Plant Physiol. (1989) 90, 1207–1213
0032-0889/89/90/1207/07/$01.00/0

Received for publication August 29, 1988
and in revised form February 3, 1989

Comparison of the Lipid Composition of Oat Root and Coleoptile Plasma Membranes[1]

Lack of Short-Term Change in Response to Auxin

Richard P. Sandstrom and Robert E. Cleland*

Portland State University, Department of Biology, P. O. Box 751, Portland, Oregon 97207 (R.P.S.) *and University of Washington, Department of Botany, KB-15, Seattle, Washington 98195* (R.E.C.)

ABSTRACT

The total lipid composition of plasma membranes (PM), isolated by the phase partitioning method from two different oat (*Avena sativa* L.) tissues, the root and coleoptile, was compared. In general, the PM lipid composition was not conserved between these two organs of the oat seedling. Oat roots contained 50 mole percent phospholipid, 25 mole percent glycolipid, and 25 mole percent free sterol, whereas comparable amounts in the coleoptile were 42, 39, and 19 mole percent, respectively. Individual lipid components within each lipid class also showed large variations between the two tissues. Maximum specific ATPase activity in the root PM was more than double the activity in the coleoptile. Treatment of coleoptile with auxin for 1 hour resulted in no detectable changes in PM lipids or extractable ATPase activity. Differences in the PM lipid composition between the two tissues that may define the limits of ATPase activity are discussed.

The plant PM[2] is a critical component of the cell, separating the cytoplasm from the apoplasm. It contains proteins involved in the selective transfer of ions and molecules across this membrane. One important protein is the H^+-ATPase, whose transport of protons to the apoplast is important both for the subsequent uptake of sugars and amino acids by proton-organic cotransport and as a component of growth regulation (28). The activity of this enzyme, as well as other PM proteins, is influenced by the composition of the lipid phase in which they are embedded. This influence is indicated both by experiments where proteins are reconstituted into synthetic bilayers of differing lipid composition (8, 17, 29) and by experiments where lipid components are partially removed by surfactants (24).

The lipid composition of the PM is characterized by high levels of sterols and modified sterols (19, 21, 22). However, both the sterols and phospholipids of PMs differ greatly from plant to plant (19, 21). One objective of this study was to determine the PM lipid composition of two organs of the same plant and to see to what extent the composition was conserved from organ to organ. The one previous study, pertinent to this one, indicated that considerable variation exists between the PM lipids of two plant organs, barley root and leaf (21).

A second objective was to determine whether a correlation existed between the PM lipid composition and the ATPase activity. Auxin causes increased excretion of H^+ from auxin-responsive tissues such as the oat coleoptile (6); this excretion is believed to be mediated by the PM ATPase (13), but the mechanism remains obscure. One possibility is that auxin causes changes in PM lipid composition, which in turn modulates ATPase activity. There have been several reports that auxin can change membrane lipid composition, especially the amounts of the various unsaturated fatty acids (11, 20). In general, however, purified plasma membranes were not used, and incubation times with auxin exceeded the time needed for a maximum stimulation of proton excretion.

A third objective of this study was to determine whether a 1 h incubation of oat coleoptiles with auxin (maximum stimulation of proton excretion [7]) leads to any change in PM lipid composition.

We will show that the lipid components as well as the ATPase activity of the coleoptile PM do not change upon exposure to auxin. We will also show that distinct differences in maximum PM ATPase activity exist between the root and coleoptile and may, in part, be the consequence of differences existing in the PM lipids between the two tissues.

MATERIALS AND METHODS

Chemicals

Sodium-ATP, dextran (mol wt 500,000), polyethylene glycol (mol wt 3,250), 6-*p*-toluidino-2 naphthalensulfonic acid (TNS), Triton X-100, and all phospholipid, glycolipid, and fatty acid standards were purchased from Sigma (St. Louis, MO). Plant sterol standards were purchased from Supelco (Bellfonte, PA) (30). Solvents were redistilled in glass and used fresh. All other chemicals were reagent grade.

[1] Supported by the National Science Foundation, grant DMB-8502021, and the U.S. Department of Energy, contract DE-AT06-76ER3019.

[2] Abbreviations: PM, plasma membrane; HPTLC, high performance thin layer chromatography; PA, phosphatidicacid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; TNS, 6-*p*-toluidino-2 naphthalensulfonic acid.

Plant Material

Oats (*Avena sativa* L. cv Victory) were grown in vermiculite as described previously (24). Briefly, the coleoptiles were grown and harvested after 4 d under continuous dim red light (to inhibit mesocotyl growth) at 25°C. At harvest, the primary leaves, enclosed in the coleoptiles, were removed and discarded. The apical 3 mm of the coleoptiles were removed and subsequently, the coleoptiles were placed in the treatment solutions. The roots were grown and harvested under the same conditions; however, harvest was 5 (instead of 4) d after planting.

IAA Experiments

Harvested coleoptiles (15–20 g) were pretreated for 4 h in 0.1 mM Mes/Tris (pH 6.0) a solution containing 0.1 mM KCl and 1% sucrose to reduce endogenous IAA. After pretreatment, the coleoptiles were split into equal weight samples and placed in the same solution plus 10^{-5} M IAA (treatment) or minus IAA (control) for 1 h at 25°C in dim red light. After 1 h in the treatment or control solutions, the tissues were removed and rinsed with distilled water.

Analytical Procedures

Extraction of microsomal vesicles, ATPase assays and protein analyses are described in Sandstrom *et al.* (24).

Phase Partitioning

The procedure has been described in Sandstrom *et al.* (24). Briefly, the microsomal pellet was subjected to three successive phase partitioning steps utilizing 6.5% (w/w) dextran (500,000 mol wt) and polyethylene glycol (3,350 mol wt) in 5 mM potassium phosphate buffer (pH 7.8), containing 250 mM sucrose and 4 mM KCl. The upper phase from the third tube, U_3, containing the purified PM vesicles, was resuspended in 10 mM Tris/HCl (pH 7.5), and 250 mM sucrose, concentrated by centrifugation at 100,000*g*, and resuspended into a smaller volume of the same solution. Marker enzymes for endoplasmic reticulum, tonoplast, Golgi apparatus, and mitochondria showed that these organelles were present in negligible amounts or absent.

Lipid Extraction and Separation

Lipids were extracted from the PM preparation by the method of Bligh and Dyer (4). U_3 PM, in amounts ranging from 0.2 to 1.0 mg protein, were extracted with 1 to 3 mL of methanol:chloroform:HCl (2:1:0.3, v/v/v). Extra chloroform and water were added to the solvent solution to get a phase separation. The chloroform phase, containing the extracted lipids, was removed, concentrated under a stream of N_2, and brought to a volume of 2 mL with chloroform:acetic acid (100:1, v/v). To separate the three major lipid classes (sterols, glycolipids, and phospholipids) in the mixture, the samples were transferred to a Sep Pak cartridge (19) attached to a 10 mL glass syringe.

After the lipid sample entered the cartridge, 2 mL of chloroform:acetic acid (100:1, v/v) were used to wash the residual lipid from the original container. The neutral lipids were eluted first with 10 mL of chloroform:acetic acid (100:1, v/v). The glycolipids were eluted next with 10 mL of acetone followed by 10 mL of acetone:acetic acid (100:1, v/v). The phospholipids were eluted last with 10 mL of methanol:chloroform:water (100:50:40, v/v/v) and recovered by effecting a phase separation with 1 mL of chloroform and 2 mL of water and removing the chloroform phase.

All of the eluted lipid fractions were concentrated to near dryness in a rotary evaporator. The concentrated neutral lipids were dissolved and stored in either hexane or chloroform. The glycolipids were dissolved and stored in chloroform:methanol (1:1, v/v). The phospholipids were dissolved and stored in either chloroform:acetic acid (100;1, v/v) or chloroform:methanol (19:1, v/v). All the samples were stored at −20°C prior to analysis. If samples were to be stored for periods greater than 1 month, then BHT was added as a precaution against oxidation.

Analysis of Lipids

Sterols

Total free sterols in the neutral lipid fraction were determined by the colorimetric method of Zlatkis and Zak (36) with cholesterol as a standard. Sterols contained in neutral lipid samples were also analyzed by GC-MS. Free sterols were separated in a Hewlett-Packard 5710 gas chromatograph on a 30 m, DB-5 column under the following conditions: injection temperature was 200°C and the column oven was programmed from 200 to 300C at 5°C/min. The column was interfaced with a VG 7070H double focusing mass spectrometer (Manchester, England) with a VG 2035 data system and the samples analyzed under the following conditions: interface and source temperatures were 280 and 200°C, respectively; electron impact e/i was 70 electron volts, and the sample was scanned from mass 500 to 50 at 1 s/decade.

Individual sterols in the neutral lipid samples were quantified by GLC with known plant sterols as standards as previously described (25). Total sterol amounts, calculated by addition of individual sterol amounts determined by gas chromatography, always gave values greater than those determined by the colorimetric method. The reason for this discrepancy is obscure and seems to be inherent in the two analytical methods. Individual glycolipid sterols from sterol glycosides and acylated sterol glycosides were analyzed by GLC after the parent compounds were separated by TLC, as described below, and hydrolyzed to remove the sugar. The fatty acids of the acylated sterols, and any free fatty acids present in this fraction were transesterified to their methyl esters and analyzed by gas chromatography as described below. Separate analyses to distinguish free fatty acids from transesterified fatty acids were not attempted.

Glycolipids

Analysis of total sugar, by the method of Roughan and Batt (23), was used to quantify total glycolipids. Individual glycolipids were separated by HPTLC, as described previously (25), identified by coelution with known standards after spraying

with TNS (16), scraped from the plate and analyzed by the above procedure. Two solvent systems were used to develop glycolipid samples on HPTLC plates (Merc) to help identify the most polar (unknown) glycolipids found in this fraction. The two systems and their compositions were chloroform:methanol:acetic acid:water (85:20:2:3, v/v/v/v) and chloroform:methanol:water (65:35:5, v/v/v). It was assumed that each mole of glycolipid contained a single mole of 6 carbon sugar. This may overestimate the amount of glycolipid present in the PM, since some of the glycolipid components may contain polymeric sugar. A more thorough analysis of the glycolipid sugars was beyond the scope of this study.

Phospholipids

Total phospholipids were analyzed colorimetrically by the method of Ames (1) after digestion in hot nitric acid. Individual phospholipids were separated by HPTLC, identified by coelution with known standards after spraying with TNS, scraped from the plate, and analyzed by the above procedures. The fatty acids from the phospholipids were transesterified to their methyl esters and analyzed by gas chromatography (below).

Fatty Acids

Fatty acids present in each of the lipid components were analyzed by GLC after they were transesterified to their methyl esters by refluxing the parent fractions in methanolic BF_3 (37). The fatty acid methyl esters were quantified by GLC in a Varian 3700 gas chromatograph with 10% cyano silicone on Chrom WAW 100/120 (Alltech CS-10) in a 6 foot by 2 mm (i.d.) glass column under the following conditions: injection, and flame ionization detector (FID) temperatures were 210, and 250°C, respectively. The column temperature was programmed at 150°C for 1 min with an increase to 190°C at a rate of 60°C/min. N_2 carrier gas, air, and H_2 flows were 18 to 20, 300, and 30 mL/min, respectively. Signal output was quantified with a Hewlett-Packard 3390 A integrator. Retention times and response factors were determined by chromatography of known fatty acid methyl esters that included heptadecanoic methyl ester as an internal standard.

Data Summary

Lipid data are represented in this paper as μmol/mg protein or mol percent. Mol percent of individual lipid components was calculated as the percentage of the total μmol/mg amount of the combined lipid classes. Values given in Tables I and II represent the mean ± SE of the n determinations indicated. Although not reported, all comparisons of means were subjected to an analysis by t-statistics for two means (H_0: $\mu_1 - \mu_2 = d$; $d = 0$). Tests for significance were determined at $P = 0.1$.

RESULTS

The data shown in Tables I and II compare the PM lipid components in oat coleoptiles with oat roots and show that the PM lipid compositions from these two tissues varied not only quantitatively but also qualitatively. In the roots, phospholipids made up the majority of the PM lipids (50 mol %), with lesser and equal amounts of sterols and glycolipids (25 mol % each; Table I). In coleoptiles, on the other hand, both phospholipids and glycolipids were present at about 40 mol %, whereas free sterols were 20 mol % of the PM lipids.

These percentages must be taken with some caution for three reasons. First, the amount of glycolipid may be overestimated, since it is based on the assumption that each molecule of glycolipid contains a single molecule of sugar. On the other hand, it might be underestimated because the glycolipid fraction contained three highly polar unknowns (which were not included in the calculated totals) that accounted for 40 mol % of the sugars in the total glycolipid from roots and 65 mol % in coleoptiles. However, it is unlikely that these are actually membrane lipids, because none of the unknowns resembled polar glycolipid standards (cerebrosides, sphingomyelin, and psychosine: sphingosyl-β-D-galactopyranoside) when developed in the two different HPTLC solvent systems. None of the three unknowns fluoresced as well as the other lipids in this study when TNS was used as the detecting agent. This result suggests that these compounds had a less hydrophobic character than the others. Degradative analyses of the unknowns were not performed for this study. As mentioned earlier, we disregarded these unknowns in the calculations of total PM glycolipids. Finally, the total fatty acid content of the neutral lipids was not included in the lipid totals for the two tissues because their analysis did not discriminate free fatty acids from bound fatty acids. However, the amounts of neutral lipid fatty acids are significantly different between the two *Avena* tissues. Their amounts were 0.2 to 0.3 μmol/mg protein in the coleoptile PM and 1.6 μmol/mg protein in the root PM. The origin of these fatty acids deserves further investigation.

There were also significant differences between the two tissues in amounts of individual lipids in each PM lipid class. For example, in the glycolipid fraction the glycocerebrosides (composed of three closely spaced bands on HPTLC) made up 26 mol % of the total PM lipid in the coleoptile, but only 10 mol % in the oat root (Table I). This high level of cerebroside in coleoptile PM also deserves further investigation. The contents of sterol glycosides and acylated sterol glycosides, on the other hand, were nearly the same for coleoptile and root PMs.

The individual phospholipid analyses presented here for both tissues show little variation from published analyses (19, 21, 22). In both the coleoptile and the root, PC and PE appeared in almost equal molar amounts and, aside from PA, were the most abundant phospholipids, while the other phospholipids (LPC, PI, PS, PG) were present in significantly reduced amounts. These results are typical of plant PM analyses (19, 21, 22, 33–35). Of interest here was the elevated amount of lysolecithin (LPC) in the oat root (1.7 mol %) when compared to the coleoptile (approximately 0.6 mol %). Lysolecithin is not a common component of plant PM lipid analyses but has been detected previously (18).

One potential problem persists with these analyses. The amount of PA present in the PM is surprisingly high. The amount of PA present in the coleoptile PM comprised the

Table I. *Lipid Composition of Plasma Membranes Isolated from Oat Coleoptiles (+ and − IAA) and Roots*

Values represent the mean ± SE. The number of determinations indicated, *n*, represents values from different extractions.

	Avena Coleoptile				Avena Root	
	−IAA		+IAA			
	μmol Pi/mg protein-min					
ATPase specific activity	1.58 ± 0.06	*n* = 10	1.56 ± 0.06	*n* = 10	3.61 ± 0.26	*n* = 4
	μmol/mg protein					
Total phospholipid	0.55 ± 0.01	*n* = 9	0.52 ± 0.02	*n* = 9	0.42 ± 0.03	*n* = 9
Total glycolipid	0.60 ± 0.12	*n* = 9	0.63 ± 0.14	*n* = 9	0.21 ± 0.03	*n* = 9
Total free sterol	0.27 ± 0.04	*n* = 9	0.27 ± 0.02	*n* = 9	0.20 ± 0.01	*n* = 9
Total lipid	1.42 ± 0.14	*n* = 9	1.42 ± 0.15	*n* = 9	0.83 ± 0.05	*n* = 9
Sterol/phospholipid	0.50 ± 0.08	*n* = 9	0.53 ± 0.04	*n* = 9	0.49 ± 0.03	*n* = 9
	mol % of Total Lipid					
Total phospholipid	41.7 ± 4.2	*n* = 9	38.8 ± 3.0	*n* = 9	50.1 ± 2.1	*n* = 9
LPC	1.0 ± 0.32	*n* = 3	0.80 ± 0.12	*n* = 3	1.7 ± 0.22	*n* = 6
PI	2.3 ± 0.35	*n* = 3	1.7 ± 0.35	*n* = 3	1.5 ± 0.27	*n* = 6
PS	3.2 ± 0.60	*n* = 3	3.0 ± 0.13	*n* = 3	4.2 ± 0.45	*n* = 6
PC	9.6 ± 1.6	*n* = 3	8.5 ± 0.98	*n* = 3	14.3 ± 1.1	*n* = 6
PG	1.8 ± 0.32	*n* = 3	2.3 ± 0.38	*n* = 3	1.3 ± 0.14	*n* = 6
PA	14.8 ± 1.2	*n* = 3	15.4 ± 0.56	*n* = 3	11.8 ± 1.9	*n* = 6
PE	9.0 ± 1.1	*n* = 3	7.2 ± 0.51	*n* = 3	15.3 ± 0.88	*n* = 6
Total glycolipid	38.9 ± 4.6	*n* = 9	40.9 ± 4.9	*n* = 9	25.3 ± 2.7	*n* = 9
ASG[a]	5.5 ± 2.3	*n* = 3	5.4 ± 2.1	*n* = 3	4.5 ± 1.3	*n* = 4
SG[b]	7.3 ± 4.1	*n* = 3	9.2 ± 2.6	*n* = 3	10.6 ± 0.68	*n* = 4
GC[c]	26.1 ± 3.7	*n* = 3	26.3 ± 6.9	*n* = 3	10.1 ± 1.1	*n* = 4
Total free sterol	19.4 ± 2.8	*n* = 9	20.3 ± 2.1	*n* = 9	24.6 ± 1.2	*n* = 9
Cholesterol	3.1 ± 0.37	*n* = 9	3.2 ± 0.34	*n* = 9	0.14 ± 0.01	*n* = 9
Brassicasterol	2.0 ± 0.23	*n* = 9	2.0 ± 0.23	*n* = 9	ND	
Campesterol	1.9 ± 0.23	*n* = 9	2.5 ± 0.62	*n* = 9	2.0 ± 0.12	*n* = 9
Stigmasterol	1.6 ± 0.17	*n* = 9	1.6 ± 0.18	*n* = 9	12.1 ± 0.48	*n* = 9
β-Sitosterol	9.2 ± 1.1	*n* = 9	9.2 ± 1.1	*n* = 9	5.1 ± 0.37	*n* = 9
Unknown	1.5 ± 0.19	*n* = 9	1.7 ± 0.21	*n* = 9	5.2 ± 0.50	*n* = 9

[a] ASG, acylated steryl glycoside. [b] SG, steryl glycoside. [c] GC, glycocerebroside.

Table II. *Fatty Acid Composition of the Lipid Classes Isolated from Avena Coleoptile (+ and −IAA) and Root*

Values represent the mean mol % ± SE from 5 or 6 determinations within each lipid class.

Lipid Class	Acyl Group					Other[a]
	16:0	18:0	18:1	18:2	18:3	
Phospholipids						
Coleoptiles						
−IAA	21.8 ± 0.4	1.3 ± 0.3	6.2 ± 0.3	47.9 ± 1.7	12.0 ± 0.3	10.9
+IAA	24.2 ± 1.3	1.4 ± 0.5	7.0 ± 0.6	48.8 ± 1.3	12.1 ± 0.5	7.3
Roots	32.6 ± 3.4	0.2 ± 0.0	1.9 ± 0.2	55.4 ± 2.8	7.9 ± 0.4	2.2
Glycolipids						
Coleoptiles						
−IAA	56.8 ± 1.9	3.6 ± 0.3	5.1 ± 2.1	21.3 ± 0.1	7.1 ± 1.4	13.7
+IAA	56.1 ± 2.8	2.5 ± 0.3	4.9 ± 0.4	24.0 ± 0.4	8.6 ± 0.9	8.3
Roots	65.6 ± 2.3	0.8 ± 0.3	2.2 ± 0.1	22.5 ± 1.3	5.1 ± 0.6	4.9
Neutral Lipids						
Coleoptiles						
−IAA	29.9 ± 2.2	6.4 ± 0.6	11.9 ± 2.5	20.3 ± 1.1	5.4 ± 0.4	26.2
+IAA	35.5 ± 1.6	7.6 ± 0.3	11.4 ± 1.2	22.9 ± 1.6	6.8 ± 0.8	12.1
Roots	51.2 ± 0.8	2.3 ± 0.1	5.0 ± 0.3	33.1 ± 1.1	6.1 ± 0.1	1.8

[a] 14:0, 15:0, 16:1, 22:0, 22:1 and other unidentified fatty acids.

highest percentage (approximately 39 mol %) of the phospholipids. It represented the third highest amount of phospholipid in the root (23 mol %) behind PC (29 mol %) and PE (31 mol %). It is often assumed that PA is an artifact in the PM whose formation results from phospholipase D activity (26, 36). However, published PM lipid analyses consistently show PA as a membrane component (18, 19, 21, 22, 27, 32, 35, 36). In this study, attempts to minimize PA formation by boiling the fresh, extracted PM in boiling isopropanol prior to lipid extraction had no effect on the PA composition. Therefore, the phospholipase degradation of the phospholipid fraction probably occurred, if at all, during the preparation of the U_3 PM fraction rather than during the lipid extraction. Various methods to reduce phospholipase activity in plant extracts have been suggested (10, 26). We made use of certain precautions (addition of EDTA and high pH), but rejected the more rigorous procedures (26) for inhibiting phospholipases for the following reasons: (a) the addition of large amounts of the organic bases choline and ethanolamine, reported to curb phospholipase activity, could alter the charge character of the phase system used in isolating the PM vesicles and thus interfere with the preparation of the U_3 fraction, and (b) other lipid analyses, including phase partitioned PM, show PA as a membrane constituent even after more rigorous procedures to limit phospholipase activity were used (18, 36). We suspect that our PA values are higher than the values *in vivo*, but to what extent we are not sure.

The individual free sterol components of the PM from the two tissues showed considerable variation. Cholesterol, while being a minor component (0.4 mol % of the sterols) in the root PM was present in an amount (16 mol %) about 40 times higher in the coleoptile. Brassicasterol was not detected in the root but made up 11 mol % of the sterols in the coleoptile. Stigmasterol comprised the highest percentage of the free sterols in the root at 49 mol % but was only 8 mol % in the coleoptile. Conversely, β-sitosterol comprised 47 mol % of the free sterols in the coleoptile but represented only 21 mol % of the free sterols in the root.

An unknown sterol we tentatively identify as 4α,14α-dimethyl-9β,19 cyclo-5α-ergost-25-en-3β-ol (5) was present in the coleoptile PM at 8 mol % of the free sterols, but was present at 21 mol % of the free sterols in the root. Our mass spectra data only allowed us to identify this compound tentatively because no standards are available. The fragmentation pattern agreed well with the published data; however, not all of the relative percentages of the major fragmentation ions matched exactly (this may be due to some contaminating material coeluting with this compound).

Cyclopropyl sterols such as this (a 9β, 19 cyclopropyl sterol) are intermediates between the sterol biosynthetic steps involving the squalene ring closure and the formation of the Δ^5 sterols commonly found in membranes (2, 3, 12). They normally do not accumulate to significant levels unless certain biosynthetic steps are blocked (2, 5). This sterol may have accumulated in the root PM because the plant growth conditions used here (inadequate root aeration or nutrient levels) may have resulted in some inhibition of sterol biosynthetic steps. The reasons it accumulated at such a high percentage is a question for further study. This compound was not present in the sterol containing glycolipids indicating it was probably not a metabolic end product.

The glycolipid sterol components reflected similar percentages as seen in the free sterols (data not shown). Stigmasterol was the prevalent sterol found in the root glycolipid fraction, whereas β-sitosterol predominated in the coleoptile. Glycolipid brassicasterol was found only in trace amounts in the coleoptile and not detected in the root PM. Cholesterol was found in the PM glycolipid fraction of both tissues.

Table II shows that the fatty acids found in the lipid fractions did not vary much between the two tissue types. In all fractions, 16:0 and 18:2 were the predominant fatty acids. In the phospholipid fraction of both the root and coleoptile PMs, the 18:2 fatty acid was the predominant acyl chain followed in order by 16:0, 18:3, 18:1, and 18:0. The 14:0 acyl chain was the predominant fatty acid classified as 'other' in the coleoptile, whereas 22:0 predominated in the root. In the PM glycolipid fraction of both tissues, the amount of 16:0 was followed in order by 18:2, 18:3, 18:1, and 18:0. In the neutral lipid fraction, the amount of 16:0 was followed in the root by 18:2, 18:3, 18:1, and 18:0; but, in the coleoptile, it was followed by 18:2 then 18:1, 18:0, and 18:3. The 14:0 and 22:0 acyl chains were the major other fatty acids in the glycolipids and neutral lipids of both tissues. The total amount of fatty acid found in other was always less in the root than in the coleoptile in all lipid fractions and always more in the −IAA-treated samples than the +IAA samples.

The specific activity of the PM ATPase (measured in the presence of optimum Triton X-100) was 2.3 times higher in oat root than in coleoptile PM. If calculated on a per mole lipid basis, the difference is even greater (nearly fourfold), since the lipid/protein ratio was 1.7 times greater in coleoptile PM than in root PM. The free sterol/phospholipid ratio was similar for both oat root and coleoptile PM (about 0.50) and considerably lower than the values reported by Rochester *et al.* (21) for either barley leaf (0.8) for root PM (2.2).

The effect of auxin on PM lipids was determined by incubating *Avena* coleoptiles with or without auxin for 1 h prior to preparation of the PM fraction. This time was chosen because it results in a maximal enhancement by auxin of both proton excretion and growth (7). The lipid analyses of the + and − auxin-treated coleoptiles showed striking similarities; there were no significant qualitative or quantitative differences between the two treatments in any of the lipids analyzed. In addition, there was no difference in the maximum specific activity of the PM ATPase from the two treatments. Therefore, we conclude that within this short time span (1 h), promotion by auxin of the acidification process in coleoptiles was due neither to any auxin induced change within any of the three lipid classes, nor to any change in total PM ATPase activity. The only lipid analyses that may have shown differences as a result of the auxin treatments were the amounts of fatty acids classified as other; however, we found that the amounts were too variable to draw meaningful conclusions.

DISCUSSION

The physiological properties of the PM must depend, to some extent, upon its lipid composition. Until recently, the exact composition of the plant PM has been uncertain for

two reasons. First, PM preparations prepared by density gradient centrifugation contained variable amounts of contaminating membranes, whose lipid compositions were certainly different (15, 22). Second, analyses have concentrated on the phospholipids or occasionally on the sterols, and have neglected the glycolipids. Recently, however, two complete lipid analyses of purified PMs, prepared by aqueous phase partitioning, have become available. Lynch and Steponkus (19) analyzed the lipids from cold-acclimated and nonacclimated rye leaves. Rochester *et al.* (21, 22) examined barley root and leaf, spinach leaf, and cauliflower inflorescence PM lipids. We have now added two more tissues; oat root and coleoptile.

The variation in lipid composition from plant organ to organ is striking. For example, free sterols vary from 7 mol % (spinach leaf) to 57 mol % (barley root). Glycolipids vary from 14 mol % (rye leaves) to 40 mol % (oat coleoptiles). The composition varies with the growing conditions (cold-acclimated *versus* nonacclimated rye leaves) and between organs on the same plant (barley leaves *versus* roots, oat leaves *versus* roots). Aside from PC and PE being the predominant PM phospholipids, it appears there is little 'conserved' lipid composition to PMs. It would be interesting to know if the composition of PMs differed between cell types in the same organ, or for a particular cell as it matures.

Despite the many differences in composition, certain common features are apparent between PMs (15, 19, 21; Table I). First, all PMs contain significant amounts of sterols, both free and glycosylated. The spinach leaf PM, which has the lowest sterol content yet measured, still contains 20 mol % total sterol. Second, all PMs contain significant amounts of cerebrosides. Finally, in all PMs the $C_{18:2}$ fatty acids exceed the $C_{18:3}$ fatty acids, while in other membranes the reverse is often true (22).

One protein contained in the PM is the H^+-ATPase. Isolated U_3 PM vesicles contain varying specific activities of this enzyme; in this study, the specific activity of the oat root PM ATPase was more than double that of the oat coleoptile PM. Differences in the lipids surrounding the ATPase may explain the varying activities of this enzyme.

There are three reasons for believing that the lipid components are important. The first comes from studies where isolated plant PM ATPases have been reconstituted into synthetic vesicles containing a single phospholipid. Radish seedling ATPase was active when reconstituted with PI, but not with PC, or PE (8), while *Vigna radiata* seedling ATPase was active with PC, PS, and PG but not with PI or PE (17). The higher content of neutral phospholipids (PC and PE) and LPC in oat root PM as compared with the coleoptile PM would be expected to give a higher ATPase activity, as judged by the results of Serrano *et al.* (29). Oat root ATPase was active when reconstituted with any phospholipid, although the activity was greater with neutral rather than acidic phospholipids.

The second reason comes from reports that PM sterols are closely associated with the ATPase (2, 14, 25). Reports exist which state that cyclopropyl sterols may influence the plant PM ATPase activity (2, 14). The root PM is greatly enriched in the putative cycloartinol-type sterol and stigmasterol when compared to the coleoptile PM, while the levels of cholesterol and β-sitosterol were reduced. Therefore, the elevated levels of the tentatively identified cyclopropyl sterol in the root PM as well as the other differences in sterol composition in the PMs between the root and coleoptile (Table I) may help to modulate the ATPase activity. Reconstitution experiments which include mixtures of phospholipids and spectrums of sterols may provide answers to this question.

The third reason comes from studies where the lipid environment around the ATPase was changed by surfactants. Removal of most of the phospholipids from oat root PM ATPase, leaving the enzyme enriched with glycolipid and sterol, resulted in enhanced activity as long as Triton X-100 was present to provide the necessary lipid environment (25). When this fraction was then treated with lysolecithin, the ATPase was solubilized and further enriched in sterols. We concluded that altering the lipid environment around the ATPase altered its activity, and that the close association of the ATPase with sterols was essential for maximal activity.

Treatment of auxin-responsive organs, such as the oat coleoptile, with auxin results in a large increase in the rate of proton excretion (6), apparently via an enhanced activity of the PM ATPase (13). Several investigations have suggested that auxin might alter the composition of membrane lipids, which might in turn alter ATPase activity. Ettlinger and Lehle (9) demonstrated change in phosphatidylinositol metabolites following exposure to auxin. Goldberg *et al.* (11) correlated the responsiveness of varying regions of the *Vigna radiata* hypocotyl for auxin-induced growth with changes in amounts of $C_{18:1}$ fatty acids in a microsomal preparation. The regions which showed the maximum growth response to auxin also showed the largest increase in $C_{18:1}$ after 4 h auxin treatment. On the other hand, Moore *et al.* (20) reported that the $C_{18:1}$ content of PC in pea epicotyl total membrane preparation decreased from 12 to 2% after 1 h auxin treatment, while the $C_{18:3}$ content increased from 12 to 28%.

In the present study, a 1 h auxin treatment of oat coleoptiles did not appear to alter the PM lipid composition or the activity of the ATPase. This suggests that reports which show changes in tissue lipid composition following exposure to auxin are occurring in membranes other than the PM. A study of effects of auxin on PM phospholipids and fatty acids composition of specific endomembranes, such as the ER and Golgi-apparatus, might be profitable.

It would appear in the coleoptile that if the increased auxin-induced proton secretion is actually due to an increased activity of the PM ATPase, then it is not due to any inherent change in maximal activity of this enzyme, since there was no difference in specific activity of the isolated ATPase between the two treatments. However, although auxin does not change the PM lipid composition or ATPase activity in the coleoptile, the data in Table I suggest that differences in the PM lipid composition between the coleoptile and root may correlate to the maximum ATPase activity possible within a specified lipid environment.

ACKNOWLEDGMENT

We would like to thank William Howald for his assistance with the mass spectrometry.

LITERATURE CITED

1. **Ames BN** (1966) Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol **8:** 115–118
2. **Benveniste P, Bladocha M, Costet MF, Ehrhard A** (1984) Use of inhibitors of sterol biosynthesis to study plasmalemma structure and functions. Annu Proc Phytochem Soc Eur **24:** 283–300
3. **Bladocha M, Benveniste P** (1983) Manipulation by tridemorph, a synthetic fungicide, of the sterol composition of maize leaves and roots. Plant Physiol **71:** 756–762
4. **Bligh EG, Dyer WJ** (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol **37:** 911–917
5. **Chiu PL, Patterson GW, Dutky SR** (1976) Effect of AY-9944 on sterol biosynthesis in *Chlorella sorokiniana*. Phytochemistry **15:** 1907–1910
6. **Cleland R** (1973) Auxin-induced hydrogen ion excretion from *Avena* coleoptiles. Proc Natl Acad Sci USA **70:** 3092–3093
7. **Cleland RE** (1976) Kinetics of hormone-induced H^+ excretion. Plant Physiol **58:** 210–213
8. **Cocucci M, Marre E** (1984) Lysophosphatidyl choline-activated, vanadate inhibited, Mg^{2+}-ATPase from radish microsomes. Biochem Biophys Acta **771:** 42–52
9. **Ettlinger C, Lehle L** (1988) Auxin induces rapid changes in phosphatidylinositol metabolites. Nature **331:** 176–178
10. **Galliard T** (1974) Techniques for overcoming problems of lipolytic enzymes and lipoxygenases in the preparation of plant organelles. Methods Enzymol **31:** 520–528
11. **Goldberg R, Prat R, DuBacq J-P** (1983) In situ and IAA-induced cell elongation is correlated to the oleoyl phosphatidylcholine content along the *Vigna radiata* hypocotyl. Plant Cell Physiol **24:** 541–549
12. **Goodwin TW** (1980) Biosynthesis of sterols. *In* PK Stumpf. EE Conn, eds, The Biochemistry of Plants: A Comprehensive Treatise, Vol 4. Lipids: Structure and Function. Academic Press, New York, pp 485–507
13. **Hager A, Menzel H, Krauss A** (1971) Versuche und Hypotheses zur Primarwirkung des Auxins beim Streckungswachstum. Planta **100:** 47–75
14. **Hartmann MA, Benveniste P** (1987) Plant membrane sterols: isolation, identification, and biosynthesis. Methods Enzymol **148:** 632–650
15. **Harwood JL** (1980) Plant acyl lipids: structure, distribution and analysis. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, A Comprehensive Treatise, Vol 4, Lipids: Structure and Function. Academic Press, New York, pp 1–55
16. **Jones M, Keenan RW, Horowitz P** (1982) Use of 6-*p*-toluidino-2-naphthalenesulfonic acid to quantitate lipids after thin-layer chromatography. J Chromatogr **237:** 522–524
17. **Kasamo K, Nouchi I** (1987) The role of phospholipids in plasma membrane ATPase in *Vigna radiata* L. (mung bean) roots and hypocotyls. Plant Physiol **83:** 323–328
18. **Keenan TW, Leonard RT, Hodges TK** (1973) Lipid composition of plasma membranes from *Avena sativa* roots. Cytobios **7:** 103–112
19. **Lynch DV, Steponkus PL** (1987) Plasma membrane lipid alternations associated with cold acclimation of winter rye seedlings (*Secale cereale* L. cv Puma). Plant Physiol **83:** 761–767
20. **Moore TS, Price-Jones MJ, Harwood JL** (1983) The effect of indoleacetic acid on phospholipid metabolism in pea stems. Phytochemistry **22:** 2421–2425
21. **Rochester CP, Kjellbom P, Larsson C** (1987) Lipid composition of plasma membranes from barley leaves and roots, spinach leaves and cauliflower inflorescences. Physiol Plant **71:** 257–263
22. **Rochester CP, Kjellbom P, Andersson B, Larsson C** (1987) Lipid composition of plasma membranes isolated from light-grown barley (*Hordeum vulgare*) leaves: identification of cerebroside as a major component. Arch Biochem Biophys **255:** 385–391
23. **Roughan PG, Batt RD** (1968) Quantitative analysis of sulfolipid (sulfoquinovosyl diglycerides) and galactolipids (monogalactosyl and diagalactosyl diglycerides) in plant tissues. Anal Biochem **22:** 74–88
24. **Sandstrom RP, deBoer AH, Lomax TL, Cleland RE** (1987) Latency of plasma membrane H^+-ATPase in vesicles isolated by aqueous phase partitioning. Increase substrate accessibility or enzyme activation. Plant Physiol **85:** 693–698
25. **Sandstrom RP, Cleland RE** (1989) Selective delipidation of the plasma membrane by surfactants: enrichment of sterols and activation of ATPase. Plant Physiol (in press)
26. **Schaller GE, Sussman MR** (1988) Phosphorylation of the plasma-membrane H^+-ATPase of oat roots by a calcium-stimulated protein kinase. Planta **173:** 509–518
27. **Serrano R** (1984) Purification of the proton pumping ATPase from plant plasma membranes. Biochem Biophys Res Commun **121:** 735–740
28. **Serrano R** (1985) Plasma membrane ATPase of plants and fungi. CRC Press, Boca Raton, FL, pp 1–174
29. **Serrano R, Montesinos C, Sanchez J** (1988) Lipid requirements of the plasma membrane ATPase from oat roots and yeast. Plant Sci **56:** 117–122
30. **Supelco** (1979) Bulletin 721 F: Esterification and Acylation. Supelco, Inc, Bellefonte, PA
31. **Uemura M, Yoshida S** (1984) Involvement of plasma membrane alterations in cold acclimation of winter rye seedlings (*Secale cereale* L. cv Puma). Plant Physiol **75:** 818–826
32. **Wright LC, McMurchie EJ, Pomeroy MK, Raison JK** (1982) Thermal behavior and lipid composition of cauliflower plasma membranes in relation to ATPase activity and chilling sensitivity. Plant Physiol **69:** 1356–1360
33. **Whitman CE, Travis RL** (1985) Phospholipid composition of a plasma membrane-enriched fraction from developing soybean roots. Plant Physiol **79:** 494–498
34. **Yoshida S, Uemura M** (1984) Protein and lipid compositions of isolated plasma membranes from orchard grass (*Dactylis glomerata* L.) and changes during cold acclimation. Plant Physiol **75:** 31–37
35. **Yoshida S, Uemura M** (1986) Lipid composition of plasma membranes and tonoplasts isolated from etiolated seedlings of mung bean (*Vigna radiata* L.). Plant Physiol **82:** 807–812
36. **Zlatkis A, Zak B** (1969) Study of a new cholesterol reagent. Anal Biochem **29:** 143–148