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Solubilization and Reconstitution of the Oat Root Vacuolar H^+/Ca^{2+} Exchanger[1]

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ABSTRACT

Calcium is sequestered into vacuoles of oat (*Avena sativa* L.) root cells via a H^+/Ca^{2+} antiporter, and vesicles derived from the vacuolar membrane (tonoplast) catalyze an uptake of calcium which is dependent on protons (pH gradient [ΔpH] dependent). The first step toward purification and identification of the H^+/Ca^{2+} antiporter is to solubilize and reconstitute the transport activity in liposomes. The vacuolar H^+/Ca^{2+} antiporter was solubilized with octylglucoside in the presence of soybean phospholipids and glycerol. After centrifugation, the soluble proteins were reconstituted into liposomes by detergent dilution. A ΔpH (acid inside) was generated in the proteoliposomes with an NH_4Cl gradient ($NH_4^+{}_{in} \gg NH_4^+{}_{out}$) as determined by methylamine uptake. Fundamental properties of ΔpH dependent calcium uptake such as the K_m for calcium (~15 micromolar) and the sensitivity to inhibitors such as *N,N'*-dicyclohexylcarbodiimide, ruthenium red, and lanthanum, were similar to those found in membrane vesicles, indicating that the H^+/Ca^{2+} antiporter has been reconstituted in active form.

The role of calcium as an activator and regulator of many biological processes is based on the ability of the cell to maintain a low concentration of this ion in the cytoplasm. This is achieved by multiple mechanisms that are thought to include pumping of calcium out of the cell across the plasma membrane as well as via sequestration of calcium in organelles such as the vacuole. Mature plant cells often contain one large central vacuole which may occupy up to 90% of the cell volume (17), and calcium concentrations in the vacuole are estimated to be up to 10,000-fold higher than those found in the cytoplasm (10, 12). Thus, the vacuole may serve as a major organelle for the sequestration and storage of calcium.

Calcium is accumulated in the vacuole of plant cells via an H^+/Ca^{2+} exchanger that is energized by a proton motive from the H^+-translocating ATPase (4, 5, 14). We have characterized the H^+/Ca^{2+} antiporter in vacuolar membrane (tonoplast) vesicles using the pH gradient established by the H^+-ATPase (14) or using imposed pH gradients (15). The antiporter has a K_m for calcium of 10 to 20 μM, and calcium transport is inhibited by low levels of DCCD,[3] ruthenium red, and lanthanum (15).

To understand the function of the transporter at the molecular level, it will be necessary to isolate and purify the protein responsible for H^+/Ca^{2+} exchange. A first step toward this goal is to develop an assay to measure transport activity after solubilization and during subsequent purification. This study describes the solubilization of the oat root vacuolar H^+/Ca^{2+} exchanger and its reconstitution into liposomes. The reconstituted calcium transport activity has similar affinity for calcium and displays the same inhibitor sensitivities as previously found for the native H^+/Ca^{2+} transporter of the oat root vacuole. To our knowledge, this is the first report of the reconstitution of a functionally active H^+-coupled transport system from plants.

MATERIALS AND METHODS

Plant Material

Oat seeds (*Avena sativa* L. var Lang) were germinated in the dark over an aerated solution of 0.5 mM $CaSO_4$. Roots were harvested after 4 d.

Preparation of Membrane Vesicles

Vesicles were prepared by a modification of an earlier procedure (15). All procedures were conducted at 4°C. Briefly, oat roots (20–60 g) were homogenized by mortar and pestle in a medium containing 250 mM sorbitol, 3 mM EGTA, 25 mM Hepes-BTP (pH 7.4), 1 mM DTT, and 0.2% BSA at a medium-to-tissue ratio of 1.5 mL/g fresh weight. After filtration through cheesecloth, the debris was rehomogenized in 1 mL/g of the original tissue weight, washed in 0.5 mL/g and filtered. The homogenate was centrifuged for 15 min at 13,000*g*, and the supernatant was centrifuged for 30 min at 60,000*g* (Beckman SW 28 rotor, r_{max}). The resulting pellet (crude microsomal pellet) was resuspended in 250 mM sorbitol, 2.5 mM Hepes-BTP (pH 7.2), and 1 mM DTT (resuspension buffer). The suspension (6 mL) was layered over a 6% dextran (w/w) cushion (10 mL) prepared in resuspension buffer. After centrifugation for 2 h at 70,000*g* (SW 28.1, r_{max}), a turbid band at the sorbitol-dextran interface was collected and is referred to as tonoplast enriched vesicles.

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[3] Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; BTP, bis-tris propane or 1,3-bis[tris(hydroxymethyl)methylamine]; ΔpH, pH gradient; MeA, methylamine; octylglucoside, octyl-β-D-glucopyranoside.

Solubilization of Oat Root Tonoplast Proteins

The final solubilization mixture (1.2 mL) consisted of the following: 4.4 mg soybean asolectin, 200 mM NH_4Cl, 50 mM K_2 oxalate, 50 mM Hepes buffered to pH 7 with BTP, 1 mM DTT, 40 mM octylglucoside, 20% glycerol, and 1 mg of tonoplast protein. The solubilization mixture was prepared as follows.

Soybean asolectin (Sigma IV-S) was dissolved in chloroform at a concentration of 200 mg/mL and stored in 0.5 mL aliquots at −20°C under N_2. To prepare lipids for use during solubilization, 4.4 mg of phospholipid were dried under N_2 gas, rotating the tube to form a thin, even film. To remove residual chloroform, the phospholipids were washed with 1 mL of cold (−20°C) diethyl ether and evaporated to dryness (rotating slowly). The lipids were dried for an additional 30 min under vacuum to remove all traces of solvent.

The phospholipids were resuspended in a solution containing 200 mM NH_4Cl, 50 mM K_2 oxalate, 50 mM Hepes-BTP (pH 7), and 1 mM DTT (buffer A). The mixture was flushed with N_2, sealed and bath-sonicated (Branson 2200 sonicator) to clarity (approximately 30 min) with periodic additions of ice to the bath to keep the temperature between 18 and 23°C. During sonication, it was important to place the tube over a point in the bath sonicator which gave the most vigorous agitation of the solubilization mixture. Octylglucoside (40 mM) was then added and the mixture vortexed for 15 s. Glycerol (20%) was added with an additional 15 s vortexing.

One mg of vesicle protein was added to the solubilization mixture prepared above. The protein was obtained from a 0/6% dextran gradient interface (50 g fresh weight). Vesicles (~2 mL) were diluted into 72 mL of buffer containing 250 mM sorbitol, 2.5 mM Hepes-BTP at pH 7.2, and 1 mM DTT, and pelleted at 90,000*g* for 45 min (SW 28, r_{max}). The pellet was resuspended in buffer A (5 mg protein/mL) and added to the phospholipid/detergent mixture. After solubilization in detergent for 20 min on ice (Total Solubilization Mixture), the soluble proteins were recovered from the supernatant after centrifugation for 1 h at 145,000*g* (type 65, r_{max}) at 4°C. Approximately 1 mL of clear supernatant was collected, and the pellet was resuspended in 1 mL of buffer A.

Reconstitution of Tonoplast Proteins into Liposomes

Reconstitution was done following the procedure of Ambudkar and Maloney (1) in a volume of 1010 μL containing 788 μL of the solubilized protein mixture and 222 μL of a phospholipid/octylglucoside mixture. The latter mixture was prepared by washing 5.0 mg of soybean lipid with diethyl ether and drying as described above. The lipids were resuspended in 178 μL of buffer A and sonicated to clarity. After sonication, 44 μL of 200 mM octylglucoside was added and the mixture was vortexed for 15 s. The phospholipid/octylglucoside mixture was added to the solubilized proteins, covered, and placed on ice for 30 min.

Proteoliposomes were formed by rapid injection (Pasteur pipet) of the ice-cold solubilized protein/phospholipid mixture into 20 mL of buffer A (22°C) minus the DTT. The solution was mixed by drawing some of the liquid into the pipet and rapidly injecting it back into the 20 mL volume (4 times total). The mixture was allowed to stand undisturbed for 20 min at room temperature. The proteoliposomes were collected by centrifugation in the cold (4°C) for 1 h, 145,000*g* (type 65, r_{max}), resuspended in 100 to 200 μL of buffer containing 200 mM NH_4Cl and 50 mM Hepes-BTP at pH 7, and stored on ice until use in transport assays.

For control experiments, proteins from either the total solubilization mixture or the high-speed pellet were reconstituted as described above for the soluble proteins. Protein-free liposomes were prepared in the same manner, but without the addition of vesicle protein or K_2 oxalate.

pH Gradient Formation

The pH gradient was generated by diluting NH_4^+-loaded proteoliposomes 200-fold into an NH_4^+-free medium containing 200 mM BTP-Cl (osmoticum) and either 50 mM Hepes-BTP (pH 7), or 50 mM BTP-Hepes (pH 8). The pH gradient formed (acid inside) could be measured as [^{14}C]MeA uptake or quenching of acridine orange fluorescence. Control experiments done in the absence of a pH gradient had equal concentrations of NH_4^+ across the proteoliposome ($NH_4^+{}_{in} = NH_4^+{}_{out}$).

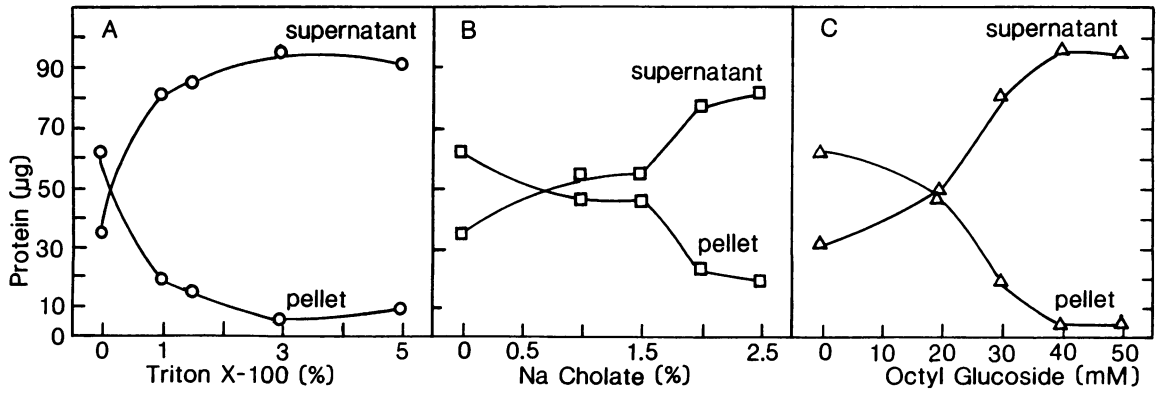

Figure 1. Effect of detergents on the solubilization of oat root tonoplast proteins. Protein (1.4 mg) was divided into 15 aliquots and each (15 μL) was solubilized in 0.5 mL of detergent in the presence of 200 mM KCl. The insoluble material was removed by pelleting at 145,000*g* (Beckman type 65, r_{max}) for 45 min. The total protein in the supernatant and pellet was determined. (A) Triton X-100; (B) sodium cholate; (C) octylglucoside.

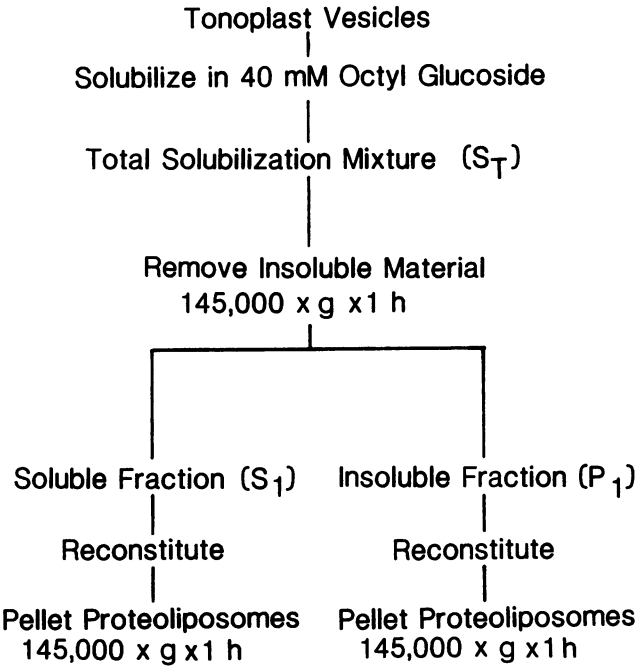

Figure 2. Scheme for the solubilization and reconstitution of the oat root vacuolar H^+/Ca^{2+} exchanger.

[^{14}C]MeA Accumulation

All assays were performed at 15°C to maintain the pH gradients. To initiate the reaction, NH_4^+-loaded vesicles were diluted 200-fold into mixtures (0.5–1.0 mL) containing the respective buffers and 20 μM [^{14}C]MeA (approximately 1 μCi). Aliquots (50–250 μL) were filtered through Millipore filters (0.22 μm pore size). The filtration procedure involved wetting a Millipore filter with 1 mL of cold wash buffer (250 mM sorbitol, 2.5 mM Hepes-BTP [pH 7.2]), filtering an aliquot of the reaction mixture, and quickly rinsing with 1 mL of the wash buffer. The filters were air dried, and the radioactivity was determined by liquid scintillation counting.

Calcium Transport

ΔpH dependent calcium accumulation was measured as uptake of $^{45}Ca^{2+}$. To initiate the reaction, NH_4^+-loaded proteoliposomes (containing 0.1–0.3 μg protein) were diluted 200-fold into a mixture containing (final concentrations) 200 mM BTP-Cl and 50 mM BTP-Hepes (pH 8) and 0.75 to 1.0 μCi/ml $^{45}Ca^{2+}$. $^{45}Ca^{2+}$ transport was measured by a filtration assay as described for MeA uptake except that nonradiolabeled calcium (0.5 mM) was added to the wash buffer to exchange away any label bound to the proteoliposomes or filter paper. As a control, calcium uptake into proteoliposomes in the absence of a ΔpH was measured concurrently.

Protein Determination

Because the high levels of phospholipids used in this procedure interferred with the Lowry protein assay, protein concentration was determined using amido black 10B proce dure as described by Kaplan and Pedersen (8) with BSA as standard.

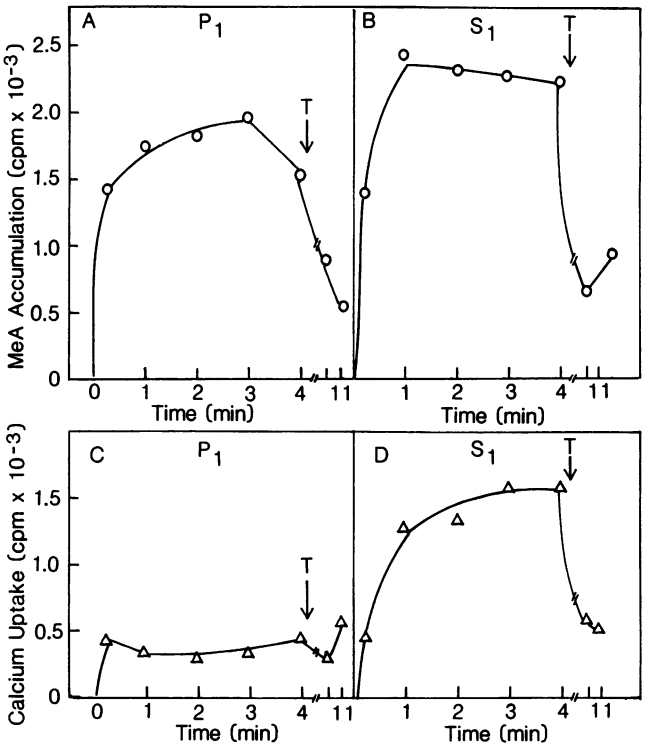

Figure 3. Time course of MeA accumulation (A, B) and calcium uptake (C, D) into proteoliposomes from soluble (S_1) and insoluble (P_1) fractions. Tonoplast vesicles were solubilized as described in "Materials and Methods." Proteoliposomes were formed by detergent-dilution in the presence of 50 mM K_2 oxalate. At time 0, 5 μl of NH_4^+-loaded proteoliposomes (containing 50 mM Hepes-BTP [pH 7] and 50 mM K_2 oxalate) was diluted into a 1 mL reaction mixture containing 200 mM BTP-Cl, 50 mM BTP-Hepes (pH 7), and either 20 μM MeA (A, B), or 10 μM $^{45}Ca^{2+}$ (C, D) at 15°C. Where indicated, 0.03% Triton (T) was added and release of MeA or calcium from the proteoliposomes was monitored.

RESULTS AND DISCUSSION

The proteins in tonoplast-enriched vesicles could be solubilized with Triton X-100, Na cholate, or octylglucoside (Fig. 1). All three detergents solubilized proteins maximally at concentrations well above their critical micelle concentrations; however, octylglucoside and cholate were chosen for further study as they have much higher critical micelle concentrations (25 and 8 mM, respectively) when compared to 0.3 mM for Triton (7). Both detergents have been successfully used in solubilization and reconstitution of calcium transport proteins from animal and bacterial cell membranes (2, 3, 6, 11, 13, 16). In the absence of detergent, 20 to 30% of the tonoplast protein was found in the supernatant and may represent loosely bound membrane proteins (9).

In experiments using imposed gradients in native membranes, we have shown that ΔpH can be formed using a K^+ gradient ($K^+_{in} > K^+_{out}$) in the presence of the K^+/H^+ exchanging ionophore, nigericin (14, 15). It was not possible to generate ΔpH consistently with similar levels of nigericin in the reconstitution experiments. One explanation might be that the ionophore is partitioning into the excess phospholipids which were added during reconstitution. Therefore, to

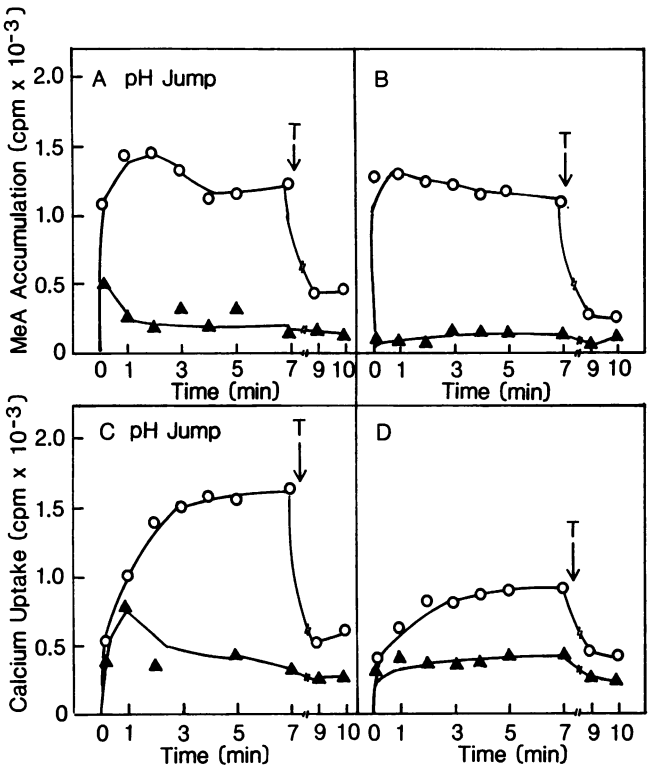

Figure 4. Effect of pH_{out} on MeA accumulation and calcium uptake in proteoliposomes. At time 0, 5 μL of NH_4^+-loaded proteoliposomes containing 50 mM Hepes-BTP (pH 7) and 50 mM K_2 oxalate was diluted into 1 mL of solution containing 50 mM buffer at pH 8 (A, C); or 50 mM buffer at pH 7 (B, D). MeA accumulation (A, B) and calcium uptake (C, D) were measured in the presence (○) or absence (▲) of NH_4^+ gradients.

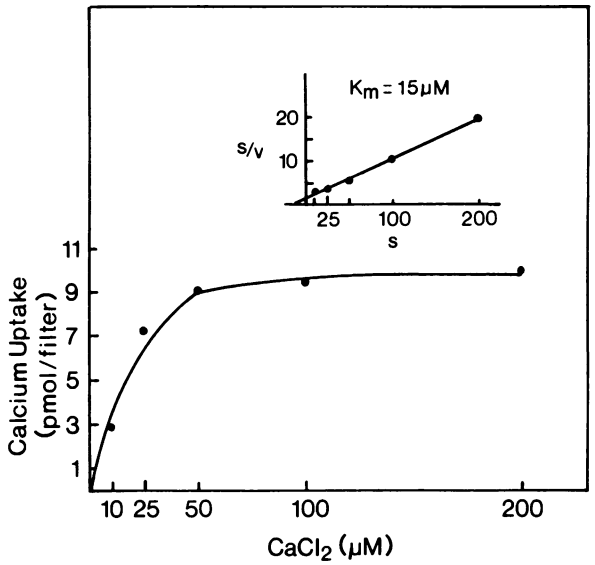

Figure 5. Calcium transport as a function of extraliposomal calcium concentration. Reaction mixtures were as described in Figure 3 with $CaCl_2$ concentrations varying from 10 to 200 μM. The specific activity of $^{45}Ca^{2+}$ was kept constant (0.075 μCi/nmol) for all calcium concentrations. Initial rates of transport were used to construct a Hanes Woolf plot of the data which showed an apparent K_m for calcium = 15 μM. Data represent the average of two to four experiments.

generate ΔpH in the reconstituted system, proteoliposomes containing NH_4Cl were diluted into NH_4^+-free buffer. NH_4Cl has the advantage of being an ionophore-free method of generating ΔpH which should be unaffected by phospholipid concentration. The ΔpH is formed by dissociation of NH_4^+ into NH_3 and H^+ and passive diffusion of NH_3 out of the vesicle leaving behind H^+ (13). The ΔpH (acid inside) was measured as accumulation of [^{14}C]methylamine or fluorescence quenching of acridine orange (not shown).

Initial attempts to reconstitute H^+/Ca^{2+} exchange activity into liposomes were unsuccessful. Proteins were solubilized in octylglucoside (1.168% or 40 mM) in the presence of glycerol (20% v/v) as a stabilant (1) and the soluble proteins (S_1) (Fig. 2) were reconstituted by a 20-fold detergent-dilution. When an aliquot of the proteoliposomes was assayed for transport activity, it was possible to measure formation of ΔpH, but there was no measurable calcium transport activity (not shown). This inability to measure calcium transport could have been a result of (a) low antiporter activity due to the relatively small number of antiporter molecules per liposome or (b) lack of solubilization of the H^+/Ca^{2+} antiporter.

To address these possibilities, we added oxalate to trap calcium inside the liposome. Including 50 mM K_2 oxalate during both solubilization and reconstitution (see "Materials and Methods"), we first tried to reconstitute the total solubilization mixture (S_T) (Fig. 2). Under these conditions, we were able to measure both ΔpH formation and calcium uptake (not shown). Subsequent reconstitution of the soluble (S_1) and insoluble (P_1) protein fractions separately (Fig. 3) provided evidence that the antiporter was solubilized by octylglucoside

Table I. *Effect of Inhibitors on ΔpH Formation and Calcium Uptake in Proteoliposomes*

Tonoplast vesicles were solubilized with 40 mM octylglucoside in the presence of 4.4 mg asolectin and 20% glycerol. Soluble material was reconstituted by detergent-dilution in the presence of 50 mM K_2 oxalate. Steady state levels of MeA accumulation and calcium uptake were measured 5 min after addition of proteoliposomes. Values reported have been corrected for binding in the absence of a pH gradient.

Addition	Transport Activity	
	[^{14}C]MeA	$^{45}Ca^{2+}$
	%	
None	100	100
Mn^{2+}		
75 μM	98	70
La^{3+}		
25 μM	94	41
75 μM	93	20
Mg^{2+}		
75 μM	91	115
DCCD		
3 μM	89	69
10 μM	75	48
Ruthenium red		
25 μM	96	77
75 μM	90	74

Table II. *Comparison of Calcium Transport Activity of the Oat Root Vacuolar H^+/Ca^{2+} Exchanger in Membrane Vesicles and Proteoliposomes*

Vacuolar membrane proteins were used as a starting material. Soluble proteins were incorporated into asolectin vesicles in the presence of 50 mM K_2 oxalate. Transport reactions were carried out for 5 min.

Fraction	Protein Yield	ΔpH dependent Calcium Uptake	
		Total Activity	Specific Activity
	mg	*nmol/min*	*nmol/mg·min*
Membrane vesicles	0.85	11.23	13.21
Proteoliposomes	0.07	5.33	75.10

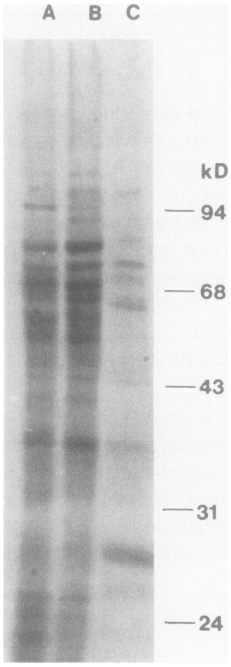

Figure 6. SDS-gel electrophoretic pattern of tonoplast membrane, soluble supernatant, and reconstituted proteins from oat roots. Proteins were run in an 8 to 14% polyacrylamide gel and subsequently silver stained. The bars indicate the positions of the following low mol wt protein standards: phosphorylase b (92,500); bovine serum albumin (68,000); ovalbumin (43,000); carbonic anhydrase (31,000); soybean trypsin inhibitor (21,000); and lysozyme (14,000). Gel lanes show proteins from: A, tonoplast membranes recovered from a 0/6% dextran interface; B, soluble supernatant (fraction S_1); C, reconstituted proteoliposomes.

and functionally active. We were able to measure ΔpH formation in proteoliposomes from both fractions (Fig. 3, A and B); however, calcium transport activity was detected only in the soluble fraction (Fig. 3D). Lack of calcium transport activity in the insoluble fraction also lends support to the idea that the calcium transport measured in the soluble fraction is due to the H^+/Ca^{2+} antiporter and not to binding of calcium to the liposomes. The reconstitution mixture contained a high concentration of phospholipids (5 mg/mL). From the MeA results, liposomes were formed in the insoluble fraction (Fig. 3A), yet neither calcium uptake nor binding were measurable (Fig. 3C).

Calcium uptake was measured in the presence or absence of a H^+ gradient across the liposome (Fig. 4, C and D). Calcium uptake was rapid in the presence of a H^+ gradient but only slight in its absence showing that a H^+ gradient is required for calcium uptake. Calcium transport increased twofold when the pH_{out} was increased from 7 (Fig. 4D) to 8 (Fig. 4C). This had the effect of combining an NH_4^+ gradient with a pH jump. Gramicidin, an ionophore which dissipates the pH gradient, caused calcium accumulated in the proteoliposomes to be released (not shown). Effective concentrations were higher than those found in membrane vesicles, most likely due to the large excess of phospholipids used for reconstitution. Therefore, Triton was used routinely to dissipate the ion gradients.

Steady state levels of calcium accumulation could be maintained for at least 7 min at 15°C. Calcium uptake was linear up to two minutes and varied as a function of extraliposomal calcium concentration. Initial rates of uptake (15 s) were used to calculate an apparent K_m for calcium of about 15 μM (Fig. 5), consistent with the values found for the antiporter in native membrane vesicles (K_m = 10–14 μM) (14, 15).

A number of specific inhibitors of H^+/Ca^{2+} exchange have been identified in native membranes by testing their effect on calcium uptake and steady state ΔpH formation (15). Ruthenium red, DCCD, and lanthanum (La^{3+}) all inhibited calcium uptake in native vesicles. When these compounds were tested on the reconstituted system (Table I), we found that, as in the native membranes, all compounds inhibited calcium uptake but had little effect on the ΔpH. At 3 μM DCCD, calcium uptake is inhibited 50% in the native membranes. In the reconstituted system, we saw only 31% inhibition. The difference in the effective concentration is not surprising as the DCCD to phospholipid ratio is important in determining the concentration. Since the phospholipid concentration was at least twofold higher than in the native membranes, a corresponding increase in DCCD concentration might be needed to see the same degree of inhibition.

Our procedure for the solubilization and reconstitution of the oat root vacuolar H^+/Ca^{2+} exchanger enhanced the apparent specific activity of the transporter (Table II). This increased activity may represent a stimulation in calcium uptake due to the presence of oxalate, or may represent a partial purification of the protein. Silver staining of the SDS-polyacrylamide gradient gel shown in Figure 6 revealed that a large number of polypeptides were solubilized by octylglucoside (lane B). After reconstitution, the total number of proteins decreased significantly (lane C), and several bands (100, 70, 60, 43, 36, and 28 kD) appear to be enriched.

In this paper we describe the solubilization of the H^+/Ca^{2+} antiporter with octylglucoside, its reconstitution, and partial purification. The protein can be incorporated into artificial liposomes which catalyze an exchange activity similar to the activity seen in tonoplast membrane vesicles suggesting that the oat root vacuolar H^+/Ca^{2+} exchanger has been solubilized and reconstituted. The ability to reconstitute an active exchanger is an important step towards the identification of the

polypeptide(s) responsible for calcium accumulation into the higher plant vacuole.

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LITERATURE CITED

1. **Ambudkar SV, Maloney PC** (1986) Bacterial anion exchange. Use of osmolytes during solubilization and reconstitution of phosphate-linked antiport from *Streptococcus lactis*. J Biol Chem **261:** 10079–10086
2. **Ambudkar SV, Lynn AR, Maloney PC, Rosen BP** (1986) Reconstitution of ATP-dependent calcium transport from *Streptococci*. J Biol Chem **261:** 15596–15600
3. **Barzilai A, Spanier R, Rahamimoff H** (1984) Isolation, purification, and reconstitution of the Na^+ gradient-dependent Ca^{2+} transporter (Na^+-Ca^{2+} exchanger) from brain synaptic plasma membranes. Proc Natl Acad Sci USA **81:** 6521–6525
4. **Blumwald E, Poole RJ** (1986) Kinetics of Ca^{2+}/H^+ antiport in isolated tonoplast vesicles from storage tissue of *Beta vulgaris*. Plant Physiol **80:** 727–731
5. **Guern J, Mathieu Y, Kurkdjian A, Manigault P, Manigault J, Gillet B, Beloeil J, Lallemand J** (1989) Regulation of vacuolar pH of plant cells. II. A ^{31}P NMR study of the modifications of vacuolar pH in isolated vacuoles induced by proton pumping and cation/H^+ exchanges. Plant Physiol **89:** 27–36
6. **Hale CC, Slaughter RS, Ahrens DC, Reeves JP** (1984) Identification and partial purification of the cardiac sodium-calcium exchange protein. Proc Natl Acad Sci USA **81:** 6569–6573
7. **Hjelmeland LM, Chrambach A** (1984) Solubilization of functional membrane proteins. Methods Enzymol **104:** 305–318
8. **Kaplan RS, Pedersen PL** (1985) Determination of microgram quantities of protein in the presence of milligram levels of lipid with amido black 10B. Anal Biochem **150:** 97–104
9. **Lai S, Randall SK, Sze H** (1988) Peripheral and integral subunits of the tonoplast H^+-ATPase from oat roots. J Biol Chem **263:** 16731–16737
10. **Macklon AES** (1984) Calcium fluxes at plasmalemma and tonoplast. Plant Cell Environ **7:** 407–413
11. **Miyamoto H, Racker E** (1980) Solubilization and partial purification of the Ca^{2+}/Na^+ antiporter from the plasma membranes of bovine heart. J Biol Chem **255:** 2656–2658
12. **Moore AL, Akerman KEO** (1984) Calcium and plant organelles. Plant Cell Environ **7:** 423–429
13. **Nakamura T, Hsu C, Rosen BP** (1986) Cation/proton antiport systems in *Escherichia coli*. Solubilization and reconstitution of pH-driven sodium/proton and calcium/proton antiporters. J Biol Chem **261:** 678–683
14. **Schumaker KS, Sze H** (1985) A Ca^{2+}/H^+ antiport system driven by the proton electrochemical gradient of a tonoplast H^+-ATPase from oat roots. Plant Physiol **79:** 1111–1117
15. **Schumaker KS, Sze H** (1986) Calcium transport into the vacuole of oat roots. Characterization of H^+/Ca^{2+} exchange activity. J Biol Chem **261:** 12172–12187
16. **Wakabayashi S, Goshima K** (1982) Partial purification of Na^+-Ca^{2+} antiporter from plasma membranes of chick heart. Biochim Biophys Acta **693:** 125–133
17. **Wiebe HH** (1978) The significance of plant vacuoles. Bioscience **28:** 327–331