Phytochemistry, Vol. 21, No. 4, pp. 859–862, 1982.
Printed in Great Britain.

0031-9422/82/040859-04$03.00/0

LIPID COMPOSITION OF A PLASMA MEMBRANE ENRICHED FRACTION OF MAIZE ROOTS

JOHN W. GRONEWALD,*‡ WAFA ABOU-KHALIL,* EVELYN J. WEBER† and JOHN B. HANSON*

*Department of Botany and †Agricultural Research, SEA, USDA, University of Illinois, Urbana, IL 61801, U.S.A.

(*Received* 26 *May* 1981)

Key Word Index—*Zea mays*; Gramineae; corn; plasma membrane; lipids; composition.

Abstract—The lipid composition of a plasma membrane enriched fraction isolated from corn (*Zea mays*) roots was examined. On a wt basis, the lipid:protein ratio was 1.11. Phospholipids comprised 60% of total lipids with the major phospholipids being phosphatidylcholine (62%) and phosphatidylethanolamine (21%). Free sterol was the major neutral lipid. The sterol:phospholipid molar ratio was 0.31. The fatty acid composition of the membrane was predominantly linoleic (60%) and palmitic (30%).

INTRODUCTION

Lipids play an important role in determining the physicochemical properties of biological membranes. It is well-documented that membrane lipid composition influences membrane fluidity [1], ion permeability and selectivity [2, 3] and the activity of membrane-associated enzymes [4 and refs. therein]. Although there have been numerous studies of the lipid composition of plasma membranes isolated from micro-organisms and mammalian tissues, such studies have been rather limited in plants [5–10]. This is partially attributable to the difficulties in isolating and purifying plasma membranes from plant tissue. At present a membrane fraction can be obtained from plant tissue which is *ca* 70% plasma membranes [11–15]. Knowledge of the lipid composition of the plasma membrane of plants would contribute to our understanding of the relationship between membrane composition and membrane function. The data reported below are a partial characterization of the lipid composition of a plasma membrane enriched fraction (PMEF) isolated from corn roots.

RESULTS AND DISCUSSION

Total lipid, phospholipid and sterol content

The PMEF isolated from corn roots has a total lipid:protein ratio of 1.11 (Table 1). PMEF isolated from other plant tissues such as oat roots [6], potato tubers [7], mushroom sporophores [5] and sugarcane leaves [9] have yielded lipid:protein ratios of 1.17, 0.71, 0.91 and 0.97, respectively. In general it appears that lipids comprise *ca* half the wt of plasma membrane fractions [10] isolated from plant tissue.

Phospholipids comprise 60% of total lipids in corn root PMEF (Table 1). On a protein basis, there was 0.668 mg phospholipid/mg protein. This value is in close agreement with that of mushroom plasma membranes ([5]; 0.664 mg phospholipid/mg protein) but slightly less than that found in plasma membranes of mature root tissue of soybean ([16]; 0.765 mg phospholipid/mg protein).

Other reports indicate that the phospholipid content of plant plasma membranes is much lower. In oat roots, phospholipids were found to comprise only 28.6% (0.334 mg phospholipid/mg protein) of total lipids [6]. Phospholipids were reported to comprise only 31% [7] and less than 1% [9] of total lipids in PMEF isolated from potato tubers and sugarcane leaves, respectively. Such low phospholipid content is surprising because most evidence suggests phospholipids play a major role in the structure and function of plasma membranes.

Recently Scherer and Morré [17] reported that soybean hypocotyl membrane fractions contain both phospholipase D and phosphatidic acid phosphatase. The sequential action of these enzymes on plasma membrane lipids during isolation of PMEF may be responsible for the low phospholipid content (measured as phospholipid phosphorus) in the above-mentioned reports.

Table 1. Total lipid, phospholipid and sterol content of a plasma membrane enriched fraction of corn roots (means±s.e.)

Lipid	mg/mg protein
Total lipid	1.11±0.12
Phospholipid*	0.668±0.023
Total sterol	0.108±0.003
Total sterol:phospholipid molar ratio†	0.31

*Quantity of phospholipids obtained by multiplying the value of phospholipid phosphorus by 25.

†Assumed average MW of phospholipid and sterol are 750 and 387, respectively.

‡Present address: U.S. Salinity Laboratory, 4500 Glenwood Drive, Riverside, CA 92501, U.S.A.

As compared to cytoplasmic or organelle membranes, there is evidence that the plasma membrane of plants [8, 11] as well as mammalian tissues [18, 19] is enriched in sterol. In corn root PMEF there are 108 μg total sterol/mg protein (Table 1). This value is slightly lower than other reports in the literature; 138–184 μg total sterol/mg protein in PMEF of oat roots [11], 117 and 152 μg total sterol in PMEF of meristematic and mature soybean roots [16], respectively, and 116–135 μg total sterol/mg protein in PMEF of corn coleoptiles [8].

PMEF isolated from corn roots have a total sterol:phospholipid molar ratio of 0.31, which compares favorably with ratios of 0.307 and 0.374 in plasma membranes isolated from meristematic and mature soybean roots, respectively. In contrast, Hodges *et al.* [11] reported a total sterol:phospholipid ratio of 1.074–1.213 for oat root PMEF. The primary reason for this disparity between roots of oats as compared to corn and soybean is the significantly lower phospholipid content found in oat roots. On a mg protein basis, the phospholipid content of PMEF from corn and soybean roots is *ca* twice that reported for oat roots. The difference may be related to species. Alternatively, there may have been greater phospholipid degradation during isolation and/or storage of oat root PMEF prior to lipid extraction.

Phospholipids

The predominant phospholipids in corn root plasma membranes are phosphatidylcholine (PC) (62%) and phosphatidylethanolamine (PE) (21%) with lesser amounts of phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidic acid (PA) (Table 2). The absence of detectable cardiolipin indicates relatively little mitochondrial contamination. PC and PE have also been reported to comprise more than 80% of the phospholipids in plasma membrane fractions isolated from potato tubers and cauliflower florets [10].

In oat root PMEF, PS was not detected and it was reported that an unknown, PC and PA were the major phospholipids [6]. Lyso-derivatives of PC and PE were also reported. It is now generally considered that large amounts of PA and lyso-derivatives in membrane fractions are artifacts due to hydrolytic activity during isolation and/or extraction [17, 20, 21]. The lack of lyso-derivatives and the small amount of PA found in this study may indicate relatively little lipid degradation. However, as pointed out by Scherer and Morré [17], low PA content does not necessarily indicate low phospholipid degradation as the sequential action of phospholipase D and phosphatidic acid phosphatase would prevent accumulation of PA. It is possible the diacylglycerol levels found in this study (Table 3) may to some extent reflect the action of these enzymes.

Glycolipids

There is a paucity of information concerning the glycolipid composition of plant plasma membranes. Glycolipids were reported to form 21% of the total lipid composition of potato tuber plasma membranes [7]. Keenan *et al.* [6] found three major glycolipids in oat root plasma membranes but no attempt was made to identify them. Digalactosyldiglyceride was the major glycolipid in sugarcane leaf plasma membranes with smaller amounts of steryl glycoside and steryl glycoside ester present [9]. Corn root PMEF contained five glycolipids which were identified as cerebroside, mono- and digalactosyldiglyceride, steryl glycoside ester and steryl glycoside.

Neutral lipids

Free sterol is the major neutral lipid in corn root plasma membranes (Table 3). In mammalian tissues cholesterol is the major sterol [22], while in higher plants, cholesterol is absent or present in very small amounts and sitosterol, stigmasterol and campesterol are the major sterols [23]. No attempt was made to identify the nature of the sterols found in this study. In corn (Table 3) and oat root [6] PMEF, free sterol comprised a little more than 30% of the neutral lipid fraction.

In addition to free sterols, smaller quantities of free fatty acids, steryl esters, mono-, di-, and triacylglycerols were found in corn root PMEF. These lipids have been reported to form part of the neutral lipid composition of the plasma membrane of oat roots, with the exception of monoacylglycerols [6], as well as rat liver [24]. Keenan *et al.* [6] reported that oat root plasma membranes were enriched in triacylglycerols, which comprised 44.7% of the neutral lipid fraction. We found triacylglycerols to comprise only 14% of the neutral lipid fraction of corn root PMEF (Table 3).

Free fatty acids comprised a small percentage (15%) of the neutral lipid fraction. Plasma membrane fractions isolated from oat roots [6], yeast [25], mushroom sporophores [5] and rat liver [24, 26] were also found to contain free fatty acids. There is some debate as to whether free fatty acids associated with PMEF are artifacts of preparation due to phospholipase activity during isolation [25]. The lack of

Table 2. Phospholipid composition of a plasma membrane enriched fraction of corn roots (means±s.e.)

Phospholipid	% Total lipid phosphorus
Phosphatidylcholine	62.2±2.7
Phosphatidylethanolamine	21.4±1.4
Phosphatidylinositol	8.4±0.6
Phosphatidylglycerol	4.9±0.8
Phosphatidic acid	1.6±0.6
Phosphatidylserine	1.5±0.3

Table 3. Neutral lipid composition of a plasma membrane enriched fraction of corn roots (means±s.e.)

Neutral lipid	% Total
Free sterol	35.8±1.9
Diacylglycerol	15.1±3.5
Free fatty acid	14.8±2.9
Triacylglycerol	14.0±2.4
Monoacylglycerol	13.0±2.8
Steryl ester	7.3±1.5

detectable levels of lyso-derivatives suggests phospholipase activity was not responsible for the free fatty acids detected in this study.

Fatty acids

The fatty acid composition of total lipids was predominantly linoleic (60%) and palmitic (30%) with lesser amounts of stearic, oleic and linolenic (Table 4). Trace amounts of palmitoleic were also detected. Linoleic and palmitic have also been reported to be the major fatty acids in PMEF isolated from potato tubers [10] and oat roots [6]. However, in oat roots, palmitic is the predominant fatty acid, comprising 48% of the total, whereas in corn root and potato tuber plasma membranes, linoleic is the major fatty acid.

EXPERIMENTAL

Plant material. Corn (*Zea mays* L.) seeds, (A619 × Oh 43) × A632, were surface-sterilized with 1% NaOCl and germinated for 3 days at 29° on paper towels saturated with 10^{-4} M $CaCl_2$. The PMEF was isolated from the primary root with 0.5 cm tip removed.

Isolation of plasma membrane. Except for minor modifications the procedure used for isolating the PMEF from corn roots was as described in ref. [27]. All operations during the isolation were carried out at 4°. 80 g root tissue were rinsed for 5 min in cold H_2O, cut into small segments with scissors and ground with a mortar and pestle for 90 sec in 240 ml of grinding medium [0.25 M sucrose, 4 mM EDTA, 25 mM *N* - tris(hydroxymethyl)methyl - 2 - aminoethanesulfonic acid (TES), pH 7.9]. Compared to the grinding medium used in ref. [27], TES, a stronger buffer in the region of pH 7.5, was substituted for Tris–MES and the EDTA concn was increased from 3 to 4 mM. These modifications were made to reduce phospholipase D activity which is stimulated by Ca^{2+} and acid pH [28]. The homogenate (pH 7.5) was filtered through 4 layers of cheesecloth and successively centrifuged at 13000 *g* for 15 min and 80000 *g* for 30 min. The microsomal fraction was suspended in 0.25 M sucrose, 1 mM TES, pH 7.5 and layered on a two-step discontinuous sucrose gradient consisting of 28 ml 45% (w/w) sucrose plus 8 ml 34% (w/w) sucrose. The sucrose was buffered at pH 7.5 with 1 mM TES. The gradient was centrifuged for 2 hr at 95000 *g* with a Spinco SW-27 rotor. The plasma membrane enriched fraction was collected at the 34–45% sucrose interface. This fraction, which is *ca* 70% plasma membrane [13], was diluted with 1 mM TES, pH 7.5 and repelleted by centrifugation for 30 min at 80000 *g*. The pellet was suspended in 1 ml H_2O and the lipids were immediately extracted as described below.

Table 4. Fatty acid composition of total lipids of a plasma membrane enriched fraction of corn roots (means±s.e.)

Fatty acid		% Total
Palmitic	16:0	30.0±2.2
Palmitoleic	16:1	Trace*
Stearic	18:0	1.6±0.3
Oleic	18:1	3.8±0.4
Linoleic	18:2	59.8±1.7
Linolenic	18:3	4.0±0.6

*Trace; % total less than 1%.

Lipid extraction. A two-step procedure was used to extract plasma membrane lipids. (a) The plasma membrane fraction, suspended in 1 ml H_2O was added to 5 ml boiling *iso*-PrOH. Except when gravimetric determinations were made or when the lipid P or sterol content of total lipid extracts were determined, the *iso*-PrOH contained 0.004% butylated hydroxytoluene (BHT) to reduce autoxidation. After boiling for 1 min, the *iso*-PrOH fraction was cooled on ice. (b) Undissolved material was then pelleted by centrifugation and re-extracted by the Bligh and Dyer [29] procedure. This two-step extraction has been reported to be effective in extracting lipids and preventing hydrolytic activity [30, 31]. The combined *iso*-PrOH and Bligh and Dyer fractions were evaporated under N_2, taken up in $CHCl_3$–MeOH (2:1) and then filtered to remove insoluble protein and polysaccharide contaminants. In those expts where lipids were not further purified by TLC before quantitative analysis, a Bligh and Dyer extraction was performed on the *iso*-PrOH fraction to ensure removal of non-lipid contaminants and this fraction combined with the original Bligh and Dyer extraction. Lipids were stored under N_2 at −20° in $CHCl_3$–MeOH (2:1) until sufficient lipid was collected for analysis.

Preparation of methyl esters and GC. Methyl esters of fatty acids were prepared by treatment of total lipid extracts with BF_3 in MeOH [32]. After transesterification, the methyl esters were further purified by TLC on Si gel G, with petrol (bp 60–68°)–Et_2O–HOAc (80:20:1). After spraying with 2′,7′-dichlorofluorescein, the methyl esters were identified by viewing under UV light and eluted from the gel with Et_2O–MeOH (7:1). The solvents were evaporated under N_2 and the methyl esters redissolved in petrol. The dye was eliminated by adding 0.5 vol. H_2O, mixing and removing the petrol layer which contained the methyl esters. The methyl ester extract was evaporated to dryness under N_2 and again taken up in a small vol. of petrol.

A FID gas chromatograph was used to analyse the methyl esters. The column, 1.8 m × 2 mm (i.d.), was packed with 15% DEGS on 80/100 mesh Chromosorb W-AW (Supelco, Inc.). Column temp. was 172° with He carrier gas, flow rate of 30 ml/min. The injector and detector temp. were 195° and 200°, respectively. A known amount of heptadecanoic acid (17:0), used as int. standard, was added to each sample prior to transesterification. The fatty acids were identified by comparing R_ts with those of pure methyl esters. The concn of each component was calcd from the peak area and expressed as a percentage of the total.

TLC for all lipid classes was performed on standard 20 × 20 cm chromatoplates coated with a 300 μm layer of Si gel G. To prevent autoxidation, BHT was added to the TLC solvent systems. When 2-D chromatography was used, BHT was added only to the first solvent system.

Polar lipids were separated from neutral lipids with petrol–Et_2O–HOAc (80:10:1). The polar lipids, which remained at the origin, were eluted from the gel twice with $CHCl_3$–MeOH–H_2O (10:9:1) and once with MeOH. The procedure of ref. [33] was used for the 2-D separation of polar lipids. The plates were developed in the first direction with $CHCl_3$–MeOH–H_2O (65:25:4), air-dried at room temp. and developed in the second direction with di-isobutyl ketone–HOAc–H_2O (80:50:10). To aid in the identification of glycolipids a 1-D separation of polar lipids was obtained with di-isobutyl ketone–HOAc–H_2O (80:50:7) [34]. Neutral lipids were fractionated utilizing a two-step development, di-isopropyl ether–HOAc (24:1) and petrol–Et_2O–HOAc (90:10:1), in one direction [35].

Qualitative analysis. Lipids separated by TLC were identified by co-chromatography with authentic standards, group-specific reagent sprays and comparison with reported R_f values. Lipids were routinely revealed with I_2 vapour. Phospholipids were visualized with the molybdenum blue reagent [36]. Ninhydrin was used to identify amino phospholipids. Glycolipids were identified with 20% $HClO_4$ [33] and sterols with H_2SO_4–HOAc [37].

Quantitative analysis of lipids separated by TLC was performed in the presence of Si gel. Phospholipids and neutral lipids were determined by P analysis [38] and dichromate oxidation [39], respectively. The procedure of ref. [40] was used to measure lipid P of total lipid extracts. Sterol content of total lipid extracts was determined colorimetrically [41] using cholesterol as a standard. Total lipid was determined gravimetrically.

Protein was determined by the method of ref. [42] with BSA as a standard.

Acknowledgements—We gratefully acknowledge the technical assistance of Lorraine Bollinger. Supported by Grants NSF(PCM 76-80886) and DOE(EY-76-5-02-0790).

REFERENCES

1. Chapman, D. (1975) *Cell Membranes. Biochemistry, Cell Biology and Pathology* (Weissman, G. and Claiborne, R., eds.) p. 13. H. P. Publishing Co., New York.
2. Hall, J. L. and Baker, D. A. (1975) *Ion Transport in Plant Cells and Tissues* (Hall, J. L. and Baker, D. A., eds.) p. 39. North-Holland, Amsterdam.
3. Kuiper, P. J. C. (1975) *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (Galliard, T. and Mercer, E. I., eds.) p. 359. Academic Press, New York.
4. Sandermann, H., Jr. (1978) *Biochim. Biophys. Acta* **515**, 209.
5. Holtz, R. B., Stewart, P. S., Patton, S. and Schisler, L. C. (1972) *Plant Physiol.* **50**, 541.
6. Keenan, T. W., Leonard, R. T. and Hodges, T. K. (1973) *Cytobios* **7**, 103.
7. Demandre, C. (1975) *Physiol. Veg.* **13**, 103.
8. Hartmann, M. A., Normand, G. and Benveniste, P. (1975) *Plant. Sci. Letters* **5**, 287.
9. Sinensky, M. and Strobel, G. (1976) *Plant Sci. Letters* **6**, 209.
10. Mazliak, P. (1977) *Lipids and Lipid Polymers in Higher Plants* (Tevini, M. and Lichtenthaler, H. K., eds) p. 48. Springer, Heidelberg.
11. Hodges, T. K., Leonard, R. T., Bracker, C. E. and Keenan, T. W. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3307.
12. Koehler, D. E., Leonard, R. T., Van der Woude, W. J., Linkins, A. E. and Lewis, L. N. (1976) *Plant Physiol.* **58**, 324.
13. Leonard, R. T. and Van der Woude, W. J. (1976) *Plant Physiol.* **57**, 105.
14. Nagahashi, G., Leonard, R. T. and Thomson, W. W. (1978) *Plant Physiol.* **61**, 993.
15. Berkowitz, R. L. and Travis, R. L. (1979) *Plant Physiol.* **63**, 1191.
16. Travis, R. L. and Booz, M. L. (1979) *Plant Physiol.* **63**, 573.
17. Scherer, G. F. E. and Morré, D. J. (1978) *Plant Physiol.* **62**, 933.
18. Colbeau, A., Nachbaur, J. and Vignais, P. M. (1971) *Biochim. Biophys. Acta* **249**, 462.
19. Harrison, R. and Lunt, G. G. (1975) *Biological Membranes: Their Structure and Function* p. 59. John Wiley, New York.
20. Kates, M. (1970) *Adv. Lipid Res.* **8**, 225.
21. Mazliak, P. (1973) *Annu. Rev. Plant Physiol.* **24**, 287.
22. Demel, R. A. and Kruyft, B. D. E. (1976) *Biochim. Biophys. Acta* **457**, 109.
23. Grunwald, C. (1975) *Annu. Rev. Plant Physiol.* **26**, 209.
24. Keenan, W. and Morré, D. J. (1970) *Biochemistry* **9**, 19.
25. Kramer, R., Kopp, F., Niedermeyer, W. and Fuhrman, G. F. (1978) *Biochim. Biophys. Acta* **507**, 369.
26. Dod, B. J. and Gray, G. M. (1968) *Biochim. Biophys. Acta* **150**, 397.
27. Leonard, R. T. and Hotchkiss, C. W. (1976) *Plant Physiol.* **58**, 331.
28. Dawson, R. M. C. and Hemington, N. (1967) *Biochem. J.* **102**, 76.
29. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
30. de la Roche, I. A., Andrews, C. J. and Kates, M. (1973) *Plant Physiol.* **51**, 468.
31. Fishwick, M. J. and Wright, A. J. (1977) *Phytochemistry* **16**, 1507.
32. Morrison, W. R. and Smith, L. M. (1964) *J. Lipid Res.* **5**, 600.
33. Lepage, M. (1964) *J. Chromatogr.* **13**, 99.
34. Nichols, B. W. (1963) *Biochim. Biophys. Acta* **70**, 417.
35. Skipski, V. P., Good, J. J., Barclay, M. and Reggio, R. B. (1968) *Biochim. Biophys. Acta* **152**, 10.
36. Vaskovsky, V. E. and Kostetsky, E. Y. (1968) *J. Lipid Res.* **9**, 396.
37. Kates, M. (1972) *Techniques of Lipidology: Isolation, Analysis and Identification of Lipids* (Work, T. S. and Work, E., eds.). North Holland, Amsterdam.
38. Parker, F. and Peterson, N. F. (1965) *J. Lipid Res.* **6**, 455.
39. Amenta, J. S. (1964) *J. Lipid Res.* **5**, 270.
40. Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466.
41. Courchaine, A. J., Miller, W. H. and Stein, D. B., Jr. (1959) *Clinical Chemistry (Winston-Salem NC)* **5**, 609.
42. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.