

Localization in Sucrose Gradients of the Pyrophosphate-Dependent Proton Transport of Maize Root Membranes

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ABSTRACT

A maize (*Zea mays* L. cv LG 11) root homogenate was prepared and centrifuged to sediment the mitochondria. The pellet (6 KP) and the supernatant (6 KS) were collected and fractionated on linear sucrose density gradients. Marker enzymes were used to study the distribution of the different cell membranes in the gradients. The distribution of the ATP- and pyrophosphate-dependent proton pumping activities was similar after 3 hours of centrifugation of the 6 KS or the 6 KP fraction. The pumps were clearly separated from the mitochondrial marker cytochrome *c* oxidase and the plasmalemma marker UDP-glucose-sterolglucosyl-transferase. The pyrophosphate-dependent proton pump might be associated with the tonoplast, as the ATP-dependent pump, despite the lack of a specific marker for this membrane. However, under all the conditions tested, the two pumps overlapped the Golgi markers latent UDPase and glucan synthase I and the ER marker NADH-cytochrome *c* reductase. It is therefore not possible to exclude the presence of proton pumping activities on the Golgi or the ER of maize root cells. The two pumps (but especially the pyrophosphate-dependent one) were more active (or more abundant) in the tip than in the basal part of maize roots, indicating that these activities might be important in growth processes.

In recent years, evidence has accumulated indicating that plant cells contain H⁺-ATPases which carry out the electrogenic transport of protons (24). Dupont *et al.* (10) reported a PPI-induced proton transport into sealed microsomal vesicles prepared from maize roots. Churchill and Sze (8) also found a PPI-dependent proton transport by microsomal membranes prepared from oat roots. Similar results were obtained with red beet membranes, collected from a 16/26% sucrose interface (2). Unfortunately, these authors did not explore the relationship between the ATP- and PPI-dependent proton transport. Rea and Poole (22) demonstrated the presence of a PPI-dependent proton pump in a tonoplast-enriched fraction from beet roots. Independently, it has been shown (5) that a PPI-driven proton transport was present in two fractions obtained from maize coleoptile microsomal membranes. The two H⁺-pyrophosphatase activities coincided with the previously characterized peaks of tonoplast and Golgi ATP-driven proton transport (7). When PPI and ATP were given together, the effect was additive, suggesting that two separate pumps were operating. The ATP-dependent proton pump was specifically inhibited by nitrate and the PPI-dependent one was inhibited by imidodiphosphate, a pyrophosphate analog (5). Two different enzymes were proved to be responsible for the two phosphohydrolase activities, after solubilization and separation by gel filtration (23, 30). A Mg²⁺-pyrophosphatase has also been shown to be associated with the tonoplast of red beet (29) and tulip (28). Reports of high concentrations of PPI in plant tissues

(5 and references therein) suggest that PPI-dependent proton pumps could play an important metabolic role.

The major objective of the present study was to fractionate membranes from tissues of maize root and to compare the distribution of the PPI-dependent proton pump with the distribution of various marker enzymes and the ATP-driven proton pump (24). Some experiments were done to study the correlation between growth and the activity of the pumps.

Maize roots were used for two reasons. Spanswick and co-workers (9, 18) have already published several papers on the localization of ATP-dependent proton pumps in maize root microsomal membranes and the same material was used for analysing root growth and its hormonal regulation (19, 20).

This work presents a procedure avoiding pelleting and resuspension of the microsomes, which can aggregate or damage organelles. Moreover, the mitochondrial fraction was found to contain half of the activity of the two pumps and was therefore fractionated. These procedures will be useful for the characterization of the PPI-dependent proton pump of maize roots. Some results will be presented on the distribution of the two pumps in the tip and the basal part of the root.

RESULTS AND DISCUSSION

Plant Material. Roots of maize (cv LG 11, Association Suisse des Sélectionneurs, Lausanne) were grown for 48 h in the dark (21 ± 1°C) (19). Primary roots of 10 to 20 mm in length were harvested and stored on ice under room lights. Homogenization and subsequent treatments were performed at 0 to 4°C. In some experiments, the tip (5 mm) and the basal part of the root were collected separately.

Homogenization. Roots (7–10 g) were chopped by hand with razor blades in 6 ml of homogenization medium (250 mM sucrose, 2 mM EDTA, 1 mM DTT, 0.1% BSA, 50 mM Tris, adjusted to pH 7.8 with solid Mes). The tissue was then ground lightly in a mortar and strained through nylon. The remaining tissue was lightly reground in an additional 6 ml of homogenization buffer. The second homogenate was filtered through nylon and combined with the first homogenate.

Unbroken cells, wall fragments, starch, and nuclei were removed by a 5-min centrifugation at 1000g (Beckman J2-21, JS-20 rotor). The pellet (1 KP)¹ and the supernatant (1 KS) were collected. In some experiments, half of the 1 KS was kept back for analysis. The majority of the mitochondria were eliminated by a 20-min centrifugation at 6000g (Beckman J2-21, JS-20 rotor) and the pellet (6 KP) and supernatant (6 KS) were collected.

Nonlinear Sucrose Gradients. The different fractions (1 KP, 1 KS, 6 KP, and 6 KS) were diluted with homogenization buffer

¹ Abbreviations: 1 KP, 1000g pellet; 6 KP, 6000g pellet; 6 KS, 6000g supernatant; 1 KS, 1000g supernatant; BTP, bis-tris propane; GSI, glucan synthase I; UDPG-ST, UDP-Glc-sterolglucosyl-transferase.

(21 ml, final volume) and layered on the top of a step gradient consisting of 5 ml of 45% (w/w) sucrose and 10 ml of 10% sucrose (each in gradient buffer: 0.5 mM EDTA, 1 mM DTT, 2.5 mM Tris, adjusted to pH 7.5 with solid Mes).

The gradients were centrifuged for 3 h at 80,000g (Kontron Centrikon T-2080, TST 28.38 rotor) and fractionated. Six ml of the interface were collected and 3 ml of each sample were diluted 3-fold (with gradient buffer) for proton pumping experiments (10% sucrose, final concentration). All the fractions were frozen in liquid N₂ and stored at -70°C.

Linear Sucrose Gradients. The 6 KP or 6 KS fractions were layered onto linear gradients consisting of a 2-ml cushion of 45% sucrose, 20 ml 10 to 45% sucrose, and a 1 ml overlay of 10% sucrose (all in gradient buffer).

The gradients were centrifuged at 80,000g for 3 h (Kontron Centrikon T-2080, TST 28.38 rotor) and fractionated into 16 portions of 1.5 ml. An aliquot of each fraction was diluted to 10% sucrose with gradient buffer for proton pumping experiments. All the fractions were frozen in liquid N₂ and stored at -70°C. Sucrose concentrations were determined by refractometry. Protein concentration was determined by the Bradford (4) method, using BSA as a standard.

Enzyme Assays. The activities of different marker enzymes were determined as previously described (6). UDPG-sterol-glucosyl-transferase was selected as the plasmalemma marker; latent UDPase and glucan synthase I as Golgi markers; NADH- and NADPH-dependent Cyt *c* reductase as ER markers, and Cyt *c* oxidase for the mitochondria. Antimycin A was added together with KCN for the NADH-Cyt *c* reductase experiments to inhibit the mitochondrial enzyme (21).

Quinacrine Fluorescence Quenching. Membrane vesicles, the appropriate salt or inhibitors and quinacrine (5 μM final concentration) were added to an assay buffer of BTP-Mes (pH 7.2) to a final volume of 0.6 ml (the final total concentration of BTP plus Mes was 25 mM).

Fluorescence was measured at room temperature with a Perkin Elmer LS-5 Luminescence Spectrometer (excitation 430 nm; emission 500 nm). After temperature equilibration, the reaction was initiated by the addition of 3 mM (final concentration) Mg-ATP or Mg-PPI (5). Later experiments showed that 0.1 mM PPI and 3 mM MgSO₄ gave higher activities. However, at 3 mM PPI, the activity was equal to 60% of the one in presence of 0.1 mM PPI and gave qualitatively similar results (data not shown). As for the PPI-dependent pump from maize coleoptiles (5), 50 mM KNO₃ (100%) gave higher activities than KCl (60%) (data not shown). At the end of the experiment, 3 μl of 1 mM monensin dissolved in ethanol (5 μM final concentration of monensin) was added to collapse the proton gradient. The initial rate of fluorescence quenching was measured (%/min) in duplicate for each experiment (5).

Treatment of the Data. All experiments were performed at least twice. Each result is the arithmetic mean of two different experiments.

Chemicals. UDP-[¹⁴C]Glc (296 Ci/mol) was obtained from the Radiochemical Centre, Amersham, U.K. ATP-Tris, UDP-Glc from Merck, Darmstadt, FRG. SDS, monensin from Calbiochem-Behring, and Antimycin A from Sigma Chemical Co. All the other chemicals were purchased from Fluka AG, Buchs, Switzerland.

RESULTS

Mitochondrial and Microsomal Fractions. The homogenate was cleared by a 5-min centrifugation at 1000g. The supernatant (1 KS) and the pellet (1 KP), containing cell wall fragments, starch, and nuclei, were collected. Half of the 1 KS fraction was centrifuged for 20 min at 6000g and the supernatant (6 KS) and the pellet (6 KP) were collected. The four fractions (1 KP, 1 KS,

6 KP, and 6 KS) were pelleted onto a discontinuous gradient composed of 10 and 45% sucrose. The membranes present at the intermediate 10/45% sucrose interface of each gradient were collected.

Table I illustrates the effects of these centrifugations on the different marker activities. Part of these activities (30–50%) was pelleted during the first centrifugation at 1000g. The second centrifugation (6000g) removed most of the mitochondrial marker Cyt *c* oxidase (80%) together with 35–50% of the other markers. Half of the ATP- and PPI-dependent proton pumping activities were also sedimented by the second centrifugation. Therefore, to prevent such major losses of activities, the two fractions (6 KS and 6 KP) were used to study the distribution of the different membranes in sucrose linear gradients.

Linear Sucrose Gradients. In Figure 1, data are shown on the distribution of five marker enzymes after centrifugation (3 h) of the 6 KS fraction on a linear sucrose gradient (10–45%). The ER marker NADH-Cyt *c* reductase was located at 1.11 g/cm³ and the mitochondrial marker Cyt *c* oxidase peaked at 1.14 g/cm³. Latent UDPase, a Golgi marker, formed a single peak at 1.13 g/cm³. GSI, another Golgi marker, peaked again at 1.13 g/cm³ with a shoulder at 1.18 g/cm³, which corresponded to the peak of the plasmalemma marker, UDPG-ST. The proteins were broadly distributed in the sucrose gradient, with a major peak on the top of the gradient, corresponding to soluble proteins and to the BSA present in the homogenization buffer.

The separation of the different markers after a 3 h centrifugation of the 6 KP fraction on a linear sucrose gradient is shown in Figure 2. The NADH-Cyt *c* reductase peaked again at 1.11 g/cm³ but presented a shoulder at higher densities. The Cyt *c* oxidase formed a single peak at 1.185 g/cm³ with an activity considerably higher than with the 6 KS fraction (Fig. 1). This high density found for the mitochondria is in agreement with the results of other authors (9, 18) using the same material. Latent UDPase exhibited a peak at 1.145 g/cm³, a density slightly higher than in Figure 1. On the contrary, UDPG-ST was shifted to a slightly lower density (1.16–1.17 g/cm³). GS I was broadly distributed between 1.145 and 1.17 g/cm³ with a major peak at 1.145 g/cm³, corresponding to the Golgi marker latent UDPase. The soluble proteins were floating on the top of the gradient, and a peak of membrane-bound proteins was recovered at 1.185 g/cm³, corresponding to the Cyt *c* oxidase activity.

The location of the ATP- and PPI-dependent proton pumps after centrifugation for 3 h of the 6 KS fraction is shown in Figure 3A. The two distributions were similar with a broad peak at 1.10 to 1.13 g/cm³.

In Figure 3B, data are given on the distribution of the two proton pumps after centrifugation for 3 h of the 6 KP fraction. The activities were broadly recovered in the gradient with a major peak at 1.12 g/cm³ for the ATP-dependent pump and at 1.13 g/cm³ for the PPI-dependent one.

Enzyme Markers and ATP- and PPI-Dependent Proton Pumps. Table II displays the distribution of the different marker enzymes and proton pumps in the mitochondrial (6 KP) and microsomal (6 KS) fractions prepared from the tip and the basal part of maize roots. The effects of the 6000g centrifugation were the same using the two root parts and they were similar to the results from the whole root (Table I). Cyt *c* oxidase (80–90%) was sedimented together with 40 to 60% of the other markers and proton pumps. The sedimentation of NADH- and NADPH-Cyt *c* reductase was higher in the tip of the root. A similar result was found for the ATP- and PPI-dependent proton pumps.

The total activities (6 KP + 6 KS) of several marker enzymes were similar in the two root parts (Cyt *c* oxidase, NADH- and NADPH-Cyt *c* reductase and latent UDPase). However, the total activities of GS I and the ATP- and PPI-dependent proton pumps

Table 1. Effect of an Initial Centrifugation Used to Separate Mitochondria and Microsomes from a Maize Root Homogenate
 The different fractions were prepared as described in "Materials and Methods." Relative fluorescence quenching of quinarine was measured after dilution of the fractions to 10% sucrose. ATP-dependent fluorescence quenching: 3 mM ATP-MgSO₄ and 50 mM KCl. PPI-dependent fluorescence quenching: 3 mM PPI, 3 mM MgSO₄, and 50 mM KNO₃.

Fraction	Protein (mg)	Cyt c Oxidase			NADH-Cyt c Reductase			Latent UDPase			Glucan Synthase I			UDPG-Sterol-Glucosyltransferase			ATP-Dependent Relative Fluorescence Quenching			PPI-Dependent Relative Fluorescence Quenching		
		Specific activity	Total activity	% quench/ fraction	Specific activity	Total activity	% quench/ fraction	Specific activity	Total activity	% quench/ fraction	Specific activity	Total activity	% quench/ fraction	Specific activity	Total activity	% quench/ fraction	Specific activity	Total activity	% quench/ fraction	Specific activity	Total activity	% quench/ fraction
1 KP	9.82	0.419	4.11	0.37	3.59	0.032	0.316	0.35	3.45	1002	9840	43.1	423	75.2	739	89.0	874					
1 KS	14.48	0.434	6.23 (100)	0.35	5.02 (100)	0.024	0.353 (100)	0.43	6.28 (100)	1015	14700 (100)	64.6	935 (100)	66.4	962 (100)	75.0	1086 (100)					
6 KP	7.71	0.646	4.99 (79.4)	0.30	2.31 (46)	0.024	0.187 (53)	0.29	2.25 (35.8)	828	6400 (43.5)	51.1	395 (42.2)	69.6	537 (55.8)	75.3	580 (53.4)					
6 KS	7.01	0.157	1.10 (17.5)	0.37	2.61 (52)	0.025	0.174 (49.3)	0.57	4.05 (64.4)	1258	8816 (60)	71.5	501 (53.6)	60.2	422 (43.8)	74.2	520 (47.9)					

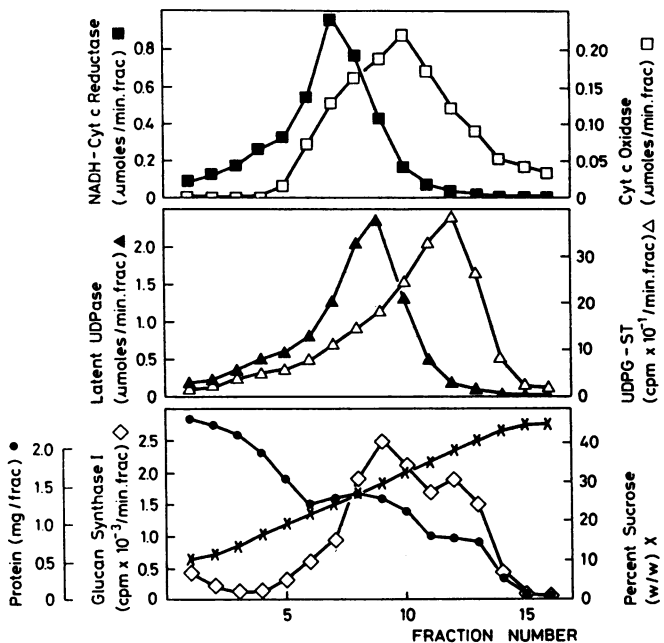


FIG. 1. Linear 10 to 45% sucrose gradient of the 6 KS fraction centrifuged for 3 h at 80,000g. NADH-Cyt c reductase (■), Cyt c oxidase (□), latent UDPase (▲), protein (●), and percentage of sucrose (×) were analyzed with 20-μl aliquots. Glucan synthase I (◇) was determined on 0.1 ml aliquots and UDPG-sterol-glucosyl-transferase (Δ) on 0.2 ml aliquots.

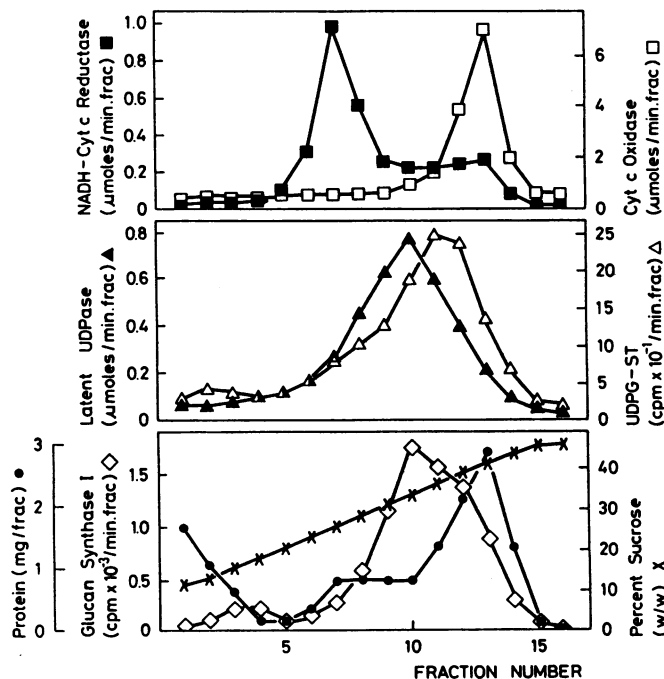


FIG. 2. Linear 10 to 45% sucrose gradient of the 6 KP fraction centrifuged for 3 h at 80,000g. NADH-Cyt c reductase (■), Cyt c oxidase (□), latent UDPase (▲), protein (●), and percentage of sucrose (×) were analyzed with 20-μl aliquots. Glucan synthase I (◇) was determined on 0.1 ml aliquots and UDPG-sterol-glucosyl-transferase (Δ) on 0.2 ml aliquots.

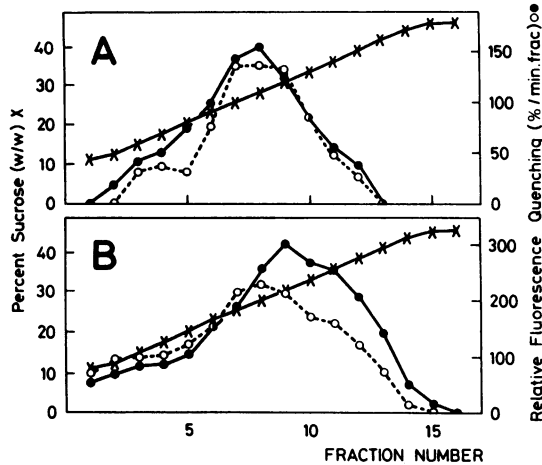


FIG. 3. Linear 10 to 45% sucrose gradient of the 6 KS (A) or the 6 KP (B) fraction centrifuged for 3 h at 80,000g. Relative fluorescence quenching of quinacrine was measured after dilution of the different fractions to 10% sucrose. ATP-dependent fluorescence quenching (O): 3 mM ATP-MgSO₄ and 50 mM KCl. PPI-dependent fluorescence quenching (●): 3 mM PPI, 3 mM MgSO₄ and 50 mM KNO₃.

were higher in the root tip. On the other hand, the UDPG-ST total activity was higher in the basal part of the root.

DISCUSSION

Most procedures for membrane preparation include a sedimentation of the mitochondria at low speed (6). Using maize roots, Nagahashi and Hiraike (15) showed that an optimal separation of microsomes and mitochondria could be achieved by a 20-min centrifugation at 6000g. These authors were using 4-d-old primary roots and reported that 93% of the mitochondria (Cyt *c* oxidase) were pelleted under these conditions. They also found a loss of 35% of the plasmalemma (pH 6.5 K⁺-stimulated ATPase) and 28% of the ER (Antimycin A-insensitive NADH-Cyt *c* reductase).

Under the same conditions, but using 2-d-old roots, we found that 80% of the mitochondria were pelleted. However, more plasmalemma (53%, UDPG-ST) and ER (46%, NADH-Cyt *c* reductase) were removed. These differences could be due to several factors as previously discussed (6). The Golgi markers latent UDPase, and GS I were also partly pelleted (36 and 43%, respectively), together with 56 and 53%, respectively, of the ATP- and PPI-dependent proton pumping activities.

This second differential centrifugation (20 min at 6000g) led to a considerable sedimentation of activities (30–50%) in the pellet and we therefore decided to fractionate the 6 KP (mitochondrial fraction) in the same way as the 6 KS (microsomal fraction), in linear sucrose gradients.

Cyt *c* oxidase, a reliable marker of the mitochondrial inner membrane showed a completely different distribution in the two linear gradients, after 3 h of centrifugation (Figs. 1 and 2). The peak of activity in Figure 2 (6 KP), corresponds to a density characteristic of intact mitochondria (21). The very low activity of Cyt *c* oxidase found in the 6 KS fraction, with a peak at lower density could be associated with the inner membrane of broken mitochondria (21), or with some intact mitochondria of lower density.

The ER marker NADH-Cyt *c* reductase peaked at the same density in the two gradients. The shoulder of activity present at higher density in the 6 KP fraction could be due to a small contribution of the antimycin A-insensitive NADH-Cyt *c* reductase associated with the outer envelope of the mitochondria (21).

UDPG-ST was selected as the plasmalemma marker for several

Table II. Distribution of Marker Enzymes from the Mitochondrial and Microsomal Fractions Prepared from the Tip (0–5 mm) and Basal Part (5–10–20 mm) of Maize Roots (10–20 mm) Two root homogenates (0–5 mm; 3.4 g fresh weight; 5 →; 8.2 g fresh weight) were prepared as described in "Materials and Methods." Relative fluorescence quenching of quinacrine was measured after dilution of the different fractions to 10% sucrose. ATP-dependent fluorescence quenching: 3 mM ATP-MgSO₄ and 50 mM KCl. PPI-dependent fluorescence quenching: 3 mM PPI, 3 mM MgSO₄ and 50 mM KNO₃.

Fraction	Protein	Cyt <i>c</i> Oxidase			NADH-Cyt <i>c</i> Reductase			NADPH-Cyt <i>c</i> Reductase			Latent UDPase			Glucan Synthase I			UDPG-Sterol-Glucosyltransferase			ATP-Dependent PPI-Dependent Relative Fluorescence Quenching			
		Specific activity	Total activity	%	Specific activity	Total activity	%	Specific activity	Total activity	%	Specific activity	Total activity	%	Specific activity	Total activity	%	Specific activity	Total activity	%	Specific activity	Total activity	%	
0–5 mm 6 KP	9.38	0.77	7.19 (84.7)	0.21	1.97 (57.8)	0.026	0.243 (59.6)	0.39	3.70 (50.3)	1446	13568 (46.2)	62.3	584 (54)	149.5	1402 (63.6)	200.1	1877 (63.4)						
0–5 mm 6 KS	4.56	0.28	1.30 (15.3)	0.32	1.44 (42.2)	0.036	0.165 (40.4)	0.80	3.66 (49.7)	3458	15772 (53.8)	109.0	497 (46)	176.2	803 (36.4)	237.8	1084 (36.6)						
5 → mm 6 KP	8.26	1.15	9.47 (91.5)	0.21	1.70 (40.8)	0.027	0.221 (41.6)	0.45	3.71 (48.7)	1112	9184 (53.8)	136.2	1125 (45.1)	78.7	649 (44.9)	55.8	460 (44.1)						
5 → mm 6 KS	5.92	0.15	0.88 (8.5)	0.42	2.46 (59.2)	0.053	0.317 (58.9)	0.66	3.90 (51.3)	1330	7876 (46.2)	231.4	1370 (54.9)	134.6	797 (55.1)	98.8	585 (55.9)						
Ratio ^a	0.98	0.82		0.82		0.76		0.97		1.72		0.43		1.52		2.83							

^a Total activity of the 6 KP + 6 KS from the tip divided by the total activity of the 6 KP + 6 KS from the basal part.

reasons. In maize coleoptiles, the distribution of UDPG-ST was shown to be clearly separated from markers of the other organelles and a clear correlation with another reliable plasmalemma marker, the vanadate-sensitive ATPase, was found (6, 7). UDPG-ST was also shown to be a convenient marker for the plasmalemma of maize roots (11). The density of the peak of UDPG-ST was slightly lower in the 6 KP fraction (Fig. 2) than in the 6 KS fraction (Fig. 1). It seemed possible that the 6000g centrifugation preferentially removed large vesicles of plasmalemma, characterized by a slightly lower density.

Latent UDPase was shown to be a reliable Golgi marker in maize root homogenates (16). In peak stems and maize coleoptiles, this marker was associated with the Golgi vesicles and cisternae (6, 25). In maize coleoptiles, GS I seemed to be preferentially associated with the Golgi cisternae; however, part of its activity coincided with the plasmalemma marker UDPG-ST (6). Therefore, GS I was not a reliable marker for the Golgi membranes. We found the same results with maize roots, where one part of the GS I activity always coincided with UDPG-ST (Figs. 1 and 2). The two Golgi markers (latent UDPase and GS I) were shifted to a slightly higher density in the 6 KP fraction compared with the 6 KS fraction. The 6000g centrifugation could have preferentially removed Golgi cisternae of large size characterized by a slightly higher density.

The activity of the two proton pumps present in the 6 KS fraction exhibited a similar distribution after 3 h of centrifugation on a linear sucrose gradient (Fig. 3A). The distribution of the Golgi markers (latent UDPase and GS I) and the ER marker NADH-Cyt *c* reductase always overlapped those of the pumps.

When the 6 KP fraction was centrifuged 3 h on a linear sucrose gradient, the distributions of the two pumps were almost similar (Fig. 3B). The PPI-dependent pump was shifted to a slightly higher density than the ATP-dependent one. Several hypotheses could be given to explain these differences. First, the two proton pumps are not associated with exactly the same population of vesicles. Second, the activity of the ATP- or PPI-dependent proton pumps are selectively decreased in some of the fractions by other enzymes competing for the same substrate (a phosphate for the ATP and PPI, and an ATPase for the ATP).

After rate-zonal centrifugation for 30 min of the 6 KS or the 6 KP fraction (7, 12), the distributions of the two pumps were again similar (data not shown). The sedimentation velocities of the two pumps were clearly different from those of the plasmalemma marker UDPG-ST and mitochondrial marker Cyt *c* oxidase, indicating that the pumps were not associated with these two membranes. Moreover, the ATP-dependent pump, present in the two fractions, was insensitive to vanadate (100 μ M), an inhibitor of the plasmalemma ATPase and only slightly inhibited by azide (500 μ M) and oligomycin (5 μ g/ml), two inhibitors of the mitochondrial ATPase (data not shown).

The distribution of a maize root ATP-dependent proton pump in linear sucrose gradients (15–45%), after 2 h of centrifugation at 80,000g, has been reported (9, 18). The pump was clearly separated from the mitochondrial markers Cyt *c* oxidase and azide-sensitive ATPase. However, part of the proton transport activity coincided with markers for the Golgi (latent UDPase and GS I), the plasmalemma (GS II and vanadate-sensitive ATPase), and the ER (NADH-Cyt *c* reductase). These authors concluded that the ATP-dependent proton pump was of tonoplast origin.

At present, no unequivocal marker has been found for the tonoplast (3, 21, 27). α -Mannosidase (3, 17) and carboxypeptidase (17, 26) activities, sometimes presented as tonoplast markers, were tested and their distributions showed them to be soluble enzymes in maize roots (A Chanson, P-E Pilet, unpublished data).

The ATP-dependent proton pump described in numerous

papers (see Sze [24] for a recent review) has been regarded as a potential candidate for a tonoplast marker. The characterization of this pump showed that it was clearly different from the plasmalemma pump and the mitochondrial ATPase. When microsomal membrane vesicles of maize coleoptiles were centrifuged in sucrose and dextran density gradients, the distribution of the pump was also separated from the ER marker NADPH-Cyt *c* reductase (13). More recently, Mandala and Taiz (14) isolated vacuoles from maize coleoptile protoplasts and measured the activity of an ATP-dependent proton transport. The characteristics of proton pumping by isolated vacuoles were similar to those of light microsomal membranes, possibly derived from the tonoplast. Moreover, the vacuoles and the light microsomal membranes exhibited similar profiles of proton transport activities after flotation in linear dextran gradients (14). However, the recent discovery of an ATP-dependent proton pump with similar characteristics, in the Golgi of maize coleoptile cells (7) and suspension-cultured cells of sycamore (1), renders the use of this activity as a tonoplast marker questionable (27).

A PPI-dependent proton pump was equally shown to be associated with the tonoplast of red beet (22, 23) and oat roots (30). In maize coleoptiles, the PPI-dependent pump was shown to be associated with tonoplast- and Golgi-rich fractions (5). Its distribution after 3 h of centrifugation in linear sucrose gradients was identical to the distribution of the ATP-dependent pump. Using the same material, Mandala and Taiz (14) showed that isolated vacuoles were able to pump protons in the presence of PPI.

In maize roots, the two pumps presented a similar distribution, indicating that the PPI-dependent pump, like the ATP-dependent one, was present on the tonoplast. However, the two pumps always overlapped the Golgi markers latent UDPase and GS I and the ER marker NADH-Cyt *c* reductase. In these conditions, it was not possible to rule out the possible partial association of these two pumps with the Golgi and ER membranes.

The two proton pumps, but especially the PPI-dependent one were more active (or more abundant) in the root tip, which corresponds to the meristems and the elongation zone (19, 20). A similar result was found for the distribution of an ATP-dependent pump present on sealed vesicles prepared from maize roots (9). The glucan synthase I was also very active in the root tip, whereas the UDPG-ST was relatively more active in the basal part.

These data raise interesting perspectives concerning the relationship between root growth and the activity of the pumps. Experiments are under way to study more thoroughly their distribution in maize roots. The PPI-dependent pump has been characterized and the results will be published in a second paper.

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