Plant Physiol. (1994) 106: 119–125

Structure and Function of the Golgi Complex in Rice Cells[1]

II. Purification and Characterization of Golgi Membrane-Bound Nucleoside Diphosphatase

Toshiaki Mitsui*, Masahiko Honma, Takashi Kondo, Noriaki Hashimoto, Shinn Kimura, and Ikuo Igaue

Department of Applied Biological Chemistry, Faculty of Agriculture, Niigata University, Niigata 950–21, Japan

Inosine diphosphatase bound to Golgi membranes was studied in rice (*Oryza sativa* L. cv Nipponkai) cells. The enzyme was solubilized with Triton X-100 from isolated rice Golgi membranes and was highly purified employing a series of chromatography steps in the presence of 20% glycerol and 0.1% Triton X-100. The apparent molecular mass of the enzyme was estimated by gel filtration column chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis to be 200 and 55 kD, respectively. The isoelectric point of the enzyme was determined to be 7.5. The optimal pH for the enzyme activity was around 7 and the enzyme required Mg^{2+} for hydrolyzing activity. IDP, UDP, and GDP were effective substrate for the purified rice Golgi membrane-bound inosine diphosphatase, whereas activity with ADP, CDP, and thymidine 5′-diphosphate was 10 to 20% of IDP. The K_m values for IDP, UDP, and GDP were 0.48, 0.50, and 0.67 mM, respectively, and V_{max} values were 1.85, 1.54, and 1.67 μmol min^{-1} mg^{-1}, respectively. These results indicate that the rice Golgi enzyme is a nucleoside diphosphatase that is specific for IDP, UDP, and GDP. Furthermore, this rice Golgi nucleoside diphosphatase stimulated the activity of glucan synthase I also localized in rice Golgi membranes. The results strongly support the view that this nucleoside diphosphatase is involved in regulation of β-glucan synthesis in the plant Golgi complex.

Suspension-cultured cells derived from the embryo of rice (*Oryza sativa* L. cv Nipponkai) seeds grow rapidly, actively secrete hydrolases such as α-amylase and chitinase, and have a well-developed Golgi complex (Kimura et al., 1993; Mitsui et al., 1993). Rice Golgi membranes contained unique structural glycoproteins that are recognized by peanut lectin (Mitsui et al., 1990; Kimura et al., 1992b). Golgi membrane glycoproteins bear an O-linked oligosaccharide moiety, and formation of these glycoproteins was prevented by brefeldin A, a compound known to destroy Golgi stacks (Kimura et al., 1993). There is little information on the structure and function of rice Golgi membrane-associated enzymes, although the existence of an isoform of UDP-Glc pyrophosphorylase was reported (Kimura et al., 1992c).

NDPase was cytochemically and biochemically identified as a Golgi membrane-bound enzyme in plant cells (Dauwalder et al., 1969; Ray et al., 1969; Gardiner and Chrispeels, 1975; Nagahashi and Kane, 1982) and has been used as an exclusive marker for Golgi membranes of higher plant cells (Goff, 1973; Morré et al., 1977; Quail et al., 1979; M'Voula-Tsieri et al., 1981). However, several NDPases have also been found in other endomembrane systems (Goff, 1973). It has been reported that mammalian microsomal NDPases can hydrolyze UDP, GDP, and IDP (Novikoff and Heus, 1963; Yamazaki and Hayashi, 1968; Pinsley and Scrutton, 1973), whereas yeast Golgi membrane-bound GDPase was highly specific for GDP (Yanagisawa et al., 1990). Since plant Golgi membrane-bound IDPases have not been purified, both the specificity of the Golgi IDPase for IDP and the role of the Golgi IDPase remain unknown. Our objective was to purify and characterize the Golgi membrane-bound IDPase from rice suspension-cultured cells.

MATERIALS AND METHODS

Plant Materials

Suspension-cultured rice cells were derived from the embryo of the rice seeds (*Oryza sativa* L. cv Nipponkai). Rice cells were cultured in LS medium (Linsmaier and Skoog, 1965) and harvested as described previously (Mitsui et al., 1990).

Chemicals

The following commercial products were used: nucleosides and nucleotides were from Yamasa (Chiba, Japan); Phosphor C-Test and *p*-nitrophenylphosphate came from Wako (Osaka, Japan); DEAE-Toyopearl 650M was obtained from Tosoh (Tokyo, Japan); Cellulofine came from Seikagaku Kogyo (Tokyo, Japan); Ampholine, Con A-Sepharose 4B, and EAH-Sepharose 4B were obtained from Pharmacia Japan; Bio-Rad protein assay and silver staining kits were from Bio-Rad Japan; molecular mass standards came from Sigma, and UDP-[U-^{14}C]Glc (13 GBq $mmol^{-1}$) came from American Radiolabeled Chemicals (St. Louis, MO).

[1] This study was supported by grant No. 05760065 to T.M. from the Ministry of Education, Science and Culture of Japan. Part of this study was carried out under the auspices of the National Institute for Basic Biology Cooperative Research Program (94–104).

* Corresponding author; fax 81–25–263–1659.

Abbreviations: GS-I, glucan synthase I; IDPase, inosine diphosphatase; LS medium, Linsmaier-Skoog medium; NDPase, nucleoside diphosphatase.

Enzyme and Protein Assays

IDPase activity was detected by measuring phosphate liberated from IDP for 10 min at 37°C in a reaction mixture consisting of 80 mM Tris-maleate-NaOH (pH 7.0), 4 mM $MgCl_2$, and 4 mM IDP. In the case of assay for crude microsomal IDPase, the reaction mixture contained 1 mM sodium molybdate (nonspecific phosphatase inhibitor) and 0.1% Triton X-100. The amount of phosphate was determined by Phosphor C-Test (Wako). Phosphor C-Test reagent (0.5 mL) was added to the reaction mixture (50 μL) and the entire mixture was incubated for 20 min at 37°C. A_{750} was measured after cooling (Mitsui et al., 1990).

The GS-I assay was essentially identical to the method described by Ray (1973). The enzyme reaction was carried out in a mixture consisting of 20 mM Tris-HCl (pH 8.0), 20 mM cellobiose, 0.5 mM $MgCl_2$, 0.3 μM UDP-[U-^{14}C]Glc (740 Bq) at 30°C. The radioactivity of [^{14}C]Glc incorporated into glucan was determined by a liquid scintillation counter (Aloka LSC-1050). Protein contents were measured by Bio-Rad protein assay dye reagent using bovine plasma albumin as a standard.

Preparation of Microsomal Membranes

Rice cells cultured for various periods (see Fig. 1) were gently homogenized in 50 mM glycylglycine-NaOH (pH 7.5), 1 mM EDTA, and 0.5 M mannitol with an ice-chilled mortar and pestle. The homogenate was passed through four layers of gauze and successively centrifuged at 1,000*g* for 10 min, 10,000*g* for 10 min, and 100,000*g* for 1 h. The final resulting pellets were used for microsomal membranes.

Enzyme Purification

All purification steps were performed at 0 to 4°C.

Step 1: Preparation of Crude Enzyme

Golgi membranes were prepared from rice cells cultured for 7 d as described previously (Mitsui et al., 1990; Kimura et al., 1992a). The Golgi membranes were suspended with 10 mM Tris-maleate-NaOH buffer (pH 6.8) containing 0.3 M NaCl and sonicated at 100 W and 28 kHz frequency for 10 min in an ice bath, and then centrifuged at 100,000*g* for 1 h to collect the Golgi membranes. The washing step with NaCl was carried out twice. The NaCl-washed Golgi membranes were suspended with 10 mM Tris-maleate-NaOH buffer (pH 6.8) and centrifuged at 100,000*g* for 1 h. The pelleted Golgi membranes were resuspended with 10 mM Tris-maleate-NaOH buffer (pH 6.8) containing 2% (w/v) Triton X-100, sonicated again, and centrifuged at 100,000*g* for 1 h. The resulting supernatant was used for a crude enzyme.

Step 2: Con A-Sepharose 4B Column Chromatography

The crude enzyme prepared from 1 kg of rice cells was loaded onto a 20-mL column of Con A-Sepharose 4B equilibrated with 50 mM Tris-maleate-NaOH buffer (pH 6.8) containing 50 mM NaCl and 1 mM $CaCl_2$ and eluted with the same buffer. Unbound fractions from the column were collected.

Step 3: DEAE-Toyopearl 650M Column Chromatography

The enzyme obtained from the Con A-Sepharose column was loaded onto an 80-mL column of DEAE-Toyopearl 650M equilibrated with 10 mM Tris-HCl (pH 7.5) and 0.1% Triton X-100. The column was washed first with 500 mL of 10 mM Tris-HCl (pH 7.5) containing 0.1% Triton X-100 and 20% (w/v) glycerol (solution 1), and then eluted with 1 L of a linear concentration gradient of 0 to 0.5 M NaCl in solution 1. The peak fractions of IDPase activity were pooled.

Step 4: EAH-Sepharose 4B Column Chromatography

The above enzyme sample was applied to a 34-mL column of EAH-Sepharose 4B equilibrated with solution 2 containing 10 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1% Triton X-100, and 20% (w/v) glycerol. After the column was washed with solution 2, it was eluted with 200 mL of a linear gradient of 0.1 to 1 M NaCl in solution 2. The active fractions were pooled and concentrated by membrane filtration (Amicon PM30).

Step 5: Cellulofine GCL-2000sf Gel Filtration Column Chromatography

The concentrated enzyme was applied to a Cellulofine GCL-2000sf column (1.5 × 108 cm) equilibrated with solution 2 and eluted at a flow rate of 4 mL h^{-1}. The purified enzyme fractions were pooled, concentrated by Amicon PM-30, and dialyzed against solution 1.

Estimation of native molecular mass of the enzyme was carried out by gel filtration. Ferritin (450 kD), catalase (250 kD), aldolase (158 kD), ovalbumin (45 kD), and chymotrypsinogen A (25 kD) were used as molecular mass standards. The purified enzyme was stable at −80°C for at least 1 month.

Native-PAGE and SDS-PAGE

Native-PAGE was accomplished according to the method described by Shuster (1971). Stacking and separating gel systems were 5% acrylamide (T = 5%, C = 2.6%) in 62.5 mM Tris-HCl (pH 6.8) and 7.5 to 15% acrylamide gradient in 0.375 M Tris-HCl (pH 8.9), respectively. A 5 mM Tris-38.4 mM Gly buffer system (pH 8.3) was used as both upper and lower electrode buffers. For electrophoresis a constant-current power supply delivered 5 mA. To detect the NDPase enzyme band on a gel after electrophoretic separation, the gel was incubated with a reaction mixture consisting of 80 mM Tris-HCl (pH 7.0), 4 mM nucleoside diphosphate, 4 mM $MgCl_2$, and lead nitrate for 5 min at 37°C, then rinsed for 1 h in repeated changes of distilled water. The enzyme band was visualized with 1% (w/v) ammonium sulfite. Protein bands on gels were visualized using a silver-staining kit (Bio-Rad).

SDS-PAGE was performed according to the method of Laemmli (1970). Protein bands on SDS gels were also visualized using silver staining. Bovine albumin (66 kD), egg albumin (45 kD), glyceraldehyde-3-phosphate dehydrogenase (36 kD), carbonic anhydrase (29 kD), trypsinogen (24 kD), trypsin inhibitor (20 kD), and α-lactalbumin (14 kD) were used as molecular mass standards.

IEF

The isoelectric point of protein was determined as described by Yotsushima et al. (1993).

RESULTS

Purification of Golgi Membrane-Bound IDPase

Suspension-cultured cells derived from rice embryo grew rapidly in LS culture medium. Rapid growth of rice cells continued for the first week of culturing, and then cell growth entered a stationary phase (Fig. 1). A high level of membrane-bound IDPase activity was expressed in rice cells for 7 d in culture, but this activity suddenly decreased after cells entered the stationary phase. Rice cells cultured for 7 d were harvested and the Golgi membrane-bound IDPase was purified.

To purify the Golgi membrane-bound IDPase, Golgi membranes were isolated from the microsomal membrane fraction of rice suspension-cultured cells by employing discontinuous glycerol gradient centrifugation as previously reported (Mitsui et al., 1990; Kimura et al., 1992a). Isolated Golgi membranes had little contamination of the other organelle membranes (Mitsui et al., 1990; Kimura et al., 1992a). Latent activity of NDPase is characteristic of the Golgi complex (Gardiner and Chrispeels, 1975; Morré et al., 1977). The latent enzyme is activated by storing isolated membranes at 0 to 4°C for approximately 4 d (Ray et al., 1969; Leonard et al., 1973; Morré et al., 1977; Ali et al., 1985). We also found the latency of rice Golgi membrane-bound IDPase; there was an approximately 2-fold increase in the enzyme activity when the Golgi membranes were stored at 0°C for 3 d (data not shown). This result supports the conclusion that this enzyme is a well-known Golgi IDPase.

Most effective solubilization of IDPase activity from the rice Golgi membranes was accomplished with 2% (w/v) Triton X-100 (data not shown). As shown in Figure 2, glycerol

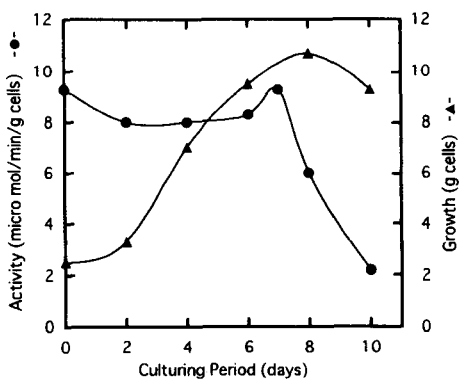

Figure 1. Change in microsomal membrane-bound IDPase activity during growth of rice suspension-cultured cells. Rice cells were cultured in LS medium (Linsmaier and Skoog, 1965) containing 3% (w/v) Suc at 30°C in the dark. Fresh weight of rice cells and IDPase activities in microsomal membrane fractions were determined for the culturing periods as indicated. The microsomal IDPase activity was measured in the presence of 1 mM sodium molybdate and 0.1% Triton X-100.

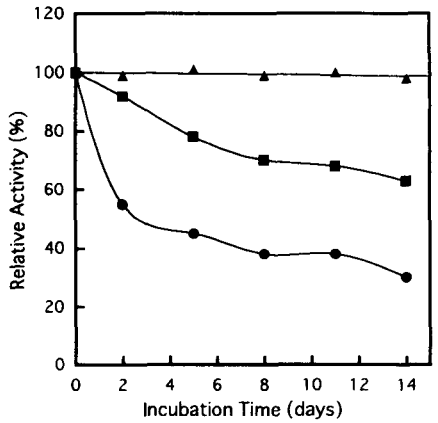

Figure 2. Effect of glycerol on the stability of rice Golgi membrane-bound IDPase. IDPase solubilized by 2% Triton X-100 from rice Golgi membranes was incubated with 0% (●), 10% (■), or 20% (▲) glycerol at 4°C for 14 d, and then the remaining activities of IDPase after incubation were measured. The activity (16 nmol min^{-1} mg^{-1} protein) of freshly prepared enzyme sample was normalized to 100%.

was necessary for stabilizing the solubilized Golgi membrane-bound IDPase. Since the activity of crude Golgi membrane-bound IDPase was kept for 2 weeks at 4°C in the presence of 20% (w/v) glycerol and 0.1% Triton X-100, this condition was used during all of the enzyme purification steps after Con A-Sepharose 4B column chromatography. A summary of the stepwise purification of rice Golgi membrane-bound IDPase using Con A-Sepharose 4B affinity column, DEAE-Toyopearl 650M column and EAH-Sepharose 4B anion-exchange column, and Cellulofine GCL-2000sf gel filtration column chromatographies is given in Table I. A final purification factor of 108 was achieved, and the specific activity of the purified enzyme was 1.73 μmol min^{-1} mg^{-1} protein. To confirm the purity of the Golgi IDPase obtained, the enzyme preparation was subjected to native-PAGE followed by active and silver stainings. A single protein band was observed on the gel and had IDPase activity (Fig. 3A), indicating that the protein band is the Golgi membrane-bound IDPase. When the enzyme preparation was subjected to SDS-PAGE, a single polypeptide band was also detected by silver staining (Fig. 3B). These results show that the rice Golgi membrane-bound IDPase was highly purified.

The apparent molecular mass of the rice Golgi membrane-bound IDPase was estimated to be 200 kD by Cellulofine GCL-2000sf gel filtration column chromatography (Fig. 4A), whereas that of the enzyme subunits was determined to be 55 kD by SDS-PAGE (Fig. 4B). These results reveal that the Golgi membrane-bound IDPase has an oligomeric structure composed of similar or identical molecular mass subunits. The isoelectric point for the enzyme was determined to be 7.5 using an electrofocusing column (data not shown).

The rice Golgi membrane-bound IDPase was a divalent cation-dependent enzyme (Table II). The catalytic activity of enzyme was most strongly activated by Mg^{2+}, and the maximum velocity of the enzyme reaction was observed at an IDP:Mg^{2+} ratio of 1 (data not shown). A stimulating effect of

Table I. *Purification of rice Golgi membrane-bound IDPase*

	Total Activity	Protein	Specific Activity	Purification	NPPase Activity
	μmol min⁻¹	*mg*	*μmol min⁻¹ mg⁻¹*	*-fold*	*μmol min⁻¹*
Crude extract	4.6	312.95	0.016	1	1.40
Con A-Sepharose	14.20	200.86	0.075	4.7	0.15
DEAE-Toyopearl	1.87	4.98	0.38	24	N.D.[a]
EAH-Sepharose	0.55	1.08	0.53	33	N.D.
Cellulofine GCL-2000sf	0.22	0.13	1.73	108	N.D.

[a] N.D., Not detected.

Mn^{2+} was nearly equal to that of Mg^{2+}, but Zn^{2+} showed an inhibitory effect. In addition, the IDPase activity was completely inhibited by 1 mM EDTA (Table II). As shown in Figure 5, the optimum pH was approximately at neutral pH. The enzyme was stable at 0 to 40°C, and most of the enzyme was inactivated by treatment at 50°C for 10 min. The effects of the phosphatase inhibitors ascorbate (1 mM), molybdate (1 mM), pyrophosphate (400 mM), KF (10 or 100 mM), NaN_3 (5 mM), and vanadate (0.1 mM) were also examined. There was no significant inhibitory effect, although 100 mM KF partially inhibited the enzyme activity (data not shown).

UDP and GDP Were Substrates for Golgi IDPase

The substrate specificity of rice Golgi membrane-bound IDPase was examined using several nucleotides and *p*-nitrophenylphosphate. Purified Golgi membrane-bound IDPase used UDP and GDP as substrate similar to IDP, whereas activity with ADP, CDP, and thymidine 5′-disphosphate was 10 to 20% compared to that of IDP (Table III). In addition, the enzyme showed little or no activity for nucleoside triphosphates, nucleoside monophosphates, and *p*-nitrophenylphosphate (Table III). These results clearly show that the rice Golgi membrane-bound IDPase is a NDPase that has a high degree of specificity toward IDP, UDP, and GDP. Values of K_m for IDP, UDP, and GDP were determined by double reciprocal plots to be 0.48, 0.50, and 0.67 mM, respectively (Fig. 6), whereas V_{max} values for IDP, UDP, and GDP were

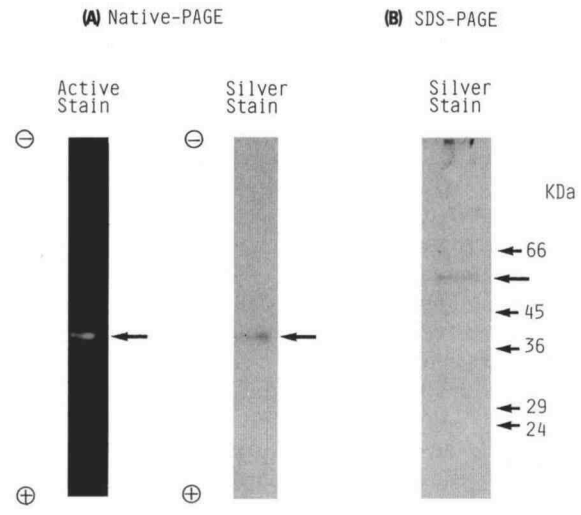

Figure 3. Native-PAGE and SDS-PAGE analyses of the purified rice Golgi membrane-bound IDPase. A, Native-PAGE. After electrophoresis, enzyme bands on gels were visualized by active staining and protein bands were detected by silver staining, as described in "Materials and Methods." B, SDS-PAGE. Polypeptide bands on SDS gels were detected by silver staining.

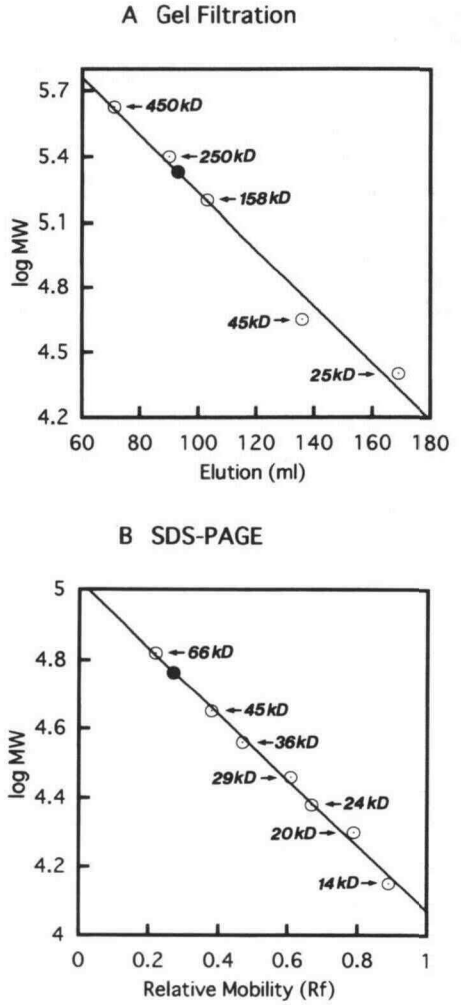

Figure 4. Molecular mass estimations of the rice Golgi membrane-bound IDPase by Cellulofine GCL-2000sf gel filtration column chromatography (A) and SDS-PAGE (B). ○, Molecular mass standards (see "Materials and Methods"); ●, distributing positions of the rice Golgi IDPase.

Table II. *Effects of various divalent cations on the activity of rice Golgi membrane-bound IDPase*

Enzyme assays were done in reaction mixtures consisting of 80 mM Tris-maleate-NaOH (pH 7.0), 4 mM IDP, and divalent cation or EDTA as indicated. The enzyme activity without adding any divalent cation (0.68 μmol min^{-1} mg^{-1} protein) was normalized to 100%.

	Relative Activity
	%
None	100
4 mM Mg^{2+}	256
4 mM Mn^{2+}	248
4 mM Co^{2+}	183
4 mM Ca^{2+}	172
4 mM Zn^{2+}	43
1 mM EDTA	0

1.85, 1.54, and 1.67 μmol min^{-1} mg^{-1} protein, respectively (Fig. 6). In addition, the effects of various nucleotides on the IDPase activity of the enzyme were examined. It was observed that 10 mM of the nucleoside triphosphates ADP, UMP, and GMP show a partial inhibitory effect against the enzyme activity, and that 20 mM ATP inhibits approximately 80% of the enzyme activity (data not shown).

Golgi NDPase Stimulates GS-I Activity

GS-I is known to be localized exclusively in the plant Golgi complex (Ray et al., 1969). GS-I catalyzes the reaction:

$$\text{Primer } \beta\text{-glucan} + \text{UDP-Glc} \rightarrow \beta\text{-1,4-Glucan} + \text{UDP}$$

Since the rice Golgi membrane-bound NDPase can hydrolyze UDP (Table III; Fig. 6), it is possible that this enzyme is involved in glucan synthesis in the Golgi complex. To clarify this possibility, GS-I activity of isolated Golgi membranes was assayed in the presence or absence of purified Golgi NDPase. The Golgi NDPase significantly stimulated the GS-I activity (Fig. 7); therefore, the Golgi NDPase may regulate glucan synthesis in the rice Golgi complex.

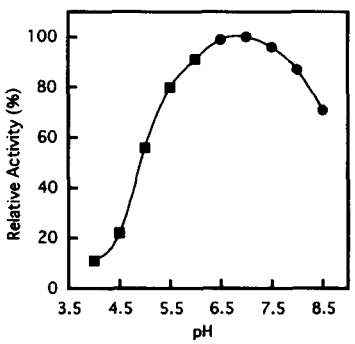

Figure 5. Effect of pH on the activity of the rice Golgi membrane-bound IDPase. The pH dependency of the enzyme was examined using buffered solutions of 80 mM sodium acetate (pH 4.0–6.0, ■) and 80 mM Tris-maleate-NaOH (pH 6.0–8.5, ●). The maximum activity of rice Golgi IDPase (1.73 μmol min^{-1} mg^{-1} protein) was normalized to 100%.

Table III. *Substrate specificity of rice Golgi membrane-bound IDPase*

Enzyme assays were done in reaction mixtures consisting of 80 mM Tris-maleate-NaOH (pH 7.0), 4 mM $MgCl_2$, and 4 mM substrate as indicated. The enzyme activity toward IDP (1.73 μmol min^{-1} mg^{-1} protein) was normalized to 100%.

Substrate	Relative Activity
	%
AMP	0
CMP	0
GMP	0
IMP	0
TMP	0
UMP	0
ADP	12
CDP	12
GDP	92
IDP	100
TDP	19
UDP	83
ATP	8
CTP	6
GTP	9
ITP	18
TTP	8
UTP	10
p-Nitrophenyl phosphate	0

DISCUSSION

Purification and characterization of Golgi membrane-bound enzymes are required for clarification of the function of the Golgi complex. However, there have been few reports concerning enzyme purification from Golgi membranes. We have established a rapid and effective method for isolating rice Golgi membranes using glycerol density gradient centrifugation (Mitsui et al., 1990; Kimura et al., 1992a). Since we are able to obtain sufficient quantities of highly purified rice Golgi membranes, the purification and characterization of rice Golgi membrane-bound IDPase, a well-known Golgi enzyme, was attempted. Rice Golgi membrane-bound IDPase was solubilized by 2% Triton X-100 and purified by a series of chromatographic separations in the presence of 20% glycerol and 0.1% Triton X-100 (Table I). The results obtained by native-PAGE and SDS-PAGE analyses of the final enzyme preparation indicated that the rice Golgi IDPase was highly purified (Fig. 3). The apparent molecular mass of the enzyme was estimated by gel filtration and SDS-PAGE to be 200 and 55 kD, respectively (Fig. 4), indicating that the enzyme has an oligomeric structure. Substrate specificity of the enzyme revealed that the rice Golgi IDPase is a NDPase that has a high degree of specificity toward IDP, UDP, and GDP (Table II). The properties of the rice Golgi membrane-bound NDPase are summarized in Table IV.

A specific NDPase has been partially purified and characterized from the soluble fraction of soybean root nodules (Doremus and Blevins, 1988). The apparent molecular mass estimated by gel filtration and SDS-PAGE suggested that the soybean NDPase exists as a dimer of 50 kD. The K_m and V_{max} with ADP was calculated to be 54 μM and 315 μmol min^{-1}

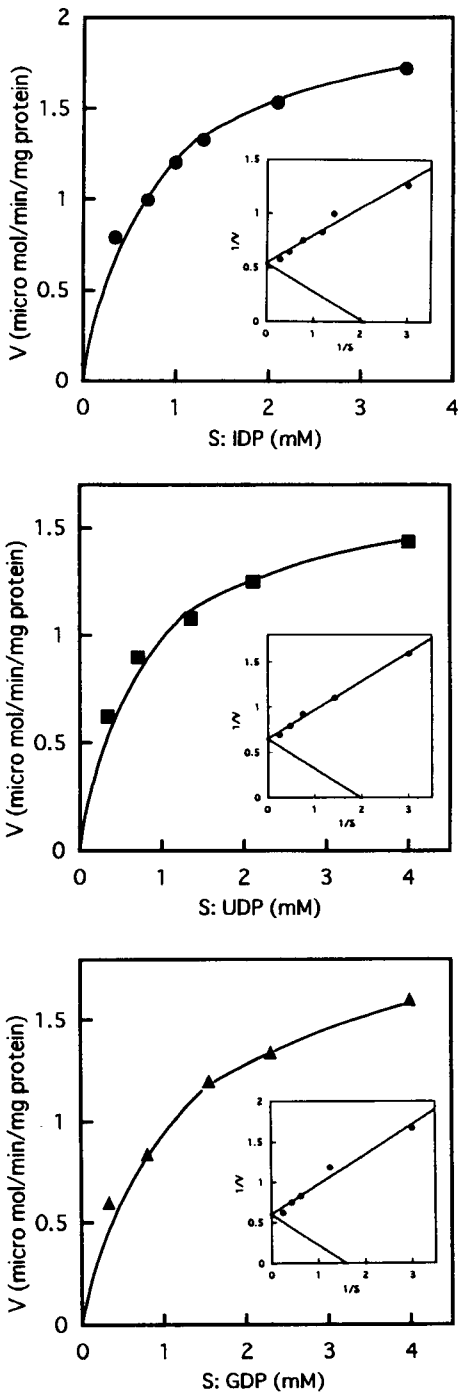

Figure 6. Effect of substrate concentration on the activity of rice Golgi membrane-bound NDPase. Top, middle, and bottom panels represent the dependencies for IDP, UDP, and GDP concentrations, respectively. Insets show double reciprocal plots. The reaction mixture consisted of 80 mM Tris-maleate-NaOH (pH 7.0), 4 mM $MgCl_2$, 0.33 to 4 mM NDP, and 0.1 μg of the enzyme.

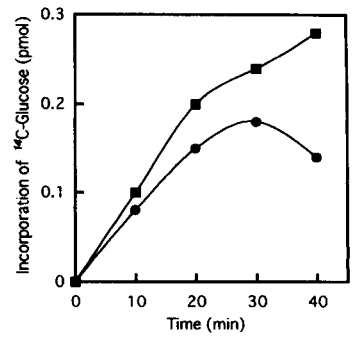

Figure 7. Stimulation of GS-I activity by NDPase in rice Golgi membranes. GS-I activities in isolated rice Golgi membranes (30 μg) were measured in the presence (■) or absence (◐) of purified Golgi membrane-bound NDPase (0.1 μg). Experimental details were described in "Materials and Methods."

mg^{-1}, respectively. Those of IDP were 19 μM and 175 μmol min^{-1} mg^{-1}, respectively. The soybean NDPase was also a divalent cation-dependent enzyme; however, the activation with Ca^{2+} was nearly twice as high as with Mg^{2+}. Judging from chemical and catalytic properties of the rice Golgi membrane-bound and the soybean soluble NDPases, it was concluded that a novel plant NDPase was purified from rice Golgi membranes. We also detected a higher NDPase activity in rice soluble fraction than that of microsomal fractions. The soluble NDPase seems to be a soybean root nodule-type enzyme (data not shown).

Membrane-bound NDPases have been purified from mammalian microsomes (Yamazaki and Hayashi, 1968; Kuriyama, 1972; Pinsley and Scrutton, 1973) and yeast Golgi vesicles (Yanagisawa et al., 1990). Native molecular masses of mammalian microsomal NDPases were estimated to be 100 to 150 kD. The mammalian enzyme activity was found to be activated by ATP and inhibited by KF and PPi (Yamazaki and Hayashi, 1968; Pinsley and Scrutton, 1973), although neither ATP nor phosphatase inhibitors affected the rice Golgi membrane-bound NDPase activity. A GDPase purified from Golgi vesicle-enriched fractions of *Saccharomyces cerevisiae* was highly specific for GDP and required Ca^{2+} for maximum activity. The yeast Golgi GDPase had high-Man-type oligosaccharide chains susceptible to endoglycosidase H, and the apparent molecular mass of the deglycosylated active enzyme was 47 kD (Yanagisawa et al., 1990). It is unclear at present whether or not the rice Golgi NDPase is a glycoprotein;

Table IV. *Properties of rice Golgi membrane-bound NDPase*

Molecular mass	
Gel filtration	200 kD
SDS-PAGE	55 kD
Isoelectric point	7.5
Substrate specificity	IDP ≅ GDP ≅ UDP > ADP, NTP
K_m (IDP)	0.48 mM
V_{max} (IDP)	1.85 μmol min^{-1} mg^{-1}
Divalent cation requirement	Mg^{2+} ≅ Mn^{2+} > Co^{2+} ≅ Ca^{2+}
Optimum pH	7
Thermal stability	0–40°C

however, the rice enzyme did not bind to the Con A-Sepharose column.

The role of Golgi membrane-bound NDPase is reportedly involved in rapid conversion of UDP and/or GDP formed by glycosyltransferases in the Golgi complex to nucleoside monophosphates (Hirschberg and Snider, 1987; Abeijon et al., 1989; Yanagisawa et al., 1990). In plant cells it has been demonstrated that the UDP-Glc-sterol glucosyltransferase isolated in a particulate fraction from etiolated pea seedlings is specific for UDP-Glc as the glucosyl donor and is inhibited by UDP, and that partial recovery from UDP inhibition is effected by preincubation of the enzyme (Staver et al., 1978). The purified rice Golgi membrane-bound NDPase was also found to have high activity toward IDP, GDP, and UDP (Table III; Fig. 6). In addition, the rice enzyme stimulated the enzyme reaction of GS-I, which produces UDP and 1,4-β-glucan from UDP-Glc as substrate (Fig. 7). This suggests that the Golgi membrane-bound NDPase is involved in the regulation of the cell wall β-glucan synthesis, which is a unique event in plant cells.

ACKNOWLEDGMENTS

We wish to thank Drs. M. Nishimura and I. Hara-Nishimura (National Institute for Basic Biology, Okazaki, Japan) for helpful discussions and suggestions. We also thank Dr. G.C. Scott-Woo for critical reading of the manuscript and helpful discussions.

Received February 28, 1994; accepted May 5, 1994.
Copyright Clearance Center: 0032-0889/94/106/0119/07.

LITERATURE CITED

Abeijon C, Orlean P, Robbins PW, Hirschberg CB (1989) Topography of glycosylation in yeast: characterization of GDP-mannose transport and lumenal guanosine diphosphatase activities in Golgi-like vesicles. Proc Natl Acad Sci USA **86:** 6935–6939

Ali MS, Nishimura M, Mitsui T, Akazawa T, Kojima K (1985) Isolation and characterization of Golgi membranes from suspension-cultured cells of sycamore (*Acer pseudoplatanus* L.). Plant Cell Physiol **26:** 1119–1133

Dauwalder M, Whaley WG, Kephart JE (1969) Phosphatases and differentiation of the Golgi apparatus. J Cell Sci **4:** 455–497

Doremus HD, Blevins DG (1988) Purification and characterization of a specific nucleoside diphosphatase from soybean root nodules. Plant Physiol **87:** 41–45

Gardiner M, Chrispeels MJ (1975) Involvement of the Golgi apparatus in the synthesis and secretion of hydroxyproline-rich cell wall glycoproteins. Plant Physiol **55:** 536–541

Goff CW (1973) Localization of nucleoside diphosphatase in the onion root tip. Protoplasma **78:** 397–416

Hirschberg CB, Snider MD (1987) Topography of glycosylation in the rough endoplasmic reticulum and Golgi apparatus. Annu Rev Biochem **56:** 63–87

Kimura S, Matsuda K, Mitsui T, Igaue I (1992a) Preparation of Golgi membrane fractions containing lectin-binding sites from suspension-cultured rice cells. Biosci Biotechnol Biochem **56:** 340–341

Kimura S, Mitsui T, Igaue I (1992b) Localization of binding sites for peanut, *Ulex europaeus*, and castor bean lectin in the rice Golgi complex. Biosci Biotechnol Biochem **56:** 1656–1657

Kimura S, Mitsui T, Matsuoka T, Igaue I (1992c) Purification, characterization and localization of rice UDP-glucose pyrophosphorylase. Plant Physiol Biochem **30:** 683–693

Kimura S, Yamada M, Igaue I, Mitsui T (1993) Structure and function of the Golgi complex in rice cells: characterization of Golgi membrane glycoproteins. Plant Cell Physiol **34:** 855–863

Kuriyama Y (1972) Studies on microsomal nucleoside diphosphatase of rat hepatocytes: its purification, intramembranous localization, and turnover. J Biol Chem **247:** 2979–2988

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685

Leonard RT, Hansen D, Hodges TK (1973) Membrane-bound adenosine triphosphatase activities of oat roots. Plant Physiol **51:** 749–754

Linsmaier EM, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. Plant Physiol **18:** 100–127

Mitsui T, Kimura S, Igaue I (1990) Isolation and characterization of Golgi membranes from suspension-cultured cells of rice (*Oryza sativa* L.). Plant Cell Physiol **31:** 15–25

Mitsui T, Ueki Y, Igaue I (1993) Biosynthesis and secretion of α-amylase by rice suspension-cultured cells: purification and characterization of α-amylase isozyme H. Plant Physiol Biochem **31:** 863–874

Morré DJ, Lembi CA, Vanderwoude WJ (1977) A latent inosine-5′-diphosphatase associated with Golgi apparatus-rich fractions from onion stem. Cytobiologie **16:** 72–81

M'Voula-Tsieri M, Hartmann-Bouillon MA, Benveniste P (1981) Properties of nucleoside diphosphatase associated with Golgi apparatus-rich fraction from maize coleoptiles. I. Study of latency. Plant Sci Lett **20:** 379–386

Nagahashi J, Kane AP (1982) Triton-stimulated nucleoside diphosphatase activity: subcellular localization in corn root homogenates. Protoplasma **112:** 167–173

Novikoff AB, Heus M (1963) A microsomal nucleoside diphosphatase. J Biol Chem **238:** 710–716

Pinsley HC, Scrutton MC (1973) Nucleoside diphosphatase from pig liver: purification and some properties. Arch Biochem Biophys **158:** 331–345

Quail PH (1979) Plant cell fractionation. Annu Rev Plant Physiol **30:** 425–484

Ray PM (1973) Regulation of β-glucan synthetase activity by auxin in pea stem tissue. 1. Kinetic aspects. Plant Physiol **51:** 601–608

Ray PM, Shininger TL, Ray MM (1969) Isolation of β-glucan synthase particles from plant cells and identification with Golgi membranes. Proc Natl Acad Sci USA **64:** 605–612

Shuster L (1971) Preparative acrylamide gel electrophoresis: continuous and disc techniques. Methods Enzymol **22:** 412–433

Staver MJ, Glick K, Baisted DJ (1978) Uridine diphosphatase glucose-sterol glucosyltransferase and nucleoside diphosphatase activities in etiolated pea seedlings. Biochem J **169:** 297–303

Yamazaki M, Hayashi O (1968) Allosteric properties of nucleoside diphosphatase and its identity with thiamine pyrophosphatase. J Biol Chem **243:** 2934–2942

Yanagisawa K, Resnick D, Abeijon C, Robbins PW, Hirschberg CB (1990) A guanosine diphosphatase enriched in Golgi vesicles of *Saccharomyces cerevisiae*. J Biol Chem **265:** 19351–19355

Yotsushima K, Mitsui T, Takaoka T, Hayakawa T, Igaue I (1993) Purification and characterization of membrane-bound inositol phospholipid-specific phospholipase C from suspension-cultured rice (*Oryza sativa* L.) cells. Identification of a regulatory factor. Plant Physiol **102:** 165–172