenizing medium, significant amounts of phosphatidic acid, about 15% of the total phospholipids, were detected in the plasma membrane. On the other hand, the content of phosphatidic acid in tonoplasts and other membrane fractions was very low. This fact may indicate that high levels of phosphatidic acid occur naturally in plasma membranes. Phosphatidylglycerol in both membranes and phosphatidylinositol in the tonoplast contained high levels of palmitic acid, which comprised more than 50% of the total fatty acids. Significant differences were observed in the sterol compositions of plasma membranes and tonoplasts. More than 90% of the sterols in the plasma membrane were unesterified, while the tonoplast was enriched in glycosylated sterols, especially acylated sterylglycosides. Ceramide monohexoside was found to be specifically located in these membranes, in particular, in the tonoplast, in which it comprised nearly 17% of the total lipids.

Lipids are recognized as major chemical components of biomembranes and play an important role in cellular activities through control of permeability, fluidity, and membrane-bound enzyme activities. In plants, considerable information on the lipid composition of chloroplasts and mitochondria (21), which are able to be easily isolated, is available. However, information is quite limited regarding the lipid composition of plasma membranes and tonoplasts, which also possess very important physiological functions including in the former, transport (22), cell wall biosynthesis (14), hormone action (24), phytochrome responses (18), and disease resistance (27), and in the latter, cellular compartmentation of lytic enzymes, acids, ions, and secondary products (11, 13). In addition, these membranes have been shown to play an important role in cold acclimation (29, 31, 33), freezing injury (26), and chilling stress (32).

Recently we have established isolation methods for plasma membranes and tonoplasts from etiolated mung bean hypocotyls (34, 35). In the present study, we have analyzed the lipid composition of both membranes and compared them. Plasma membranes are characterized by their high content of phosphatidic acid and tonoplasts by their high content of ceramide monohexoside.

MATERIALS AND METHODS

Plant Materials. Seeds of mung bean (*Vigna radiata* [L] Wilczek) were imbibed and germinated at 26°C in the dark on a stainless steel mesh installed at one-third depth of a square plastic box (20 × 30 × 16 cm). About 3 L of 1 mM $CaSO_4$ solution in distilled H_2O were added to the level of the seeds on the stainless steel mesh. After germination for 4 d, hypocotyls were excised from the etiolated seedlings.

Preparation of Membranes. Plasma membranes and tonoplasts were isolated from hypocotyl tissues as reported earlier (34, 35). Briefly, the tissues were homogenized in a Polytron PT30 at a medium speed setting in a medium containing 0.25 M sucrose (for plasma membrane isolation) or 0.25 M sorbitol (for tonoplast isolation), 75 mM MOPS[3]/KOH, pH 7.6, 5 mM EGTA, 5 mM EDTA, 10 mM KF, 1.5% PVP, 0.5% defatted-BSA, 2 mM PMSF, 4 mM SHAM, 10 μg/ml BHT, and 5 mM $K_2S_2O_5$. The homogenates were passed through four layers of gauze and submitted to differential centrifugation. The 3,600 to 156,000*g* pellets and 12,500 to 156,000*g* pellets were used as the starting materials for the isolation of tonoplasts and plasma membranes, respectively. Tonoplast membranes were collected at the interface of the sample load and the top of the sucrose layer after a linear sucrose density gradient (35). Plasma membranes were purified after a two-polymer phase partition technique and subsequent discontinuous sucrose gradient centrifugation of the plasma membrane-

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[3] Abbreviations: MOPS, 3-(*N*-morpholino)-propanesulfonic acid; BHT, butylated hydroxytoluene; PMSF, phenylmethylsulfonyl fluoride; SHAM, salicylhydroxamic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; PI phosphatidylinositol; PA, phosphatidic acid; CMH, ceramide monohexoside.

enriched upper phase on 32 and 43% sucrose layers as reported elsewhere (34). The membrane fraction which partitioned into the lower phase after phase partitioning was enriched in ER and unidentified yellow colored membrane materials and is designated as 'lower phase fraction.'

Mitochondria were isolated on a linear sucrose density gradient (15–45%, w/w). The 1,500 to 12,500*g* membrane fraction was first submitted to a phase partition to remove plasma membranes and the resultant lower phase enriched in mitochondria was recovered as a pellet. The pellet was resuspended in 0.25 M sucrose-5 mM MOPS/KOH (pH 7.3) and loaded onto a linear sucrose density gradient. After centrifugation at 26,000 rpm for 2 h in a Hitachi SW-2 rotor, mitochondria were banded at the 40 to 41% sucrose layer.

Extraction and Analysis of Membrane Lipids. Lipids were extracted from the isolated membranes (equivalent to 5 mg protein) according to Bligh and Dyer (2) except that isopropanol was used instead of methanol. The lipid extracts were separated on two-dimensional TLC with solvent mixtures of acetone/benzene/methanol/water (80:30:20:10, by volume) in the first dimension and chloroform/acetone/methanol/acetic acid/water (100:40:20:30:10, by volume) in the second dimension. After development, spots were located with iodine vapor or by spraying with 30% H_2SO_4 and subsequent heating. Lipids were identified by cochromatography with authentic samples.

Phospholipids and sterols were quantified according to the method described previously (33). The area corresponded to each phospholipid on the TLC was scraped into a test tube and directly ashed by addition of 70% $HClO_4$ with heating. The liberated Pi was analyzed by the Fiske-Subbarow reagent. Ceramide monohexoside and galactolipids were quantified as described by Roughan and Batt (23) (phenol-H_2SO_4 method). Glucose and galactose were used as standards, respectively. Ceramide monohexoside was also quantified by measuring the content of methylated sugars released after hydrolysis with 5% methanolic HCl at 100°C for 6 h as described by Fujino *et al.* (4–6). After hydrolysis, fatty acid methylesters and long chain bases were extracted with hexane and ether, respectively, and aliquots of the residual aqueous layer were analyzed for methylated sugars by the anthrone method (1). Methyl glucose was used as standard. The latter method gave a slightly lower value than the former method.

For the analysis of fatty acids, each phospholipid separated by two-dimensional TLC was transmethylated in 0.5 N sodium methoxide solution at 50°C for 15 min. The fatty acid methylesters were analyzed by GC using an FS-WCOT (FFAP) capillary column (0.25 mm × 10 m) purchased from Gasukuro Kogyo Co., Ltd.

***In Vitro* Degradation of Membrane Phospholipids.** Membrane samples (1 mg/ml) were incubated at 30°C for 30 min in a medium containing 2 mM $CaCl_2$ and 20 mM sodium acetate buffer (pH 5.0), in a final volume of 0.8 ml. The reaction was terminated by cooling to 0°C and by the addition of 0.2 ml glacial acetic acid. Lipids were extracted from the membrane suspension as described above. The lipid extracts were separated by one-dimensional TLC using a solvent mixture containing chloroform/methanol/acetic acid (65:25:8, by volume) or on two-dimensional TLC as described above. Each phospholipid class was quantified as described above.

Protein Analysis. Protein was analyzed by the method of Bradford (3).

Chemicals. Phospholipid standard samples were obtained from Sigma. Sterols and galactolipids were extracted and purified from spinach leaves. CMH purified from rice stalks was kindly provided by Dr. Y. Fujino, Obihiro University of Agriculture and Veterinary Medicine. Silica gel plates, Merck 5721, were used for TLC.

RESULTS

Figure 1 shows a two-dimensional thin-layer chromatogram of tonoplast lipids. The major phospholipids are PC and PE. PS, PG, PI, and PA are the minor phospholipid components. Free sterols (S) and sterylglycosides (SG, ASG) are the major neutral lipids. Although the quantities are small, mono- and digalactosyldiglycerides (MDG, DGD), and unidentified less polar lipids are detectable on the TLC. CMH, which was identified by cochromatography with a CMH sample purified from rice stalks, which was kindly provided by Dr. Fujino of Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan, was detected in the tonoplast in relatively large amounts. As will be described below, the CMH was also detected in plasma membranes.

Table I summarizes the content of major lipid classes in plasma membranes and tonoplasts. The content of total lipids per mg protein basis was higher in the tonoplasts than in the plasma

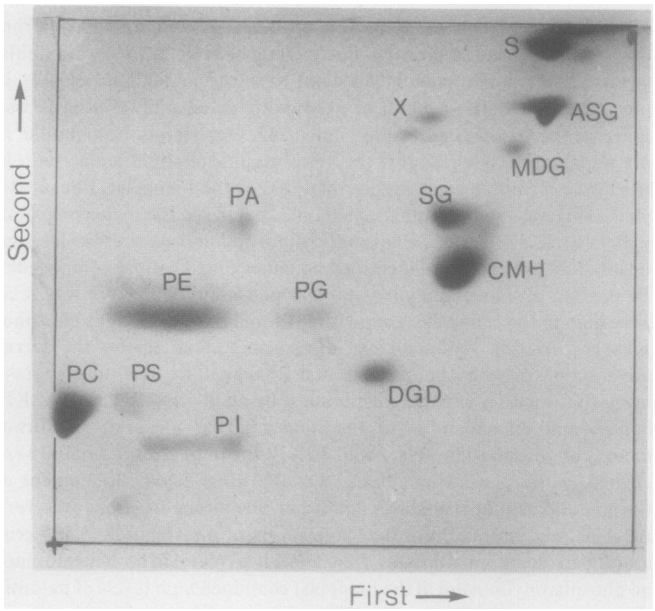

FIG. 1. Two-dimensional thin-layer chromatogram of tonoplast total lipids. The solvent mixture was acetone/benzene/methanol/water (80:30:20:10, by volume) for the first dimension and chloroform/acetone/methanol/acetic acid/water (100:40:20:30:10, by volume) for the second dimension. Spots were developed by spraying with 30% H_2SO_4 and subsequent heating. Spots were identified as PC, PE, PI, PS, PG, PA, free sterol (S), acylated sterylglycoside (ASG), sterylglycoside (SG), monogalactosyl diglyceride (MDG) and digalactosyldiglyceride (DGD). X: unidentified lipids.

Table I. *Lipid Composition of Plasma Membranes and Tonoplasts Isolated from Etiolated Mung Bean Hypocotyls*

The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

Lipid Class	Plasma Membranes	Tonoplasts
	$\mu mol \cdot mg^{-1}$ protein	
Phospholipids	1.29	1.93
Sterols	1.15	1.05
Ceramide monohexoside	0.18	0.63
Monogalactosyldiglyceride	Trace	0.04
Digalactosyldiglyceride	0.02	0.13
Total	2.64	3.78
Sterol/Phospholipid ratio	0.89	0.54

membranes. Total sterol to phospholipid ratio was higher in the plasma membrane than in tonoplasts, i.e., 0.89 *versus* 0.54, while the content of CMH was much higher in the tonoplasts, amounting to 16.6% on molar basis.

Table II shows the mole percentage of individual lipids which were separated and identified by two-dimensional TLC. Nearly half the total lipids in both membranes consisted of phospholipids. Although PC and PE were the major phospholipids in both membranes a relatively high amount of PA, *i.e.* nearly 15% total phospholipids, was detected in the plasma membranes. On the other hand, the content of PA was low in the tonoplasts, *i.e.* less than 2% of total phospholipids. Tonoplasts and plasma membranes contained high contents of sterols, 28 and 43% of total lipids, respectively. Most of the sterols in the plasma membranes were not glycosylated, but about 35% of the sterols in the tonoplast were glycosylated. Both the plasma membranes and tonoplasts contained relatively high levels of CMH, especially the tonoplast, in which the content amounted to 17% of the total lipids. As shown in Table III, CMH was present at higher concentrations in plasma membranes and tonoplasts than in other membranes, including mitochondria and the membrane fraction which partitioned into the lower phase after a phase partition of the crude microsomal fraction. This latter fraction was enriched in ER and unidentified yellow colored membrane materials.

Table II. *Mole Percent Composition of Lipids in Plasma Membranes and Tonoplasts Isolated from Etiolated Mung Bean Hypocotyls*

The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

Lipids	Plasma Membranes	Tonoplasts
	mol %	
Phospholipids		
PI	2.6	5.7
PS	1.5	2.2
PC	16.0	23.7
PE	18.6	16.0
PG	2.2	2.3
PA	8.0	1.1
Subtotal	48.9	51.0
Sterols		
Free sterols	39.5	18.2
Acylated sterylglycoside	1.5	7.4
Sterylglycoside	2.3	2.3
Subtotal	43.3	27.9
CMH	6.8	16.6
Monogalactosyldiglyceride	0.2	1.0
Digalactosyldiglyceride	0.8	3.4
Total	100.0	99.9

Table III. *Relative Content of CMH in Various Membrane Fractions*

The content was expressed as the molar ratio of CMH to phospholipids. Mitochondria were purified on a linear sucrose density gradient as described in "Materials and Methods." The lower phase refers to membrane fractions which partitioned into the lower phase of the phase partition system, and which are enriched in ER and unidentified yellow colored membrane materials. The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

Membrane Fraction	Mole Ratio of CMH to Phospholipids
Plasma membranes	0.140
Tonoplasts	0.326
Mitochondria	0.050
Lower phase	0.088

The fatty acid composition of phospholipids from plasma membranes and tonoplasts are presented in Table IV and V. PG, PI, and PE contained high levels of palmitic acid, and consequently had relatively low ratios of unsaturated to saturated acids. PS, PC, and PA contained high levels of polyunsaturated fatty acids. The fatty acid composition of PA was not exactly the same as that of PC or PE. The content of 20:3 in PA was low compared with that of PC or PE. In general, tonoplast phospholipids, especially PG and PI, were less unsaturated than those of plasma membranes. The fatty acid composition of PA in tonoplasts was quite different from that of the other phospholipids, in that it contained relatively high amount of 20:2.

Most plants contain a soluble phospholipase D (7, 19), which may degradate phospholipids into PA during homogenization of tissues and subsequent steps of membrane fractionation. Table VI compares the protective effect of various homogenization media. When 5 mM EGTA was included in the homogenizing medium as a chelator of Ca^{2+} ion, the PA content of isolated plasma membranes was about 20%, whereas the PA content of tonoplasts isolated in the same medium (except that sucrose was replaced by sorbitol) was less than 2%. As reported elsewhere (7), inclusion of 5 mM each of EGTA and EDTA together with 10 mM KF in the homogenizing medium reduced significantly the PA content in the isolated plasma membranes to 16%. Choline and ethanolamine have been reported to act as potent inhibitors of phospholipase D (25). The protective effect of these compounds, together with the chelators and KF, during homogenization was found to be insignificant, resulting in only a small reduction in the PA content. When lipids were extracted from mung bean hypocotyls, directly boiled in isopropanol, and were analyzed, 3.4% of PA was still detectable. When the same experiment was performed with chopped hypocotyls, the PA content of plasma membranes was not changed, suggesting that wounding does not affect the PA content. Based on these facts, it might be considered that the plasma membrane of mung bean hypocotyls has a naturally high content of PA.

Table VII shows that *in vitro* degradation of phospholipids into PA occurs in various membranes, catalyzed by a membrane-bound phospholipase D. All these membranes have a membrane-bound phospholipase D, but the activity as assessed by the formation of PA on mg protein basis was significantly higher in tonoplasts and the lower phase fraction than in plasma membranes. The membrane-bound phospholipase D, however, was not active on the endogenous phospholipids at pH 7.3 even in the presence of Ca^{2+} ion (results not shown). Thus, the high content of PA in plasma membranes could not be simply ascribable to the specific activation of the membrane-bound phospholipase D *per se* during the membrane preparation, because the pH was kept above 7.3 throughout the preparation. As demonstrated by the results in Table VIII, the membrane-bound enzyme showed a relatively higher specificity for PC than PE in every membrane fraction, especially in the lower phase fraction.

DISCUSSION

There have been detailed reports concerning the composition of phospholipids and sterols in plant plasma membranes (8, 10, 28, 29, 31, 33, 36). However, no quantitative analysis has been made on other lipid components including ceramide lipids. In some plants, sterylesters, diglycerides, fatty acids, and triglycerides were reported to be present in the plasma membrane-enriched fraction (8). However, this must be due to contamination of the isolated plasma membranes by other membranes. As reported here (34), plasma membranes isolated from etiolated mung bean hypocotyls were very pure, as assessed by phosphotungstic acid-CrO_3 staining and electronmicroscopic observation,

Table IV. *Fatty Acid Compositions of Phospholipids in Mung Bean Plasma Membranes*

The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

	PI	PS	PC	PE	PG	PA	Total
				mol %			
16:0	43.2	18.5	26.1	42.0	67.1	35.3	35.0
18:0	7.3	6.9	8.7	4.2	5.7	4.6	5.9
18:1	8.7	5.9	11.6	8.0	5.6	9.0	9.2
18:2	16.0	19.3	24.4	23.4	8.5	25.4	21.4
18:3	22.2	26.2	23.3	19.3	7.3	24.2	19.0
20:1		14.4	1.2	0.9		0.7	1.6
20:2	2.6	0.8	0.4	0.3	4.2	0.7	1.6
20:3		0.4	3.5	1.5	2.0	0.1	2.2
22:1		8.3	1.1	0.4	0.5	0.5	1.1
Unsaturated/saturated	0.98	2.94	1.87	1.16	0.37	1.51	1.44
Percent of total phospholipids	5.3	3.0	33.0	38.0	4.4	16.3	

Table V. *Fatty Acid Composition of Phospholipids in Mung Bean Tonoplasts*

The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

	PI	PS	PC	PE	PG	PA	Total
				mol %			
16:0	50.6	24.0	31.5	43.3	79.1	34.0	39.4
18:0	4.8	6.6	8.5	4.3	3.6	5.0	6.2
18:1	6.6	8.0	11.9	7.7	3.3	8.5	9.1
18:2	15.0	22.1	24.3	23.4	5.8	16.5	22.2
18:3	20.5	27.7	21.3	18.2	6.1	15.4	19.8
20:1	0.6	9.2	0.9	1.1	0.6	5.0	1.5
20:2	1.7	3.0	0.7	0.7	1.7	11.2	1.2
20:3		0.1	0.1	1.8			0.8
22:1		6.2	2.2	2.6		0.1	2.1
Unsaturated/saturated	0.81	2.27	1.50	1.10	0.21	1.56	1.19
Percent of total phospholipids	11.1	4.3	46.4	31.4	4.6	2.1	

Table VI. *Phospholipid Composition of Mung Bean Plasma Membranes Obtained by Using Different Isolating Media*

Five mM EGTA, 5 mM EDTA, 10 mM KF, 4% (w/v) choline or 4% (w/v) ethanolamine were added to the homogenizing medium (pH 7.6), respectively. Lipids were extracted and separated on two-dimensional TLC. Total lipids were extracted from intact hypocotyls by boiling in isopropanol, followed by further extraction with chloroform/methanol (2:1, v/v). The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

Phospholipids	EGTA	EGTA + EDTA + KF	EGTA + EDTA + KF + Choline + Ethanolamine	Lipid Extract from Intact Hypocotyls
		mol %		
PI	3.9	5.3	7.5	10.1
PS	2.3	3.0	4.0	3.9
PC	31.8	33.0	32.4	42.9
PE	38.6	38.0	38.7	31.2
PG	2.4	4.4	2.8	4.2
PA	21.1	16.3	14.6	3.4
CL	0	0	0	4.3

specific naphthylphthalamic acid binding activity and membrane marker enzyme assays including vanadate-sensitive ATPase.

The reports on lipid composition of tonoplasts are quite limited. Marty and Branton (12) have quantified the phospholipids of beet root tonoplasts, but only qualitative analysis was made for other neutral lipid components. Verhoek *et al.* (30) have reported a detailed lipid composition of oat tonoplasts, in which glycosylated sterols and cerebroside are the predominating glycolipids. However, as they stated, the tonoplast fraction was considerably contaminated by chloroplast envelopes, as assessed by the presence of relatively high amounts of galactolipids. In our previous study, tonoplasts were isolated from etiolated mung

Table VII. *In Vitro Degradation of Phospholipids by Membrane-bound Phospholipase D*

Aliquots of membrane samples (1 mg protein equivalent) were incubated in 20 mM sodium acetate buffer (pH 5.0) and with or without 2 mM $CaCl_2$ in a final volume of 0.8 ml at 30°C for 30 min. After incubation, 0.2 ml of glacial acetic acid was added and lipids were extracted according to Bligh and Dyer (2). The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

Membrane Sample	Formation of Phosphatidic Acid	
	$-CaCl_2$	$+CaCl_2$
	$\mu mol \cdot mg^{-1}$ protein $\cdot h^{-1}$	
Plasma membranes	0.326	0.574
Tonoplasts	0.856	1.390
Lower phase	0.960	0.962

Table VIII. *Degradation of PC and PE after Incubation of Various Membranes at pH 5.0 with Addition of 2 mM $CaCl_2$*

The experimental results shown are the average of duplicated measurements. Errors of analysis were estimated to be within 5%.

Membrane Samples	Degradation during 30 Min Incubation	
	PC	PE
	%	
Plasma membranes	41.5	32.5
Tonoplasts	36.8	22.8
Lower phase	78.7	35.8

bean hypocotyls by homogenization in a buffer medium containing sorbitol and by subsequent linear sucrose density gradient centrifugation of the crude membrane fraction (35). Tonoplasts were recovered from the sample/sucrose interface after the centrifugation with high purity as assessed by electronmicroscopic observation and membrane marker enzyme assays.

Lipids were protected against peroxidation by the addition of SHAM, BHT, and potassium metabisulfite to the homogenizing medium. BHT was included throughout the whole process. As is well documented, many plants have a high activity of both soluble and membrane-bound phospholipase D which may give rise to the degradation of phospholipids into phosphatidic acid during homogenization (7). The addition of 5 mM EGTA to the homogenizing medium, in the present study, was not sufficient to protect against the degradative activity and phosphatidic acid comprised about 20% of total phospholipids in the isolated plasma membrane. As recommended by Galliard (7), further additions of 5 mM EDTA and 10 mM KF, as inhibitors of phosphatidic acid phosphatase, reduced the PA content from 20 to 16%. Additions of choline and ethanolamine, which are potent inhibitors for phospholipase D, into the EDTA-EGTA medium gave no further protection, suggesting that the phospholipase D activity may have been sufficiently controlled by the addition of EDTA, EGTA, and KF. However, there still remains a possibility that these chelators do not protect against *in situ* degradation of membrane phospholipids by a membrane bound phospholipase D.

Significant degradation of phospholipids into PA was observed in some membranes, including plasma membranes, tonoplasts and the lower phase fractions, upon *in vitro* incubation in a medium containing 20 mM sodium acetate buffer (pH 5.0) and 2 mM $CaCl_2$ as the activator, suggesting that the membrane-bound phospholipase D was active in these membrane fractions under the conditions employed. If the phospholipase D bound to these membranes is equally active during membrane isolations, it would be reasonable to think that phospholipids in these membranes should be commonly attacked by the enzyme. However, phospholipids in tonoplasts, the lower phase fraction and mitochondria were observed to be intact, being well protected by the addition of 5 mM EGTA to the homogenizing medium (data not shown). The content of PA in these membranes was always less than 2%. In the *in vitro* experiment, PC and PE were preferentially degraded by the membrane-bound phospholipase D. However, an inverse relation between the contents of PA and PI plus PS was observed in plasma membranes isolated using different media, while the relative contents of PC and PE were stable (Table VI). The reason for this observation is not clear.

In a preliminary experiment, the content of plasma membranes in etiolated mung bean hypocotyls was estimated to be around 12% of all membranes on the basis of phospholipid content. Together with the fact that the concentration of PA in plasma membranes and other membranes were respectively 15 and 2%, then the average concentration of PA in intact cells may be expected to be around 3.7%. This estimate agrees well with the PA content in total phospholipids extracted from intact hypocotyl tissues (3.4%). Based on these results, it may be considered that the plasma membrane of mung bean hypocotyls may normally contain such a high percentage of PA. According to Whitman and Travis (36), plasma membranes of meristematic and mature soybean roots contain respectively 2.0 and 8.9% of PA. On the other hand, the plasma membrane-enriched fraction prepared from maize roots has been reported to contain 1.6% of PA (8). Accordingly, the content of PA in plasma membranes may vary with plant species, type of tissue, and physiological state including development, aging, senescence, and probably responses to environmental stresses and diseases.

As shown in two-dimensional thin-layer chromatogram of tonoplast lipids (Fig. 1), small amounts of galactolipids were also detected in plasma membranes. Although a slight contamination of plastid envelopes in these membranes cannot be totally excluded, galactolipids may also be minor components of these membranes. It has been demonstrated that wheat plasma membrane contains both monogalactosyl and digalactosyl diglycerides which are responsible for the protoplast aggregation caused by soybean agglutinine which is specific to galactose residues (9).

Plant plasma membranes are known to be enriched in sterols (10, 28, 29, 31, 33). Forty-five molar percent of the total lipids in mung bean plasma membrane were sterols. More than 90% of the sterols were free sterols, in contrast to tonoplast sterols, 35% of which were glycosylated. The difference in the amount of glycosylated sterols between these membranes may have an important role to determine the physiological functions.

CMH is known to be widespread in plants, although the content is usually low (4–6, 17). It has been reported that plasma membrane and tonoplast contain CMH (8, 12, 15, 30). In oat tonoplasts, the cerebroside was reported to account for about 20% of all lipids. In mung bean hypocotyls, the glycolipids were found to be concentrated in plasma membranes and tonoplasts, especially in the latter where glycolipids comprised nearly 17% of the total lipids. Therefore, these lipids may have an important role in determining the function of those membranes. The chemical structures of the CMH extracted from seeds of leguminous plants and rice stalks have been reported (5, 6, 17). Most of the lipids are esterified by saturated and hydroxylated long carbon-chain fatty acids. Preliminary analysis of the ceramide lipids in mung bean plasma membranes and tonoplasts indicate that they are also esterified by highly saturated long chain fatty acids, suggesting a high melting point. Further detailed chemical analysis of the glycolipids is required.

The degree of unsaturation of tonoplast phospholipids was found to be lower than that of the plasma membranes. PI and PG in both membranes contained high levels of palmitic acid

and as a result the degree of unsaturation was quite low. In tonoplast, these phospholipids comprise more than 15% of total phospholipids and, therefore, they may give rise to phase separations at low temperatures. According to Murata's group, there is a good correlation between chilling-sensitivity of plants and the content of PG molecular species with highly saturated fatty acids (16). Most of the PG, however, is localized in plastids and thus studies of the phase separation of lipid bilayers has been mostly concerned with plastid membranes. The present study, however, suggests that phase separation may occur in other membranes including plasma membranes and tonoplasts, because of their content of highly saturated fatty acids in PG and PI. In addition, PE in both membranes contains more than 40% of palmitic acid, and since PE is nonlamellar forming lipid preferring to adopt the hexagonal II phase (20), it also may be involved in the mechanism of chilling sensitivity of mung bean plants. For this reason, more detailed analysis of the molecular species will be very important to assist our understanding of the effect of these lipids on the physicochemical properties of plasma membranes and tonoplasts, as affected by temperature.

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