Lysophosphatidylcholine specifically stimulates plasma membrane H^+-ATPase from corn roots

V.K. Pedchenko, G.F. Nasirova and T.A. Palladina

Institute of Plant Physiology and Genetics, Ukrainian Academy of Sciences, Kiev, USSR

Received 24 September 1990

The plasma membrane H^+-ATPase activity from corn seedling roots is shown to be stimulated 3- to 4-fold by the addition of lysophosphatidylcholine (lysoPC). This effect clearly differs from that of other detergents by both the magnitude and the absence of inhibition at higher concentrations. LysoPC decreases the apparent K_m for MgATP, increases V_{max} of the ATPase reaction but does not change its pH optimum. On the contrary, the acid phosphatase activity associated with plasma membranes is not influenced by lysoPC. A lysoPC stimulation is also demonstrated for the solubilized preparation of the H^+-ATPase. It is assumed that lysoPC stimulation of the plant plasma membrane H^+-ATPase is not only due to permeabilization of the vesicles for MgATP, but also to direct action on the enzyme.

Lysophosphatidyl choline; Enzyme activation; H^+-ATPase; Plant plasma membrane

1. INTRODUCTION

The plant plasma membrane H^+-ATPase is a proton pump hydrolyzing ATP and generating a protonic potential. This enzyme belongs to the E1–E2 family of transport ATPases, controls cell elongation and nutrients uptake and shows close similarity to the plasma membrane H^+-ATPase of fungi [1,2]. When considering different ways of regulation, the effects of lipids on this integral enzyme are of special interest.

The lipid dependence of the plasma membrane H^+-ATPase activity has been previously demonstrated, both on partially delipidated [3] and solubilized preparations [4,5]. The enzyme reactivation by the different phospholipids revealed that the lysoPC was the most powerful activator in plasma membrane preparations of plants and yeast [4,6].

LysoPC is a non-denaturing detergent which is especially suitable for H^+-ATPase solubilization [7], and therefore the enzyme stimulation in vesicular preparations by this detergent could be explained as a result of an increase in permeability which enhances substrate accessibility to the active site of the enzyme [8]. Alternatively, lysoPC could directly stimulate the enzyme activity.

Correspondence address: V.K. Pedchenko, Plant Physiology and Genetics Institute, Ukrainian Academy of Sciences, Vasilkovskaya 31/17, Kiev, 252022, USSR

Abbreviations: lysoPC, lysophosphatidylcholine; PMSF, phenylmethylsulfonyl fluoride; MES, morpholynoethane sulfonic acid; zwittergent 3–14, *N*-tetradecyl-*N*-*N*-dimethyl-3-ammonia-1-propane-sulfonate; DTT, dithiothreitol; SDS, sodium dodecylsulfate; TX-100, Triton X-100; p-NPP, 4-nitrophenyl phosphate; p-NP, 4-nitrophenol; CMC, critical micellar concentration

The present paper is devoted to the investigation of the lysoPC stimulation of the plasma membrane H^+-ATPase activity from corn seeding roots.

2. MATERIALS AND METHODS

Corn (*Zea mays* L., hybrid Dneprovsky 247 MV) was grown on 1 mM $CaSO_4$ solution in the dark at 26°C for 8 days. Plasma membrane preparations were isolated from etiolated corn seedling roots by centrifugation of the microsomal fraction (80 000×*g* pellet) in the discontinuous sucrose gradient [9] in the presence of 0.5 mM PMSF and 10 mM DTT.

The activity of H^+-ATPase was assayed mainly according to [10] in 1 ml of the incubation medium containing 20–50 μg of membrane protein, 50 mM KCl, 5 mM sodium azide, 0.2 mM ammonium molybdate and 50 mM MES (pH 6.50, adjusted with Tris). The reaction was started by the addition of an equimolar mixture of $MgSO_4$ and ATP up to the final concentration of 3 mM and was carried out at 30°C for 15 min. After the addition of 1 ml of 0.2 M sodium acetate buffer (pH 4.20) containing 0.15% ammonium molybdate, 0.5% SDS and 0.2 ml of $SnCl_2$ solution (19 mg $SnCl_2 \times 2H_2O$ and 0.1 ml of acetic acid adjusted to 25 ml with distilled water) color development continued for 10 min. After the addition of 0.3 ml 2.2% citric acid to prevent additional color development due to non-enzymatic ATP hydrolysis, absorbance was measured at 750 nm. The acid phosphatase activity was assayed in a final volume of 1 ml containing 20–50 μg of membrane protein in 50 mM MES (pH 5.0, adjusted with Tris). The reaction was started by the addition of an equimolar mixture of $MgSO_4$ and p-NPP to a final concentration of 2.5 mM. After 15 min incubation at 30°C the reaction was stopped with 2 ml 0.2 M NaOH containing 0.5% SDS, and p-NP production was measured at 400 nm.

Solubilization of H^+-ATPase was carried out with lysoPC [7] or zwittergent 3–14 [5] and supernatants were used without further purification.

Kinetic parameters of the hydrolytic reaction were estimated by a non-linear regression program [11]. The protein concentration was measured according to Bradford [12]. All experiments were repeated 4–8 times in triplicate and average values ± SE presented.

LysoPC and MES were obtained from Sigma; Tris, Triton X-100, SDS, sodium cholate, sodium azide, PMSF and octyl glucoside from Serva and zwittergent 3–14 from Calbiochem.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

Table I

Effect of different detergents on the plasma membrane H^+-ATPase activity

Detergent	CMC (mM)[a]	Conc. (mM)	Activity (μmol/mg protein/h)	Rel. activ. (%)
LysoPC	0.03	-	12.98 ± 1.34	100.0
		0.01	17.56 ± 0.54	135.3
		0.02	23.76 ± 1.20	183.1
		0.05	26.13 ± 3.41	201.3
		0.10	35.74 ± 4.46	275.3
		0.20	41.12 ± 4.86	316.8
		0.49	42.17 ± 4.72	324.9
		0.98	39.22 ± 4.73	302.2
TX-100	0.30	-	13.37 ± 1.43	100.0
		0.16	19.33 ± 1.11	144.5
		0.40	17.15 ± 0.83	128.3
		0.80	10.99 ± 1.61	82.2
		1.60	10.03 ± 1.95	75.0
Zwittergent 3-14	0.30	-	8.91 ± 0.96	100.0
		0.28	16.06 ± 1.12	180.2
		0.55	3.47 ± 0.86	38.9
		1.38	3.03 ± 0.30	34.0
Octyl glucoside	25	-	12.82 ± 0.75	100.0
		5	12.99 ± 0.61	101.3
		10	15.39 ± 0.74	120.0
		25	3.72 ± 0.20	29.0
Sodium cholate	14	-	7.99 ± 0.91	100.0
		2	9.54 ± 0.92	119.4
		5	14.55 ± 0.78	182.1
		10	9.78 ± 1.36	122.4
		20	2.09 ± 0.50	26.2
SDS	33	-	10.50 ± 0.80	100.0
		0.07	12.64 ± 0.50	120.3
		0.17	4.28 ± 0.20	40.7
		0.35	2.89 ± 0.25	27.5

[a]Data from [13].

3. RESULTS AND DISCUSSION

The effects of several detergents on the H^+-ATPase activity of plasma membrane preparations have been tested (Table I). All detergents used stimulated the enzyme activity at concentrations below their CMC, presumably due to permeabilization of right-side out vesicles for mgATP. The effect of lysoPC clearly differed from other detergents by the highest order of magnitude, strong stimulation at the low detergent/protein ratio and the absence of the inhibitory action at high concentrations, which is in agreement with the data of other groups [13,14]. This 3–4-fold stimulation apparently could be explained, not only by membrane permeabilization, but also by direct interaction of lysoPC with the enzyme molecule. This suggestion was further supported by experiments with combined action of detergents. When lysoPC was supplied to the reaction mixture containing Triton X-100 at the concentration generally employed for studies of the ATPase latency, an additional stimulation was observed (not shown).

The lysoPC stimulatory effect was also shown with solubilized H^+-ATPase preparations (Table II). Stimulation in such latency-free systems is strong evidence for a direct lysoPC effect on the enzyme. This effect was higher in preparations solubilized with zwittergent 3-14 than with lysoPC in which lysoPC was already presented in a sub-optimal concentration. Reactivation of the zwittergent 3-14 solubilized enzyme by lysoPC

Table II

Effect of lysoPC on the H^+-ATPase solubilized with lysoPC and zwittergent 3-14

Detergent used for solubilization	ATPase activity (μmol/mg protein/h)	
	− lysoPC	+ 50 μM lysoPC
LysoPC	58.69 ± 6.65	67.64 ± 2.98
Zwittergent 3-14	25.27 ± 3.48	45.09 ± 6.10

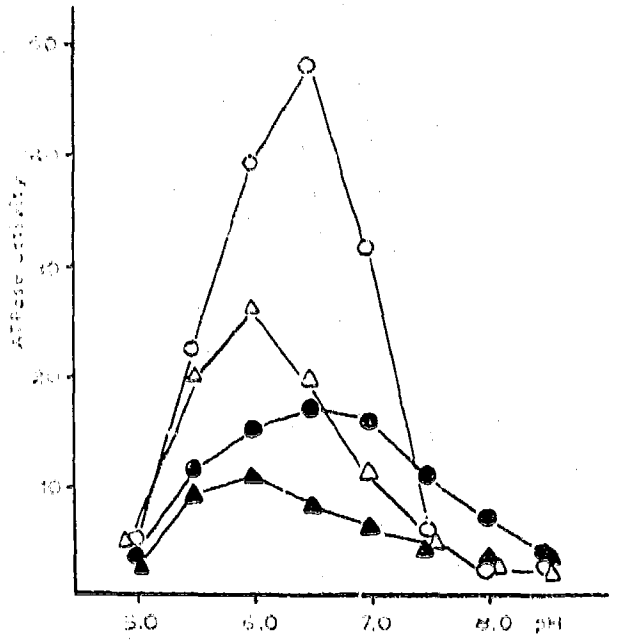

Fig. 1. Effect of lysoPC and vanadate on pH dependence of plasma membrane H^+-ATPase. (●), no additions; (○), 0.025% lysoPC; (▲), 100 μM vanadate; (△), 0.025% lysoPC + 100 μM vanadate. Plasma membranes were preincubated with lysoPC and vanadate for 10 min at 30°C. The pH of the reaction medium was adjusted with Tris. (ATPase activity, μmol·mg protein^{-1}·h^{-1}).

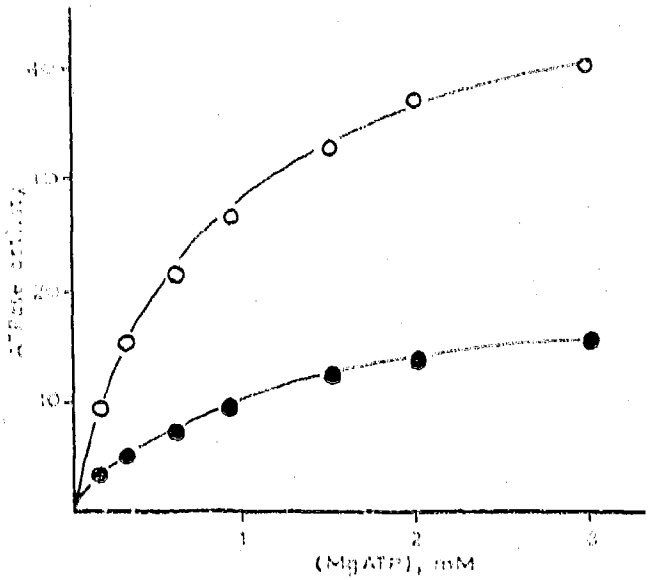

Fig. 2. Effect of MgATP concentration on plasma membrane H^+-ATPase activity. (●), no additions; (○), 0.025% lysoPC. Plasma membranes were preincubated with lysoPC for 10 min at 30°C.

exceeded that obtained by soybean phospholipids (not shown).

We have investigated the influence of lysoPC on some properties of H^+-ATPase. The maximal stimulation by lysoPC occurred at pH 6.5, which coincided with the pH optimum of the enzyme, but the effect reversed to inhibitory at pH > 7.0 (Fig. 1). LysoPC also did not change the sensitivity of the enzyme to its specific inhibitor vanadate.

We also found that lysoPC had no effect on the acid phosphatase activity of plasma membrane (Table III). These data confirmed the specific action of this phospholipid on the plasma membrane H^+-ATPase.

No deviations from Michaelis kinetics were found in the range 0.15–3 mM of MgATP both with and without lysoPC (Fig. 2). LysoPC increased both V_{max} (from 20.5 to 50.4 μM/mg/h) and affinity of enzyme to sutstrate as concluded from the apparent K_m reduction (from 0.70 to 0.52 mM). This observation supports the hypothesis proposed by Serrano [15] that lysophospholipids could induce a transition of H^+-ATPase to the active state. It has been recently shown that the stimulatory effect of lysoPC on the H^+-ATPase hydrolytic function is accompanied by an increase of the active H^+ transport [16].

Specific stimulation of the H^+-ATPase by lysoPC shows that it is impossible to determine the vesicles' sidedness, based on the stimulation of the enzyme activity by lysoPC as it has been shown previously [8]. The specific effect of the lysoPC on the plasma membrane ATPase, demonstrated above, suggests its possible regulatory role in vivo. This assumption can be confirmed by the correlation between lysoPC levels and ATPase activity in the plasma membrane of oat roots and coleoptiles [17]. Recently the new lipid factor structurally similar to lysoPC, namely 1-*O*-alkyl-2-acetyl-*sn*-glycerol-3-phosphocholine, has been isolated from plant tissues, which increased proteinkinase and H^+-ATPase activities [18]. Stimulation by this substance of both hydrolytic and transport activity of H^+-ATPase was comparable to the effect of lysoPC [16]. Endogenous phospholipase A_2 producing lysoPC was found in plasma membranes of oat roots and its participation in the regulation of ATPase was suggested [14].

Table III

Comparison of H^+-ATPase and acid phosphatase activities of plasma membrane from corn roots

Additions	ATPase (μmol/mg protein/h)	Acid phosphatase (μmol p-NP/mg protein/h)
None	16.42 ± 1.49	10.42 ± 1.04
+ 50 μM lysoPC	46.95 ± 2.97	11.85 ± 1.03
+ 0.1 mM vanadate	5.86 ± 0.41	8.14 ± 1.04

REFERENCES

[1] Serrano, R. (1989) Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 61–94.
[2] Sussman, M.R. and Surovy, T.K. (1987) Oxford Surv. Plant Mol. Cell. Biol. 4, 47–70.
[3] Brauer, D. and Shu, J.-T. (1989) Plant Physiol. 89, 867–874.

[4] Dufour, J.-P. and Goffeau, A. (1980) J. Biol. Chem. 255, 10591–10598.
[5] Vara, F. and Serrano, R. (1982) J. Biol. Chem. 257, 12826–12830.
[6] Serrano, R., Montesinos, C. and Sanchez, J. (1988) Plant Sci. 56, 117–122.
[7] Serrano, R. (1984) Biochem. Biophys. Res. Commun. 121, 735–740.
[8] Grouzis, J.-P., Gibrat, R., Rigaud, J. and Grignon, C. (1987) Biochim. Biophys. Acta 903, 449–464.
[9] Leonard, R.T. and Van der Woude, W.J. (1976) Plant Physiol. 57, 105–114.
[10] Rathbun, W.B. and Betlach, M.V. (1969) Analyt. Biochem. 28, 436–445.
[11] Page, A. (1987) Comput. Applic. Biosci. 3, 49–51.
[12] Bradford, M.M. (1976) Analyt. Biochem. 72, 248–254.
[13] Navarrete, R. and Serrano, R. (1983) Biochim. Biophys. Acta 728, 403–408.
[14] Palmgren, M.G., Sommarin, M., Ulvskov, P. and Larsson, C. (1990) Biochim. Biophys. Acta 1021, 133–140.
[15] Monk, B.C., Montesinos, C., Leonard, K. and Serrano, R. (1989) Biochim. Biophys. Acta 981, 226–234.
[16] Palmgren, M.G. and Sommarin, M. (1989) Plant Physiol. 90, 1009–1014.
[17] Sandstrom, R.P. and Cleland, R.E. (1989) Plant Physiol. 90, 1207–1213.
[18] Scherer, G.F.E., Martiny-Baron, G. and Stoffel, B. (1988) Planta 175, 241–253.