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The Role of Phospholipids in Plasma Membrane ATPase Activity in *Vigna radiata* L. (Mung Bean) Roots and Hypocotyls[1]

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ABSTRACT

Root and hypocotyl plasma membrane H^+-ATPases were partially purified from deoxycholate-solubilized fractions of microsomes in mung bean (*Vigna radiata* L.) plants in the presence of glycerol. Certain properties of the ATPases and the manner in which phospholipids affect their activity were compared. Root ATPase was similar to hypocotyl ATPase with respect to substrate specificity, salt stimulation, pH dependence, K_m for ATP·Mg^{2+} and inhibitor sensitivity, except for inhibition by vanadate. Both purified ATPases required phospholipids for their activation. Optimum concentrations of exogenously added phospholipid mixture (asolectin) to hypocotyl and root ATPase mixture were 0.03% and 1.0%, respectively. Root ATPase activation did not decrease if more than 1.0% asolectin was added. Qualitatively, phosphatidylserine and phosphatidylcholine brought about greater ATPase activation than other phospholipids. The hypocotyl ATPase was activated by phosphatidylinositol, phosphatidylserine and phosphatidylglycerol to a greater extent than the root ATPase. Root, but not hypocotyl ATPase, was slightly inhibited by the addition of phosphatidylinositol, phosphatidylethanolamine, and phosphatidic acid. The hypocotyl plasma membrane contained phosphatidylinositol + phosphatidylserine, phosphatidylglycerol and phosphatidic acid, and unsaturated fatty acids in greater abundance than the root plasma membrane. The differential activation of the plasma membrane ATPases may arise from these differences.

Lipids significantly determine the physicochemical properties of biological membranes. In various biological systems, lipid composition has been studied in relation to the development of soybean roots (27), seed germination (6, 8, 29), cell elongation (9), phytochrome (11, 19), temperature sensitivity (20, 22, 23, 31), membrane permeability (27), senescence (10), salt tolerance (25), water stress (26), and the activity of membrane-associated enzymes (24).

The effects of polar lipids on the plasma membrane ATPase activity of plants have been investigated by Cocucci and Marré (7) using radish seeds. Their findings indicated a close relation between seed germination and lipid-induced increase in ATPase activity (6). In a previous paper (14), a partially purified ATPase from DOC[2]-solubilized fractions of plasma membranes in mung bean roots was found to require phospholipids for activation and to possess proton translocating activity (13, 15). During purification of plasma membrane ATPase, distinct differences were noted in phospholipid requirements for root and hypocotyl ATPases. There are still relatively few studies of plant membrane lipids and their effects on enzymes.

In the present study, the effect of adding phospholipids to partially purified ATPase was examined and the phospholipid compositions of plasma membranes in roots and hypocotyls of mung bean plants were compared to each other. The *in vivo* relation of phospholipids to ATPase activity in plasma membranes is discussed.

MATERIALS AND METHODS

Plant Material. Mung bean (*Vigna radiata* L.) seeds were germinated in the dark at 32°C on thin absorbent cotton on a 0.75% agar plate in an enamelled tray. Following cultivation for 75 h, the root and hypocotyl were excised from each plant and chilled in aerated cold distilled H_2O.

Isolation of Plasma Membranes. Approximately 200 g fresh weight of excised roots and hypocotyls were separately homogenized with a chilled mortar and pestle in 300 ml of grinding medium containing 0.25 M mannitol, 25 mM Hepes-Tris (pH 7.3), 2 mM EGTA, 1 mM DTT, and 1% BSA. The homogenate was filtered through four layers of gauze and the filtrate was centrifuged at 1,500*g* for 10 min. The supernatant was centrifuged at 10,000*g* for 30 min and the resulting supernatant, further centrifuged at 80,000*g* for 30 min for preparation of a microsomal pellet. The pellet thus obtained was suspended in 1.0 ml of the grinding medium and the suspension was layered over a dextran step gradient consisting of 8% dextran T-70 (bottom) and 1% dextran T-70 (top) (Pharmacia Fine Chemicals, Uppsala, Sweden) made in the gradient mixture containing 1 mM DTT, 2.5 mM Hepes-Tris (pH 7.3) and 1 mM EDTA to remove the tonoplast (18). The step gradients were centrifuged for 2 h at 120,000*g*. Tonoplast-enriched membranes were localized in the 1 to 8% dextran interface and the plasma membranes were enriched in the 8% dextran pellet. The 8% dextran pellet was suspended in 1 ml of grinding medium and layered over a two-step sucrose gradient consisting of 30 and 40% (w/w) sucrose

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[2] Abbreviations: DOC, deoxycholate; DCCD, *N,N'*-dicyclohexylcarbodiimide; DES, diethylstilbestrol; LPC, lysophosphatidylcholine; PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; VO_4, orthovanadate; PS, phosphatidylserine.

made in the gradient mixture. The sucrose step gradient was centrifuged for 2 h at 80,000*g*. Plasma membrane-enriched fractions were collected from the 30 to 40% sucrose interface. They were then diluted with suspending medium containing 0.25 M mannitol, 1 mM DTT, 2.5 mM Hepes-Tris (pH 7.3), and 1 mM EDTA and centrifuged 140,000*g* for 40 min. The resulting pellet was suspended in suspending medium to a concentration of 4 to 6 mg/ml and stored at −80°C for up to 1 week without significant loss in activity.

Solubilization and Purification of Plasma Membrane ATPase. A two-step procedure using the DOC and zwittergent 3-14 (Calbiochem) was employed to solubilize ATPase from the plasma membrane as described previously (14). Briefly, loosely bound membrane proteins were removed by treatment with 0.1% DOC followed by solubilizing the ATPase from a 0.1% DOC pellet with 0.1% zwittergent plus 1% DOC in the presence of 45% glycerol. The solubilized material was further purified by centrifugation through a glycerol gradient (45–70%).

ATPase Assay and Protein Determination. ATPase activity was routinely measured at 38°C for 30 min with 1 to 5 μg of protein for each assay as described previously (14). Phosphate was determined by the method described previously (13). Protein was determined by the method of Bradford (4) using BSA as the standard.

Lipid Extraction. Total lipids were extracted from the membrane suspension according to the method of Bligh and Dyer (2). To inactivate the membrane associated phospholipases, the plasma membrane suspension was boiled and then cooled to room temperature. After that 20 volumes of $CHCl_3$:methanol (2:1, v/v) were added to the mixture and the lipids were extracted for 30 min. The extracts were washed twice with 1% (w/v) NaCl, evapolated to dryness under N_2, redissolved in 100 μl $CHCl_3$:methanol (9:1 v/v), and stored overnight under N_2 at −20°C.

For TLC, a Silica Gel 60 F_{254} (0.5 mm, Merk Ltd, Darmstadt, Germany) was activated by heating at 120°C for 60 min and then cooled to room temperature. Compositional analysis of the phospholipids was performed using two dimensional TLC. The plates were developed in the first dimension with $CHCl_3$:methanol:water (65:25:4, v/v) and dried 20 min under vacuum. The second solvent was $CHCl_3$:methanol:ammonia: isopropylamine (65:35:0.5:5, v/v). The lipids were identified by co-chromatography using phospholipid standards. For analysis of fatty acid content, the total phospholipids were transmethylated in 10% sulfuric acid in methanol at 40°C for 16 h. Fatty acid methylesters were analyzed by GC (Shimadzu GC-9A, Tokyo, Japan) with Chromatopack (Shimadzu C-R2A, Tokyo, Japan).

Lipids. The asolectin used in all experiments was a phospholipid mixture (Associated Concentrates Inc., New York) containing approximately 40% PC. Purified phospholipids were purchased as powder: PC, PG, PE, PA, and LPC from Avantipolar Lipids, Inc. (Birmingham, AL); PI and PS from Sigma. A 5% phospholipid stock solution was prepared in distilled water and sonicated in a bath sonicator (Kokusai Denki Co., Type UA-100, Tokyo, Japan) to near clarity prior to use.

Chemicals. ATP was obtained from Boehringer Mannheim (W. Germany) as a disodium salt and converted to the Tris salt by ion exchange chromatography through a Dowex 50W. Sodium orthovanadate of Fisher Chemical Co. (Montreal, Canada) was kindly provided by Carolyn W. Slyman of Yale University. The concentration of the stock solution of vanadate was verified spectrophotometrically using the extinction coefficient determined by Cantley *et al.* (5). DTT was from Wako Chemical Co. (Osaka, Japan); DCCD from Aldrich Chemical Co. (Milwaukee, WI), and tyrocidine from the United States Biochemical Corp. (Cleveland, OH). Each of these chemicals was dissolved in 1% ethanol just before use. All other chemicals were of analytical grade.

RESULTS

Purification of Plasma Membrane ATPases from Roots and Hypocotyls. The plasma membrane ATPases of mung bean hypocotyls and roots were solubilized with a two-step procedure using DOC and zwittergent 3-14 and further purified by centrifugation through a glycerol gradient (45–70%). Finally, about 10% of the ATPase activity was recovered and the specific activity found to be 3 to 6 μmol Pi/mg protein·min and to increase about 10- to 12-fold (14).

Effects of Various Inhibitors on Plasma Membrane ATPases from Roots and Hypocotyls. The specific activity of partially purified plasma membrane ATPase of hypocotyls in the presence of 0.03% asolectin was always higher than that from the roots. The effects of various inhibitors on ATPase are shown in Table I. The ATPase was insensitive to oligomycin and azide, mitochondrial ATPase inhibitors and to ouabain, a specific inhibitor of Na^+-K^+-ATPase. Slight inhibition was observed by ammonium molybdate, an inhibitor of nonspecific phosphatase and nitrate, an inhibitor of tonoplast ATPase. DES, DCCD, tyrocidine, and gramicidin S, an inhibitor of membrane-bound ATPases, strongly inhibited the enzyme. Root ATPase and hypocotyl ATPase differed in their sensitivity toward VO_4, a potent inhibitor of H^+-ATPase. Hypocotyl ATPase was less sensitive to VO_4 than root ATPase (Fig. 1). Root and hypocotyl ATPases were sensitive to VO_4, with half maximal inhibition at 1 and 5 μM, respectively.

Effects of Salts on Plasma Membrane ATPases from Roots and Hypocotyls. The effects of salts on root and hypocotyl ATPases are presented in Tables II and III. These salts stimulated the activity of both enzymes to essentially the same extent. They were activated by divalent cations in the presence of 50 mM KCl in the following order: $Mg^{2+} > Mn^{2+} > Cu^{2+} > Co^{2+}$ (Table II). Stimulation due to monovalent cations had the following order: $Rb^+ = NH_4^+ > K^+ > Na^+ > Li^+ >$ choline $> Cs^+$ (Table III). Maximal stimulation by KCl varied from 30 to 80%, depending on the conditions of preparation and tissue material. ATPase activity decreased with increasing concentration of Cs^+. Purified ATPase preparations resulted in an even greater decrease (Fig. 2).

Substrate Specificity and pH Optimum of Plasma Membrane ATPases from Roots and Hypocotyls. Table IV illustrates the

Table I. *Effects of Inhibitors on Plasma Membrane ATPases Purified from Roots and Hypocotyls*

ATPase activity was measured in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), 0.03% asolectin, and various concentrations of inhibitors as indicated. Numbers in parentheses indicate percent of control.

Inhibitor	ATPase Activity	
	Root	Hypocotyl
	μmol Pi/mg protein·min	
None	2.80 (100)	3.52 (100)
Ammonium molybdate (100 μM)	2.36 (84.3)	3.07 (87.1)
Oligomycin (10 μg/ml)	2.80 (100)	3.83 (108.8)
KNO_3 (50 mM)	2.49 (89.0)	3.07 (87.1)
NaN_3 (1 mM)	2.78 (99.2)	3.48 (98.8)
DES (100 μM)	0.35 (12.6)	0.62 (17.5)
DCCD (100 μM)	1.06 (37.8)	1.54 (43.8)
VO_4 (50 μM)	0.24 (8.6)	0.66 (18.7)
Tyrocidine (100 μM)	1.54 (55.1)	1.73 (49.1)
Gramicidin S (100 μM)	0.31 (11.0)	0.45 (12.9)
Ouabain (50 μM)	2.78 (99.3)	3.47 (98.5)

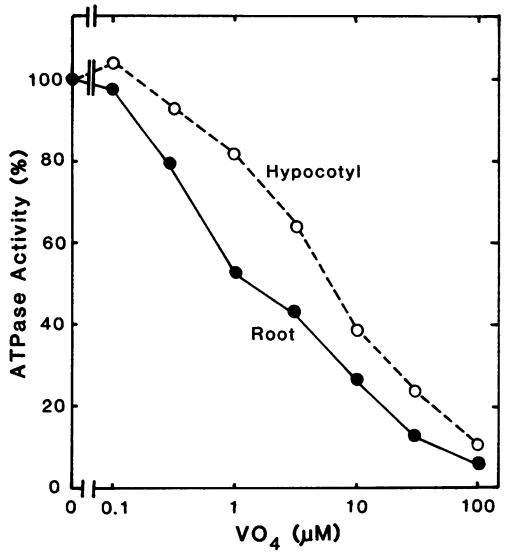

FIG. 1. Effects of VO_4 on plasma membrane ATPases from roots and hypocotyls. ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM $MgSO_4$, 50 mM KCl, 30 mM Mes-Tris (pH 6.5) and various concentrations of VO_4 as indicated. Control activities of plasma membrane ATPases from roots and hypocotyls in the absence of VO_4 were 2.41 and 4.66 μmol Pi/mg protein·min, respectively.

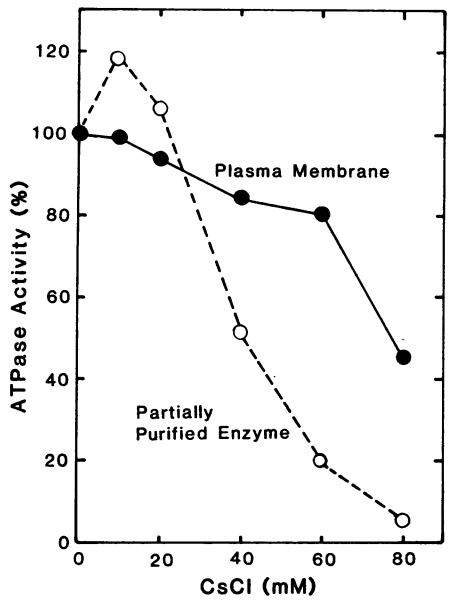

FIG. 2. Effects of $CsCl_2$ on ATPase activity in plasma membrane vesicles and ATPase partially purified from a hypocotyl plasma membrane. ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM $MgSO_4$, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), and various concentrations of $CsCl_2$. The assay solution containing purified ATPase preparation included 0.03% asolectin. Control activities of the ATPase in plasma membrane vesicles and purified ATPase from the plasma membrane were 0.69 and 3.51 μmol Pi/mg protein·min, respectively.

Table II. *Effects of Divalent Cations on Plasma Membrane ATPases Purified from Roots and Hypocotyls*

ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM divalent cations, 50 mM KCl, 0.03% asolectin, and 30 mM Mes-Tris (pH 6.5).

Cation	ATPase Activity	
	Root	Hypocotyl
	μmol Pi/mg protein·min	
KCl	0.15 (4.5)	0.23 (5.9)
+$MgSO_4$	3.42 (100)	3.85 (100)
+$MnSO_4$	1.45 (42.6)	1.52 (39.6)
+$CoCl_2$	0.40 (11.6)	0.43 (11.2)
+$ZnSO_4$	0.38 (11.0)	0.27 (6.9)
+$CaCl_2$	0.38 (11.0)	0.14 (3.7)
+$CuSO_4$	0.41 (12.0)	0.83 (21.6)

Table III. *Effects of Monovalent Cations on Plasma Membrane ATPases Purified from Roots and Hypocotyls*

ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM $MgSO_4$, 50 mM monovalent cations, 0.03% asolectin, and 30 mM Mes-Tris (pH 6.5).

Cation	ATPase Activity	
	Root	Hypocotyl
	μmol Pi/mg protein·min	
$MgSO_4$	1.98 (72.6)	2.65 (78.2)
+KCl	2.73 (100)	3.40 (100)
+RbCl	3.26 (119.4)	3.89 (114.6)
+CsCl	0.22 (8.1)	0.23 (6.7)
+NaCl	2.38 (87.1)	2.47 (72.7)
+LiCl	1.81 (66.1)	2.43 (71.5)
+Choline-Cl	1.43 (52.4)	1.91 (56.4)
+NH_4Cl	3.06 (112.1)	3.89 (114.6)

Table IV. *Activity of Partially Purified Plasma Membrane Enzymes with Various Substrates*

Enzymic activity was assayed in the presence of 3 mM $MgSO_4$, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), and 0.03% asolectin in the presence of 3 mM substrate.

Substrate	ATPase Activity	
	Root	Hypocotyl
	μmol Pi/mg protein·min	
AMP	0.02 (0.8)	0.04 (1.2)
ADP	0.22 (8.7)	0.33 (10.2)
ATP	2.54 (100)	3.21 (100)
GTP	0.68 (26.8)	0.56 (17.3)
UTP	0.93 (36.6)	0.85 (26.9)
CTP	0.24 (9.4)	0.29 (9.0)
β-Glycero-P	0.09 (3.5)	0.08 (2.6)
p-Nitrophenyl-P	0.02 (0.8)	0.08 (2.6)

substrate specificity of plasma membrane ATPases. Root ATPase was similar to hypocotyl ATPase with respect to hydrolysis of nucleotide phosphates. Hydrolysis efficiency was of the following order: ATP > UTP > GTP > CTP followed by other substrates. The pH optimum of both ATPases was about same, the optimum being from 6.5 to 7.0 (Fig. 3).

Kinetics of Plasma Membrane ATPases from Roots and Hypocotyls. Straight lines were obtained from Lineweaver-Burk plots with ATP·Mg^{2+} for root and hypocotyl ATPases. The enzymes were essentially the same with respect to K_m at 1.7 mM (Fig. 4). The V_{max} values were 5.0 and 6.2 μmol Pi/mg protein·min for roots and hypocotyls, respectively.

Effects of Added Phospholipids on Root and Hypocotyl ATPases. Figure 5 shows the effects of asolection on the inhibition of ATPase activity due to VO_4. The inhibition of hypocotyl ATPase by VO_4 decreased with increasing concentration of asolectin, though its effect on root ATPase was small. Thus, asolectin

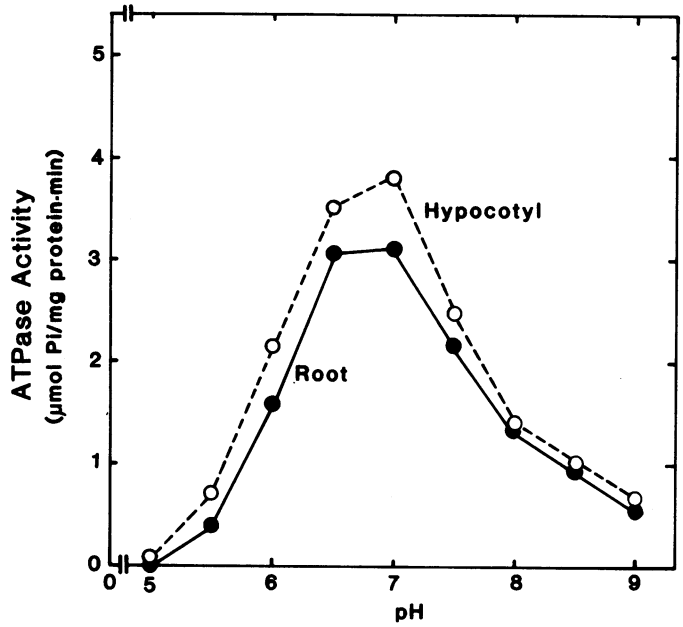

FIG. 3. pH dependence of plasma membrane ATPases from roots and hypocotyls. ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM $MgSO_4$, 50 mM KCl, and 0.03% asolectin. The assay pH was adjusted by varying the ratio of Tris-Mes.

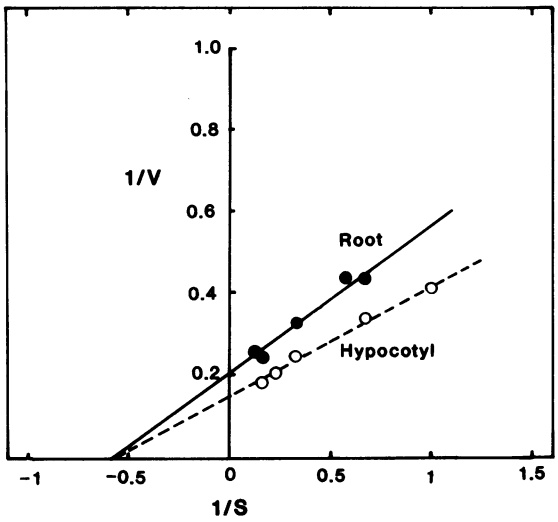

FIG. 4. Lineweaver-Burk plots for the hydrolysis of ATP. ATPase activity was assayed in the presence of 50 mM KCl, 30 mM Mes-Tris (pH 6.5), and various concentrations of Tris-ATP·$MgSO_4$. Velocity (V) is expressed as μmol Pi/mg protein min and substrate concentration (S), as mM. Kinetic constants were calculated by linear regression analysis.

prevents, to some extent, inhibition due to VO_4 suggesting the likelihood of interaction between VO_4 and phospholipids.

Activation of ATPase activity by added phospholipid was investigated (Fig. 6). A phospholipid mixture or purified phospholipid was added at specified concentrations directly into the assay reaction mixture prior to introduction of the enzyme. Maximal hypocotyl ATPase activation was noted when 0.03 to 0.05% asolectin was added; as the concentration became higher, the activation gradually decreased. Activation of root ATPase increased with increasing asolectin concentration, becoming optimum at 1.0 to 1.2%. Above this concentration, no decrease in activation was noted. The effects of several added phospholipids on the activation of root and hypocotyl ATPases are given in Table V. Slight activity was observed without phospholipid. In a

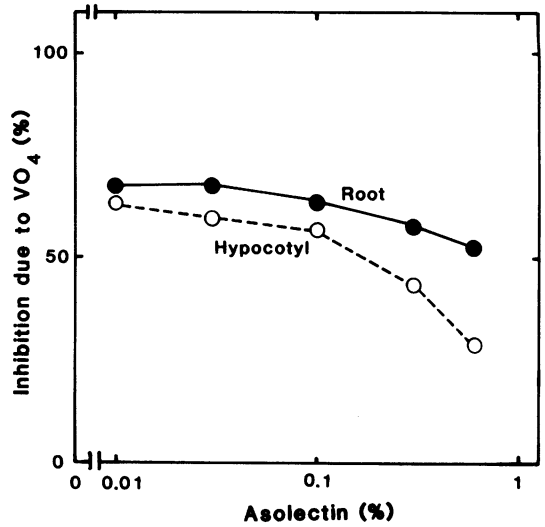

FIG. 5. Effects of asolectin on inhibition of ATPase activity due to VO_4. ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM $MgSO_4$, 50 mM KCl, 30 mM Mes-Tris (pH 6.5) with or without 10 μM VO_4, and various concentrations of asolectin as indicated.

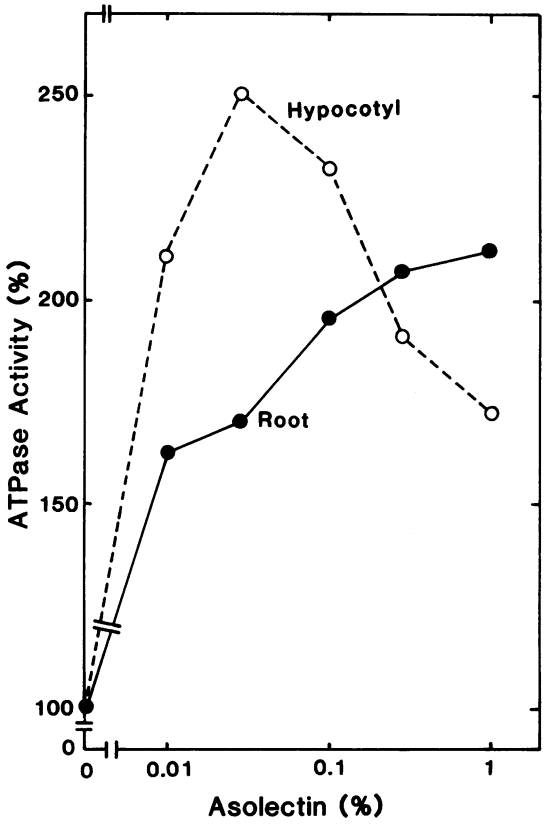

FIG. 6. Stimulation of the activity of plasma membrane ATPases from roots and hypocotyls by asolectin. ATPase activity was assayed in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), and various concentrations of asolectin as indicated. Control activities of plasma membrane ATPases from roots and hypocotyls in the absence of added asolectin were 1.3 and 2.0 μmol Pi/mg protein·min, respectively.

phospholipid mixture, asolectin from Associated Concentrations Inc. was noted to cause by far the greatest activation. This may have been due to qualitative differences in phospholipid composition. In fact, the *in vitro* activation of ATPase activity was

Table V. *Phospholipid Activation of Plasma Membrane APTases Purified from Roots and Hypocotyls*

ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM $MgSO_4$, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), and 0.05% phospholipid and expressed as percent of the activity in the absence of added phospholipid.

Phospholipid Added	ATPase Activity	
	Root	Hypocotyl
	μmol Pi/mg protein·min	
None	0.56 (100)	0.82 (100)
Soybean phosphatide extract	1.38 (246.4)	3.24 (397.1)
Asolectin	2.09 (373.6)	4.28 (524.0)
LPC	0.87 (155.0)	1.65 (202.5)
PS	1.07 (191.4)	2.72 (333.8)
PI	0.46 (82.1)	1.47 (180.4)
PE	0.41 (72.9)	0.92 (112.3)
PC	1.25 (224.3)	2.70 (331.4)
PG	0.82 (145.7)	2.39 (292.6)
PA	0.36 (63.6)	0.76 (92.6)

Table VI. *Phospholipid Composition of Plasma Membranes from Roots and Hypocotyls*

Phospholipid	Root	Hypocotyl
	mol %	
PC	29.2	30.3
PE	12.7	10.7
PI + PS	6.1	9.8
PG	2.1	2.7
PA	10.5	15.4

Table VII. *Fatty Acid Composition of Plasma Membranes from Roots and Hypocotyls*

Membrane Fraction	Fatty Acids						Unsaturated/ Saturated
	16:0	18:0	18:1	18:2	18:3	20:0	
	mol %						
Root	46.6	8.2	4.4	15.8	23.1	1.9	0.76
Hypocotyl	36.9	7.0	7.1	23.4	24.3	1.3	1.21

dependent on added purified phospholipids. Root ATPase activity appeared to be stimulated by phospholipids in the following order: PC > PS > LPC > PG; it was inhibited by PI, PE, and PA. Hypocotyl ATPase activity was activated by phospholipids in the order, PS > PC > PG > LPG > PI > PE > PA. No inhibition due to added phospholipids could be observed. Root ATPase appeared to particularly differ with hypocotyl ATPase in its activation by PI, PG, PC, and PS. Another, although the possibility might be unlikely, it cannot be ruled out that a small amount of phospholipids in both solubilized ATPase preparations was differentially retained even after treatment with excess detergents and the differential phospholipid activation between root and hypocotyl ATPase may be due to differential extent of solubilization and removal of endogenous lipids.

Phospholipid and Fatty Acid Content in Root and Hypocotyl Plasma Membranes. Table VI shows the phospholipid compositions in hypocotyl and root plasma membrane samples. PC and PE are the major phospholipid components in the plasma membrane. The content of PI + PS, PG and PA in the hypocotyl plasma membranes was greater than that in the root plasma membranes. The PA content in both membranes was from 15 to 11%. There is the possibility that phospholipase D cleavage causes an increase in PA.

Table VII gives the fatty acid composition of phospholipids in both plasma membranes. The ratio of unsaturated/saturated fatty acids in root plasma membrane was less than that in hypocotyls, indicating the former to contain considerable amounts of saturated fatty acids. This membrane was relatively enriched in 16:0, 18:0, and 20:0 content, but to a lesser extent in 18:1 and 18:2 content. This may be related to membrane functions such as temperature sensitivity and seed germination.

Table VIII shows the fatty acid composition of each phospholipid in both membranes: 16:0, 18:2, and 18:3 were the major fatty acid components in PC, PE, PA, and PI + PS. The major fatty acid in PG was 16:0. No 16:1 content could be detected. The ratio of unsaturated/saturated fatty acids in both plasma membrane indicated the phospholipids (except for PG) of the hypocotyl plasma membranes to contain a much higher unsaturated fatty acid content than that of the root plasma membranes. In particular, the 18:1 and 18:2 content of PA, PC, and PI + PS was excessive.

DISCUSSION

Root and hypocotyl plasma membrane H+-ATPase were solubilized in the same manner by DOC plus zwittergent 3-14 in the presence of glycerol. In these preparations, the extent of delipidation of the two solubilized ATPases is not assessed, however, it is known that DOC cause removal of more than 90% of the endogenous phospholipids (16), presumably by formation of mixed detergent-phospholipid micelles (21).

Plasma membrane ATPase (3, 6, 7, 14) and tonoplast ATPase (1) purified from DOC- or cholate-solubilized fractions require phospholipid for *in vitro* activation. Phospholipid is absolutely indispensable for the activation of partially purified plasma membrane ATPase from the roots and hypocotyls of mung bean (Fig. 6). The quantitative (Fig. 6) and qualitative (Table V) requirements of this enzyme in the roots differed from those in the hypocotyls. Quantitatively, the optimum concentration of added phospholipid to a hypocotyl ATPase solution was 0.03% and activation of the enzyme gradually decreased as this concentration increased (Fig. 6). Root ATPase activation did not decrease with an increase in phospholipid concentration. ATPase activation by phospholipid and the specific activity of hypocotyl ATPase in the presence of phospholipid generally exceeded those in the roots. ATPase activity in plasma membrane vesicles from hypocotyls was also greater than that in roots (data not shown). These findings indicate hypocotyl ATPase to be more sensitive to phospholipid. Of the phospholipids, PI, PG, PC, and PS induced the activation of hypocotyl ATPase to a greater extent than that of root ATPase. PI, PE, and PA acted to inhibit root ATPase (Table V). This indicates that the response of two

Table VIII. *Fatty Acid Composition of Phospholipids from Plasma Membranes from Roots and Hypocotyls*

Phospholipid	Fatty Acids						Unsaturated/ Saturated
	16:0	18:0	18:1	18:2	18:3	20:0	
	mol %						
Root							
PC	37.3	8.5	3.6	19.8	29.5	1.3	1.12
PE	69.2	9.4	5.2	36.2	40.8	1.0	1.03
PI + PS	45.9	5.0	2.5	14.7	30.5	1.3	0.91
PG	75.1	9.1	2.0	5.7	8.0	n.d.	0.19
PA	36.7	5.6	2.8	21.8	32.4	0.7	1.33
Hypocotyl							
PC	31.9	8.3	7.4	26.1	25.9	0.3	1.47
PE	40.7	5.1	5.8	26.1	21.8	0.6	1.16
PI + PS	40.0	5.0	4.5	21.3	27.1	2.1	1.12
PG	76.6	7.2	2.6	6.0	6.9	0.4	0.18
PA	25.5	4.0	6.1	30.7	33.1	0.6	2.32

ATPases to hydrophilic moiety of phospholipids are different from each other. These observations, namely the specificity of the head group requirement can be reconciled in the view point that the head groups of fatty acids may provide a specific "functional" requirement such as the recognization of the functional sites of the enzymes (16). Phospholipid fatty acid chains provide the appropriate environments such as low dielectric. The difference of the fatty acid composition and the rate of unsaturated/saturated fatty acids (Table VII) may reflect the difference of microenvironment in hydrophobic domain. Furthermore, the hypocotyl plasma membrane fraction contained more PI + PS and PG (Table VI) and unsaturated fatty acids in the phospholipids (Tables VII and VIII) than the root plasma membrane fraction. Thus, hypocotyl and root plasma membranes contain different amounts of phospholipids and fatty acids. These differences may possibly give rise to ATPase activation which in turn may have an effect on cold acclimation (30) and seed germination (6).

The effect of phospholipid on VO_4-inhibition is evident from Figure 5. VO_4-inhibition of hypocotyl ATPase was suppressed to a greater extent by added phospholipid. Taking into consideration that VO_4 is less sensitive to hypocotyl ATPase (Fig. 1), these data indicate that interactions quite likely occur between phospholipid and VO_4. The vanadate competes with phosphate for binding, and is highly effective as an inhibitor because, vanadate can easily adopt a stable structure, which resembles the transition state of the phosphate during reaction (17). Thus, phospholipids may affect, for example, the ratio of phosphorylated to dephosphorylated enzyme.

Hypocotyl ATPase was similar to root ATPase with respect to substrate specificity (Table IV), salt stimulation (Tables II and III), pH dependence (Fig. 3), K_m for $ATP \cdot Mg^{2+}$ (Fig. 4), inhibitor sensitivity, except for inhibition by VO_4 (Table I). Thus, the ATPase moiety of plasma membranes from roots and hypocotyls is basically the same and differences in both ATPase activities may possibly be accounted for on the basis of activation levels.

In summary, hypocotyl plasma membranes contain PI + PS, PG, PA, and unsaturated fatty acids (18:1, 18:2) in amounts greater than those in root plasma membranes. Plasma membrane ATPase activities from hypocotyls and roots were abolished upon removal of membrane lipids by DOC extraction, but were restored by addition of phospholipids such as PS and PC. Purified plasma membrane ATPase from hypocotyls require more PI, PG, PC, and PS for activation than that from roots. This requirement difference may be an indication of the organ-specificity of plasma membrane ATPase activity *in vivo*. Plasma membrane ATPase consists of amphipathic structure and its activity may thus be regulated by interactions between the active site(s) of the enzyme moiety and hydrophobic lipid environment of the plasma membrane (12).

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