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Association of H^+-Translocating ATPase in the Golgi Membrane System from Suspension-Cultured Cells of Sycamore (*Acer pseudoplatanus* L.)

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ABSTRACT

The Golgi complex and the disrupted vesicular membranes were prepared from suspension-cultured cells of sycamore (*Acer pseudoplatanus* L.) using protoplasts as the starting material and employing linear sucrose density gradient centrifugation followed by osmolysis (Ali *et al.* [1985] Plant Cell Physiol 26: 1119–1133). The isolated Golgi fraction was found to be enriched with marker enzyme activities and depleted of the activity of a typical mitochondrial marker enzyme, cytochrome *c* oxidase. Golgi complex, and vesicular membranes derived thereof were found to contain the specific ATPase (specific activity of about 0.5 to 0.7 micromoles per minute per milligram protein). Inhibitor studies suggested that the ATPase of Golgi was different from plasma membrane, tonoplast and mitochondrial ATPases as it was not inhibited by sodium vanadate, potassium nitrate, oligomycin and sodium azide. The sensitivity to *N*-ethylmaleimide further distinguished the Golgi ATPase from F_0 to F_1 ATPase of mitochondria. The internal acidification was measured by monitoring the difference in absorbance at 550 nanometers minus 600 nanometers using neutral red as a probe. The maximum rate detected with Golgi and disrupted membrane system was 0.49 and 0.61 optical density unit per minute per milligram protein, at pH 7.5, respectively, indicating that the proton pump activity was tightly associated with the Golgi membranes. In both cases, the acidification was inhibited 70 to 90% by various ionophores, indicating that the proton pump was electrogenic in nature. Both the Golgi ATPase activity and ATP-dependent acidification were profoundly inhibited by *N,N'*-dicyclohexylcarbodiimide, which also indicate that the two activities are catalyzed by the same enzyme.

The ATP-driven proton pumping activities, which have several different physiological functions such as (a) to drive secondary active transport, (b) to control cellular volume and rigidity, and (c) to regulate activities of intracellular enzymes, has been reported in various cell types, as well as in cell organelles from both plant and animal origin (33). The extensively studied H^+-translocating ATPase has been described in mitochondria (11), chloroplasts (40), lysosomes (32), coated pits (45), vacuoles (21), and plasma membranes (42). From characteristic inhibitor studies, it is now well established that there are two basically different ATP-driven proton pumps in microsomal vesicles from various plant tissues (8, 14, 38). One is mainly present in the vesicles derived from plasma membranes (33). It forms a phosphorylated acyl intermediate, which is sensitive to orthovanadate (23, 34). The second type is represented by the proton pump of tonoplast, insensitive to vanadate but sensitive to potassium nitrate (6, 24, 25).

The study of the H^+-ATPase of the Golgi membranes is an interesting area of cell biological research when one considers the intracellular traffic and sorting processes carried out by this organelle (15). However, our knowledge about the presence of such an enzyme is limited in the case of the plant Golgi system. Glickman *et al.* (17) have reported the presence of an electrogenic proton pump, having chloride transport ability, in a highly purified preparation of rat liver Golgi apparatus. The requirement for an ATP-driven proton pump for the processing of newly synthesized protein by the Golgi complex has been suggested by Zhang and Schneider (46). Barr *et al.* (5) have recently demonstrated NADH and ATP-dependent acidification of the Golgi membranes from mouse liver using neutral red as a probe. Virk *et al.* (43) have observed ATP-dependent Ca^{2+} accumulation by Golgi vesicle preparations from the lactating mammary gland of rat.

In plants, there are only few reports regarding the presence of an H^+-translocating ATP-ase associated with Golgi membranes. The association of an ATP-dependent H^+-ATPase with the Golgi membranes of corn coleoptiles has been described by Chanson *et al.* (12, 13). Binary and Racusen (7) characterized an ATPase in isolated secretory vesicles from a liquid suspension culture of *Avena sativa*.

We have recently reported the isolation of highly purified Golgi complex, enriched with some Golgi marker enzymes, from sycamore suspension-cultured cells (2). We have undertaken further steps to prepare the vesiculated membranes from the Golgi preparations. The present paper reports the presence of a proton pump in the purified organelle and the vesiculated membranes, using neutral red uptake as a criterion of acidification. It is found that ATP is involved in the acidification of Golgi vesicles as observed by others (5, 13, 46). Furthermore, the H^+-ATPase is found to be clearly distinguishable from those of the mitochondria. A possible role of the Golgi system in the biosynthesis and secretion of laccase-type polyphenol oxidase by the sycamore cells has been discussed previously (1).

MATERIALS AND METHODS

Chemicals. K^+-ATP, Mg^{2+}-ATP, BSA, gramicidin D, monensin, and oligomycin were purchased from Sigma. Nigericin was purchased from Eli Lilly Co. DCCD,[1] sodium azide, orthovanadate, neutral red, and all other chemicals of reagent grade were

[1] Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; GF, Golgi fraction; IDPase, inosinediphosphatase; M, mitochondrial fraction; NEM, *N*-ethylmaleimide; PNP, *p*-nitrophenol.

purchased from Wako Chemical Co. (Tokyo, Japan).

Plant Material. Sycamore cells were cultured under the previously described conditions (2). Usually 3-d old cells were used for the preparation of the organelle.

Preparation of Golgi and Mitochondria. The isolation procedure of the GF involved protoplast preparation, followed by disruption, and subsequent differential centrifugation at 10,000*g* for 10 min. The 10,000*g* pellet was suspended in 2 ml of buffer containing 50 mM glycyl-glycine (pH 7.5) and 1 mM EDTA and subjected to a linear sucrose density gradient centrifugation as described previously (2). Although we have achieved the reasonably satisfactory separation of Golgi from mitochondria, in order to isolate more homogeneous organelle preparation, each fraction was collected separately and then pelleted by centrifugation at 30,000 rpm for 1 h using a R-30 rotor in a Beckman model L5-50 ultracentrifuge. In the case of the Golgi, only the GF_2 fraction (*cf.* Ref. 2) was subjected to the second sedimentation. After suspending the resulting pellets of the Golgi fraction and the mitochondria in 1 ml of the same buffer, the fractions were applied to a linear sucrose gradient (15–50%, w/w) and centrifugation was carried out under the same conditions as described for the first centrifugation.

Golgi and mitochondrial fractions obtained after centrifugation were collected separately, using an ISCO density gradient fractionator (Model 640) in 0.6 ml fractions (see Fig. 1).

Preparation of Vesiculated Golgi Membranes and Submitochondrial Particles. To prepare Golgi vesicles, enzymically active GF (described in "Results") were pooled and subjected to osmotic lysis. In a typical experiment, to GF (2 ml) in 25% sucrose was added 5 volumes of a chilled buffer containing 50 mM glycyl-glycine (pH 7.5) and 1 mM EDTA. The suspension was gently stirred for 30 min and centrifuged at 30,000 rpm for 1 h using a Beckman R-30 type rotor. The resulting pellet was suspended in 1 ml of the same buffer, homogenized gently using a Teflon homogenizer, and the total homogenates were applied to a linear sucrose gradient (15–40%, w/w). The gradients were centrifuged at 21,000 rpm for 10 h in a Beckman model L5-50 ultracentrifuge with an SW-25.3 rotor at 4°C. Fractionation was performed as described previously.

Submitochondrial particles were prepared according to the method of Tzagoloff and Meagher (41), using pure M (Fig. 1) as starting material.

Enzyme Assays. ATPase (17) activity was assayed by measuring the released Pi, using 1 ml of phosphate Kit (Phosphor C Reagent, Wako Chemical Co., Tokyo), after incubation for 10 min at 37°C in a medium (total volume 100 μl) containing 12 mM Mes-Tris (pH 7.0), 5 mM $MgCl_2$, 30 mM KCl, 5 mM K-ATP, and 10 μl of gradient mixture.

α-Mannosidase activity (10) was assayed by measuring the liberation of PNP from *p*-nitrophenyl-α-mannopyranoside. The reaction mixture contained (in a total volume of 0.5 ml) 100 μl of the gradient mixture, 10 mM Na-acetate buffer (pH 5.3) and 1 mM *p*-nitrophenyl-α-mannopyranoside was incubated at 37°C for 1 h. The reaction was stopped by adding 0.8 ml of 0.25 M Na_2CO_3-$NaHCO_3$ buffer (pH 10.1) and the *A* at 400 nm was determined.

Glucan synthetase II activity was measured as a marker of plasma membranes, following the method described by Ray (30). The reaction mixture contained in a total volume of 100 μl: Tris-HCl (pH 8.1), 40 mM cellobiose, 4 mM EDTA, 1 mM DTT, 0.5 mM UDP-glucose, 33 nCi UDP-[^{14}C]glucose (7.2×10^4 dpm) and 70 μl of GF. The incubation was continued at 30°C for 30 min and other assay procedures were the same as before (2).

IDPase (15) and Cyt *c* oxidase (18) were assayed by the published methods.

Protein Analysis. Protein content was analyzed using Bio-Rad protein reagent and globulin as a standard.

Proton Pump Activity Assay. Proton pump activity was measured by using neutral red as a probe according to the method of Barr *et al.* (5). The reaction mixture contained in a final volume of 1 ml; 30 to 60 μg of the Golgi complex membranes or vesiculated Golgi membranes suspended in 0.1 ml sucrose (0.25 M), 12.5 μM neutral red in final concentration, 0.85 ml sucrose buffered with BSA (0.25 M sucrose containing 1.3 mg BSA/ml, pH 7.5) and 1 mM Mg^{2+}-ATP. The mixture of Golgi membranes and neutral red was equilibrated for 1 min with stirring, followed by the addition of sucrose buffered with BSA in order to secure that the neutral red was first accumulated by the Golgi vesicles. After an equilibration period of 5 min 1 mM Mg^{2+}-ATP was added. The absorbance change (550 nm − 600 nm) was followed using a Hitachi model 557 spectrophotometer in the dual beam mode at 25°C. The inhibitory effect of DCCD was tested under the identical assay conditions.

Inhibitor and Uncoupler Studies. Ten μl of inhibitors or uncouplers were added to the reaction mixture. Stock solutions were prepared in absolute ethanol. Control treatments also contained the same volume of ethanol. The concentrations of inhibitors and uncouplers are indicated in the legends to the figures and tables.

RESULTS

Characterization of the Golgi Membranes. In a linear sucrose density gradient centrifugation (15–50%), GF and M were well separated as shown in the upper inset of Figure 1. The distribution pattern of the Golgi marker enzymes, latent IDPase (31) and α-mannosidase (35), is illustrated in Figure 1B. Both enzyme activities of the organelle showed a single coincident peak at 1.12 g/cm^3. The recovery of the enzyme activities and their sedimentation profile were quite reproducible (2). The GF was totally depleted of the mitochondrial marker enzyme (Cyt *c* oxidase), which occurred as a single distinctive peak at 1.18 g/cm^3 after centrifugation on a linear sucrose gradient (Fig. 1C). We have also measured the IDPase activities in the M (Fig. 1B) as well as the Cyt *c* oxidase activities in the GF (Fig. 1C). In each case, only negligible activities were detected. The results indicated that GF and M were well separated and mutually uncontaminated. The association of α-mannosidase activity with vesicular membranous structures prepared from the Golgi by the osmolytic method is presented in Figure 3B. As can be seen, the activity was more broadly distributed and sedimented around a density of 1.12 g/cm^3. Under the conditions employed a single white band was obtained (upper inset of Fig. 3). The sealed nature of the fragmented membranous preparations can be verified from the acidification experiments as described later.

ATPase Activity. Both the original GF and the disrupted vesicular Golgi membranes exhibited ATPase activities as illustrated in Figures 2A and 3A. The specific activities were about 0.44 μmol/min·mg protein, and 0.66 μmol/min·mg protein, respectively. We have concluded that this activity was not due to nonspecific phosphatase, as the presence of 1 mM sodium molybdate, did not inhibit the reaction (16). It is important also for us to exclude a contamination of plasma membranes in the GF, since K^+-ATPase activity is known to be associated with the plasma membrane. As shown in Figure 2A, glucan synthase II, a marker of plasma membrane (30), was totally absent in GF; and we can reasonably conclude that the isolated GF was not contaminated by plasma membrane.

Figure 2B illustrates the distribution pattern of ATPase activity in the M fraction in the presence and absence of oligomycin. The specific activity was 0.16 μmol/min·mg protein. It can be seen that the mitochondrial ATPase was strongly inhibited by oligomycin, whereas the Golgi ATPase was only slightly inhibited (Fig. 2A).

As the ATPase of mitochondria is located in the inner mem-

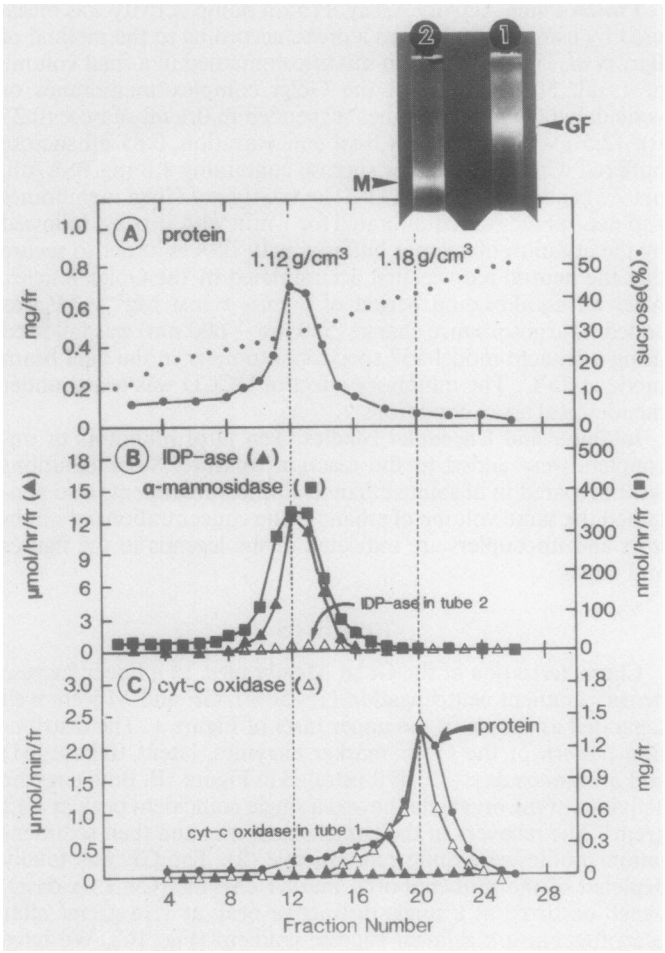

FIG. 1. Distribution patterns of mitochondrial and Golgi marker enzymes. Pellets (10,000*g*) of the disrupted protoplasts were applied to a linear sucrose gradient (15–50%, w/w) and centrifuged for 3 h in a Beckman L5-50 ultracentrifuge using an SW 25.3 rotor. The mitochondria and Golgi fractions from the gradient were sedimented by centrifugation at 30,000 rpm for 1 h using a R-30 rotor; each resulting pellet was suspended in 1 ml of 50 mM glycyl-glycine, 1 mM EDTA (pH 7.5) and applied to a 15 to 50% linear sucrose gradient. Each tube was then centrifuged under the same condition as described above to separate Golgi (GF) and mitochondria (M) as shown in tube (1) and (2) of the top panel. Fractionation pattern of tube (1) is given in A protein (●) and sucrose concentration (•), and that of tube (2) in C (protein [●] and Cyt *c* oxidase [▲]). A, Protein (●) and sucrose concentration (•); B, IDPase (▲) and α-mannosidase (■); and C, Cyt *c* oxidase (▲) and protein (●) of mitochondrial fraction. The top panel shows the separation of mitochondria (M) and Golgi (GF) fractions. Panel B, IDPase activities assayed for fractions of tube (2) are shown (△), and in panel C Cyt *c* oxidase activities assayed for fractions of tube (1) are shown (▲). For details of the enzyme assays, see text.

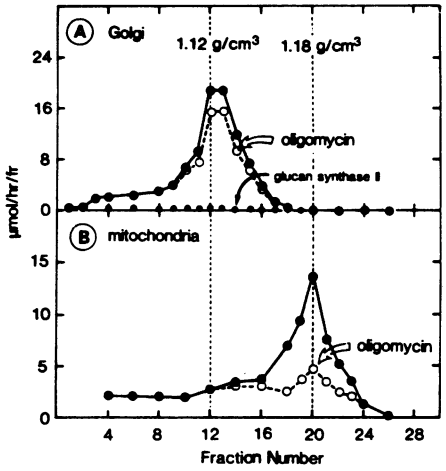

FIG. 2. Distribution patterns of ATP-ase in mitochondria and Golgi complex. Experimental conditions employed were essentially the same as those described in Figure 1. For details of the enzyme assay, see the text. A, Golgi ATPase, and B, mitochondrial ATPase. In panel A glucan synthase II activities assayed are given (●) to show that GF is not contaminated with plasma membrane (see text).

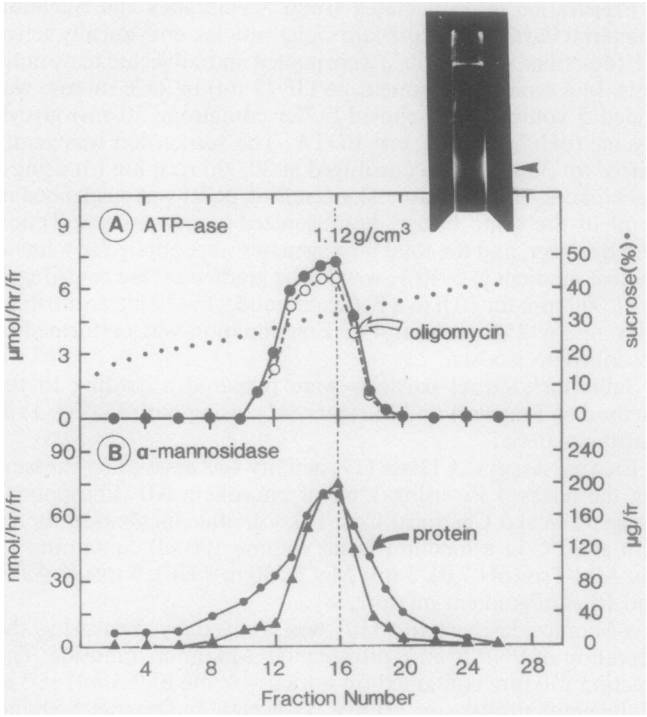

FIG. 3. Distribution patterns of ATP-ase in disrupted Golgi vesicular membranes. Enzymically active GF fractions, tube nos. 11 to 14 in Figure 1B, were pooled and subjected to osmolysis. The pellets collected by centrifugation were suspended in 1 ml of the same buffer as described previously, gently homogenized using a Teflon homogenizer and applied to a 15 to 40% linear sucrose gradient. Centrifugation pattern (10 h) using an SW 25.3 rotor in a Beckman L5-50 ultracentrifuge is presented in the top panel. Fractionation pattern of the tube is given in A (ATPase [●]) and B (α-mannosidase [▲] and protein [•]). Other experimental details are given in the text.

branes, it is essential to prepare the submitochondrial particles to expose the active site of the enzyme to exogenous reagents. For this purpose the intact mitochondria were subjected to ultrasonic disintegration (41). The different responses of submitochondrial ATPase and Golgi ATPase to some characteristic inhibitors can be seen from the results presented in Table I. DCCD, which is known to inhibit H^+-ATPase, strongly inhibited both ATPases but a much higher concentration was needed to inhibit the Golgi ATPase in comparison to the mitochondrial enzyme. As shown in Table I, a very low concentration of oligomycin (1 μg/reaction mixture) was sufficient to inhibit the mitochondrial ATPase (83%), whereas the Golgi enzyme was unaffected (see also Fig. 2). The thiol reagent NEM was inhibitory to the Golgi ATPase (52%), but showed no effect on the mitochondrial ATPase. Unlike the mitochondrial ATPase the Golgi

Table I. *Inhibitory Effects of Various Compounds on ATPase Activities of Submitochondrial Particles and Golgi*

Inhibitors	Concentration	Inhibition	
		Submitochondrial particles	Golgi
		%	
DCCD	50 μM	89	20
	500 μM	—[a]	78
Oligomycin	1 μg[b]	83	ND[c]
	10 μg[b]	—[a]	10
Sodium azide	1 mM	50	ND
Sodium molybdate	1 mM	ND	ND
N-Ethylmaleimide	1 mM	ND	52
Sodium vanadate	50 μM	5	ND
Potassium nitrate	100 mM	34	ND

[a] Not measured. [b] Per reaction mixture. [c] Not detectable.

ATPase was not inhibited by NaN_3. Sodium molybdate and orthovanadate showed no effect on either ATPase under identical assay conditions. Potassium nitrate (100 mM), a well known inhibitor on the tonoplast-type H^+-ATPase (24, 25, 35), showed no effect on the Golgi ATPase but slightly (34%) inhibited the mitochondrial ATPase.

ATP-Dependent Acidification of Golgi Membranes. Since the Golgi ATPase was distinguishable from the tonoplast, plasma membrane, and mitochondrial ATPases and a reasonable inhibitory effect of DCCD was observed, we assayed ATP-dependent acidification using neutral red as an indicator, which is a sensitive spectrophotometric method recently reported by Barr *et al.* (5). The principle of the method is the following: when the external phase of the organelle concerned is strongly buffered by a nonpermeating buffer, *e.g.* BSA, the internal acidification due to the hydrolysis of ATP could be detected in the presence of neutral red which traps a proton, provided there is no leakage of H^+ from the sealed vesicles. Consequently, an absorbance change occurs at two characteristic wavelengths and the overall spectral change can be monitored by a dual beam spectrophotometer (20).

The addition of Mg^{2+}-ATP caused the accumulation of H^+ inside the isolated Golgi membranes and disrupted Golgi membranes vesicles (Fig. 4, A and B). The observed H^+ accumulation was inhibited by the ionophore, nigericin (10 μM). Table II lists the effect of various ionophores on the rate of absorbance change of both membranes in the presence of neutral red. Nigericin, gramicidin D, and monensin (all 10 μM) inhibited the process of H^+ accumulation 70 to 90% in both isolated and disrupted Golgi membranes. It is crucial for us to get a proof that the membrane acidification and ATPase are catalyzed by the same enzyme entity, and for this purpose we have tested the effect of DCCD. We have found the inhibitory effect of DCCD on the ATP-induced acidification of Golgi but at a relatively lower concentration (Table II), a finding being similar to that observed in other systems (26, 44). The reason for the discrepancy between the effects of DCCD on ATPase activity and on acidification is not clear. It may be due to the fact that ATPase is not a single system.

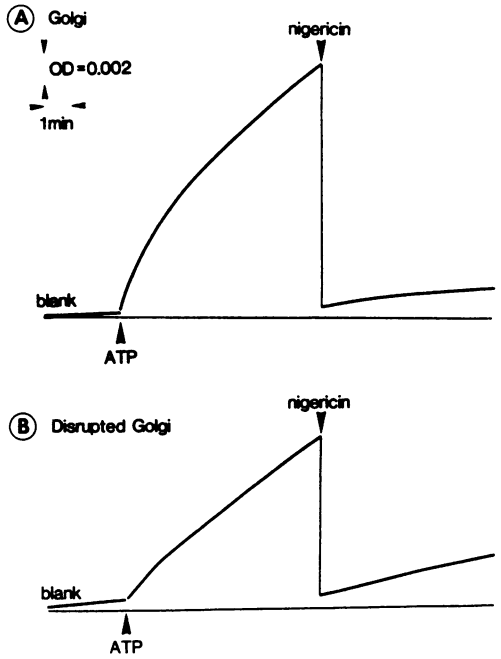

FIG. 4. ATP-induced acidification of Golgi (A) and disrupted vesicular membranes (B). Details of the double-beam spectrophotometric assay of the ATP-driven acidification of Golgi (A) and disrupted vesicular membranes (B) in the presence of neutral red are described in text. The absorption increment, OD (550–660 nm), due to 1 mM Mg^{2+}-ATP addition and the inhibitory effect caused by 10 μM nigericin are retraced from the original chart. The preparative method of isolating the disrupted Golgi vesicular membranes by osmolysis is described in "Materials and Methods."

DISCUSSION

The Golgi complex plays a role in the complex and diverse sorting processes of various macromolecular compounds after their separation from the ER and the subsequent intracellular transport of these compounds to their final destination at the cell surface. Several lines of evidence have suggested that metabolic energy is definitely required in order to accomplish this important function by the Golgi complex. Jamieson and Palade (19) have found that the transport of secretory protein of pancreatic exocrine cells can be blocked by respiratory inhibitors and by inhibitors of oxidative phosphorylation. Monensin and FCCP were found to reduce the appearance of viral membrane glycoproteins at the cell surface (22). Tartakoff (37) postulated that the normal compressed structure of the Golgi cisternae might represent the operation of an ion pump and that monensin might cause an enhancement of cation permeability and result in the dilation of the cisternae. Recently Anderson and Pathak (3) have demonstrated that cisternae and vesicles associated with the *trans* face of the Golgi apparatus of human fibroblasts were acidic compartments using immunocytochemical method. Indeed it has been reported that monensin inhibits the secretion of laccase in sycamore cells and causes swelling of the Golgi cisternae (9).

Table II. *Inhibitory Effects of Various Compounds on the ATP-Induced Acidification of Golgi Membranes in the Presence of Neutral Red*

Experiment	Inhibitors	Relative Rate	Inhibition
		OD (550-600 nm) · mg protein^{-1} · min^{-1}	%
1	Golgi	0.49	
	+ 50 μM nigericin	0.02	96
	+ 10 μM gramicidin D	0.10	80
	+ 10 μM monensin	0.04	92
2	Disrupted Golgi membranes	0.61	
	+ 50 μM nigericin	0.17	72
	+ 10 μM gramicidin D	0.06	90
	+ 10 μM monensin	0.05	92
3	Golgi	0.42	
	+ 10 μM DCCD	0.34	19
	+ 20 μM DCCD	0.09	79
	+ 50 μM DCCD	0.03	93
4	Disrupted Golgi membranes	0.61	
	+ 10 μM DCCD	0.49	20
	+ 20 μM DCCD	0.12	80
	+ 50 μM DCCD	0.0	100

The acidification of the Golgi apparatus from mouse liver by ATP or NADH and its inhibition by uncouplers and ionophores have been reported by Barr *et al.* (5). A 3-fold accumulation of newly synthesized proteins in the Golgi complex of the rat liver, which was caused by DCCD, implicates the involvement of an ATP-driven proton pump in protein secretion (46).

In our present investigation we have demonstrated an ATP-driven proton pump in the highly purified and morphologically preserved Golgi and the osmotically disrupted vesicular membranes from sycamore suspension-cultured cells. We have prepared the vesiculated Golgi membranes, because isolated vesicles have several advantages over intact organelles in studying transport mechanisms (35).

The H^+-translocating ATPase associated with the Golgi was clearly different from the mitochondrial ATPase in its responses to various characteristic inhibitors such as oligomycin, NaN_3, and NEM. At a low concentration, oligomycin had no effect on the GF H^+-ATPase but a high concentration caused a slight inhibition (Fig. 2A and Table I). Sodium azide (1 mM) did not affect the GF H^+-ATPase but at a concentration higher than 1 mM it stimulated the activity due to its uncoupling effect (21). GF H^+-ATPase was susceptible to the effect of NEM. H^+-ATPases from rat liver Golgi (17), lysosomes (26), and bovine brain coated pits (32) were also inhibited by NEM. It is now recognized that Golgi H^+-ATPase contains a sulfhydryl group and is clearly distinguishable from F_0 to F_1 ATPase of mitochondria (17). Orthovanadate inhibited both Ca^{2+}-ATPase and Na, K-ATPase (27); it was not effective against either the mitochondrial or Golgi H^+-ATPase. GF H^+-ATPase was not affected by treatment with 100 mM potassium nitrate. It thus further ruled out the possibility of contamination with tonoplast type ATPase (Table I).

The GF H^+ATPase was inhibited by DCCD at a relatively high concentration (0.5 mM) (Table I), which is similar to the effect on the Golgi ATPase isolated from rat liver (46) and the vacuolar membrane ATPase from yeast (21). From the observed inhibitory effect of GF ATPase by DCCD, we have tentatively concluded that the enzyme is a proton translocating ATPase. Also, despite different dose-response (DCCD) of ATP-dependent acidification of Golgi membranes with that of ATPase, we tentatively conclude that the two activities are catalyzed by the same enzyme (*cf.* Table II). There are numerous reports describing the usefulness of DCCD to characterize the nature of H^+-ATPase (4, 28, 35, 39, 46). Recent investigation of Chanson and Taiz (13) has reported the presence of H^+-ATPase in the Golgi from corn coleoptiles by measuring [^{14}C]methylamine uptake and quinacrine fluorescence quenching techniques. Effects of some inhibitors they have observed are similar to our present studies.

The ATP-driven acidification in the presence of neutral red was used to assess the proton pump activities of two types of Golgi membranes. The fact that ATP-induced accumulation of protons led to the development of a ΔpH was confirmed by adding nigericin, a protonophore, which rapidly collapses the ΔpH by exchanging protons for potassium or sodium (29) (Fig. 4, A and B). Monensin and gramicidin D were also found to cause a rapid discharge of neutral red (Table II). It is well known that these ionophores induce swelling of the Golgi apparatus membranes and interfere with their normal secretory function. It has been reported by Sze (35) that an electrogenic H^+-translocating ATPase might have the following properties: (a) stimulation of ATP hydrolysis in the presence of ionophores followed by simultaneous dissipitation of ΔpH, (b) generation of an electrical gradient, and (c) generation of a pH gradient. Our present findings have shown dissipitation of ΔpH by various ionophores. In spite of the fact such experiments alone do not provide convincing evidences for electrogenic or electroneutral transport, the ionophores effect is judged to be a useful and simple assay method, because the inhibitory effect is directly correlated with the presence of functional electrogenic H^+-ATPase (6).

The mitochondrial proton pump is used to drive the formation of ATP from ADP and Pi. The Golgi proton pump, as reported here, is conceivably involved in the covalent modification and secretion of protein at the expense of ATP. The elucidation of the crucial role of such a proton pump in relation to the secretory mechanisms operating in the sycamore cell is an interesting area of our future research. As our previous study has demonstrated the inhibitory effect of monensin on the intracellular transport of laccase molecules accompanies the dramatic dilation of the Golgi cisternae (9).

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