Plant Physiol. (1978) 62, 766–772

Membrane-associated Glycosyl Transferases in Cotyledons of *Pisum sativum*

DIFFERENTIAL EFFECTS OF MAGNESIUM AND MANGANESE IONS[1]

Received for publication March 6, 1978 and in revised form June 12, 1978

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ABSTRACT

In crude particulate fractions isolated from pea (*Pisum sativum*) cotyledons, the transfer of radioactivity from GDP-[^{14}C]mannose to glycolipid appears to be preferentially stimulated by Mn^{2+} while the transfer to lipid-free residue is enhanced by Mg^{2+}. In contrast, the transfer of radioactivity from UDP-N-acetyl-[^{14}C]glucosamine to glycolipid shows preferential stimulation by Mg^{2+} while the transfer to lipid-free residue prefers Mn^{2+}. These results are accounted for by the differential stimulation by Mg^{2+} and Mn^{2+} of glycosyl transferases associated with subcellular membranes which were separated by isopycnic sucrose density centrifugation.

Particulate fractions isolated from developing pea cotyledons can transfer labeled sugar from nucleoside diphosphate sugars to lipid components and to components which remain in the residue following lipid extraction (2, 17). Earlier reports have indicated a preferential stimulatory effect by Mg^{2+} and Mn^{2+} on the transfer of radioactivity from GDP-[^{14}C]Man[2] to lipid intermediates and glycoproteins (4, 12, 13, 15, 18, 21). In contrast, very little information is available on the divalent cation effect on UDP-[^{14}C]-GlcNAc transferase activities.

Recent evidence has shown that GDP-Man transferases are associated with ER membranes isolated from cells of plants (17), animals (6), and yeast (11). Furthermore, a UDP-GlcNAc transferase was localized in RER membranes isolated from pea cotyledons (17). These investigations were performed without considering a preferential stimulation of various glycosyl transferases by different divalent cations.

This report describes the differential influence of Mg^{2+} and Mn^{2+} on UDP-GlcNAc and GDP-Man transferase activities and demonstrates that glycosyl transferases associated with cellular membranes, separated by sucrose density gradient centrifugation, show preferential responses to the divalent cations.

MATERIALS AND METHODS

Plant Material. Pea seeds (*Pisum sativum* cv. Burpeeana) were germinated and grown as described by Basha and Beevers (1). Pea cotyledons 21 days postanthesis were used for all experiments.

Preparation of Crude Particulate Fraction. Seeds were harvested and the testa and embryos were removed. The crude particulate fraction was prepared as described by Beevers and Mense (2). Optimal Mg^{2+} and Mn^{2+} requirements for transferase activity in this fraction were determined by incubating 150 μl of the pellet suspension with 100 μl of 50 mM Tris-HCl (pH 7.0) containing 10 mM KCl, 5 mM 2-mercaptoethanol, 2 μl of ^{14}C-labeled nucleoside diphosphate sugar, and 48 μl of H_2O or 48 μl of a $MgCl_2$ or $MnCl_2$ solution. The specific activities of the labeled nucleoside diphosphate sugars are given below. Incubation was at 37 C for 60 min with the reaction terminated by the addition of 0.7 ml of H_2O and 2.5 ml of chloroform-methanol (2:1, v/v). Further lipid extraction and analysis of the lipid-free residue were described previously (2).

Sucrose Gradients. Membrane fractions used in linear sucrose density gradients were prepared by sedimenting particulate material from 250 to 13,000*g* and 13,000 to 40,000*g* (17). The crude pellets were either washed in GM (0.5 M sucrose + 5 mM 2-mercaptoethanol + 30 mM Tris-MES [pH 7.5]) or GM + 5 mM EDTA and pelleted at the initial force (17). Crude pellets were overlaid on identical gradients (15–60% sucrose, w/w) and centrifuged to equilibrium at 81,000*g* for 15 hr. Preparation of sucrose gradients and fractionation were described elsewhere (17).

Enzyme Assays for Isolated Membrane Fractions. Inosine diphosphatase (IDPase) activity of pH 7.5 was determined as described by Leonard and VanDerWoude (14). NADH-Cyt *c* reductase activity was assayed spectrophotometrically by following the reduction of Cyt *c* at 550 nm (10).

Glycosyl transferase activities were determined either by measuring incorporation of label from nucleoside diphosphate sugar into trichloroacetic acid-insoluble material or by assaying incorporation into lipid intermediates and lipid-free residues using an extraction procedure described earlier (2). Although we use the singular form of GDP-Man transferase and UDP-GlcNAc transferase when we speak of trichloroacetic acid-precipitable material, it should be kept in mind that these precipitates represent an aggregate of glycosyl transferase activities. UDP-N-acetyl-[U-^{14}C]glucosamine (300 mCi/mmol, 10 μCi/400 μl) and GDP-[U-^{14}C]mannose (179 mCi/mmol, 50 μCi/2 ml) were used as substrates to measure activities of UDP-GlcNAc and GDP-Man transferases. Membrane fractions (0.3 ml) were incubated with 0.1 ml of substrate mix plus 1 μl of radioactive nucleoside diphosphate sugar as described by Nagahashi and Beevers (17).

When lipid extractions were performed on isolated membrane fractions, the fractions used were combined around three distinct areas of a sucrose gradient. One area had an average density of 1.123 g/cc (28–30% sucrose, w/w) and contained IDPase activity which may be associated with Golgi apparatus membranes. A second area, at an average density of 1.165 g/cc (36–38% sucrose, w/w), contained RER which was identified morphologically and by antimycin A-insensitive NADH-Cyt *c* reductase activity (17).

[1] Research was supported by National Science Foundation Grants BMS575-05722 PGM76-05722 A01 and by funds provided by the University of Oklahoma Faculty Research Council.

[2] Abbreviations: Man: mannose; GlcNAc: N-acetylglucosamine; GM: grinding mix.

The third area, with a density of 1.201 g/cc (44–46% sucrose, w/w), contained unidentified membranes with an associated Mg^{2+}-requiring GDP-Man transferase (17). Lipid-free residues were either counted directly or treated with protease and then counted.

Protease Digestion. The incubation mixture contained 0.2 M NaCl in 0.1 M K^+-phosphate buffer (pH 7.5) plus 0.75 mg of protease (type VI) and an aliquot of lipid-extracted membrane residue. The digestion mixture was overlaid with toluene to prevent bacterial growth. Incubation was at 38 C and after 40 hr, 1 mg of protease was added and digestion was continued for an additional 40 hr. Samples were filtered and counts retained on Whatman GF/A glass fiber filters after digestion were compared to controls incubated under identical conditions without protease. To obtain enough labeled lipid-free residue, the glycosyl transferase assays were scaled up and 3 μl of labeled GDP-Man were used/0.3 ml of membrane preparation.

RESULTS

Crude Particulate Fraction. Both Mg^{2+} and Mn^{2+} stimulated the transfer of radioactivity from GDP-[^{14}C]Man into the $CHCl_3$-CH_3OH (2:1)-soluble lipid fraction (Fig. 1). Low concentrations of Mn^{2+} were more effective than similar concentrations of Mg^{2+}. Transfer of label to the oligosaccharide lipid ($CHCl_3$-CH_3OH-H_2O, 1:1:0.3) was not affected by either divalent cation and this result is consistent with previous reports (5, 9).

Transfer of label from GDP-[^{14}C]Man to the lipid-extracted residue was stimulated by concentrations of $MgCl_2$ up to 16 mM. Above 16 mM Mg^{2+}, no further stimulation was observed. In contrast, Mn^{2+} only produced a slight stimulation at very low concentrations. These results agree with the report of Lehle *et al.* (12) which showed that the transfer of label from GDP-Man to a lipid-extracted polymer preferred Mg^{2+} over Mn^{2+}.

Mn^{2+} was more effective than Mg^{2+} in stimulating the transfer of label from UDP-[^{14}C]GlcNAc into the $CHCl_3$-CH_3OH-H_2O (1:1:0.3)-soluble lipid and lipid-extracted residue (Fig. 2). At concentrations below 8 mM, Mn^{2+} was the preferred divalent cation for the transfer to $CHCl_3$-CH_3OH (2:1)-soluble lipid but at higher concentrations, Mg^{2+} appeared to give a slightly better enhancement of transferase activity. Although the transfer of label to lipid-extracted residue steadily rose in the presence of increasing concentrations of Mg^{2+}, Mn^{2+} was more effective in stimulating transfer to the residue over the same concentration range.

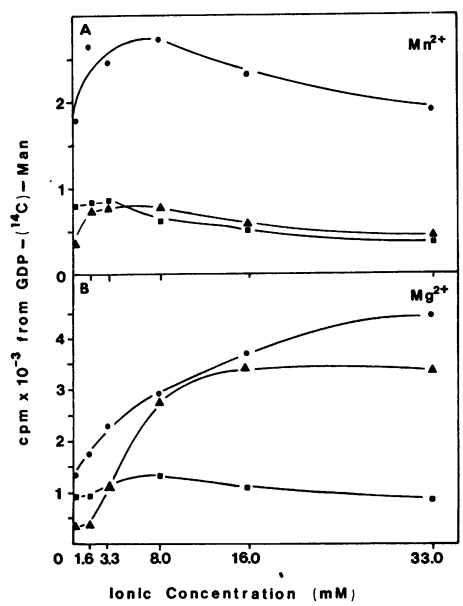

FIG. 1. Divalent cation effect on crude particulate GDP-Man transferase activities isolated from pea cotyledons. A: GDP-Man transferase activities were assayed in the presence of Mn^{2+}. Crude particulate fraction was extracted with $CHCl_3$-CH_3OH (2:1) and $CHCl_3$-CH_3OH-H_2O (1:1:0.3). Radioactivity was determined in both lipid fractions as well as in the lipid-free residue. (●——●): 2:1; (■——■): 1:1:0.3; (▲——▲): residue. B: same as A except Mg^{2+} was used.

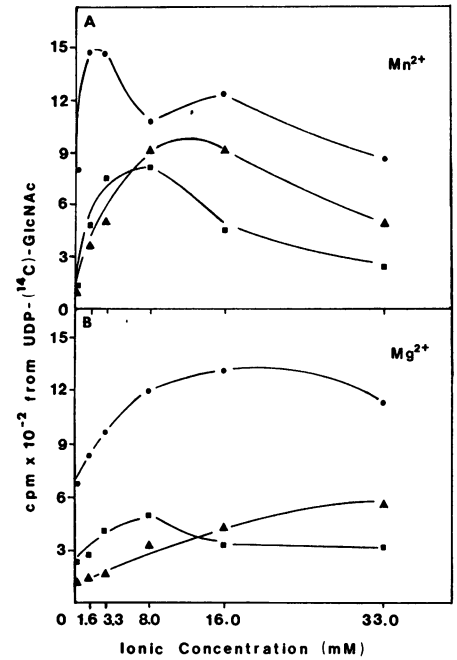

FIG. 2. Divalent cation effect on crude particulate UDP-GlcNAc transferase activities isolated from pea cotyledons. A: UDP-GlcNAc transferase activities were assayed in the presence of Mn^{2+}. Crude particulate fraction was extracted with $CHCl_3$-CH_3OH (2:1) and $CHCl_3$-CH_3OH-H_2O (1:1:0.3). Radioactivity was determined in both lipid fractions as well as in the lipid-free residue. (●——●); 2:1; (■——■): 1:1:0.3; (▲——▲); residue. B: same as A except Mg^{2+} replaced Mn^{2+}.

Linear Sucrose Density Gradient Analysis of 13,000 to 40,000*g* Pellet. After isopycnic centrifugation of the 13,000 to 40,000*g* fraction in a linear sucrose gradient, the major peak of NADH-Cyt *c* reductase activity (RER marker) corresponded to maximum absorbance at 280 nm at a density of 1.145 g/cc (Figs. 3A, 4A, and 5A). This was less than the density reported previously for RER (17). When 5 mM $MgSO_4$ was added to the grinding medium and the pellet was washed in the same solution prior to sucrose density centrifugation, the peak density of the RER marker and absorbance trace was 1.165 g/cc (Fig. 3B), identical to our previous report (17). The lighter density of the RER in Figures 3A, 4A, and 5A was apparently due to a lower level of endogenous Mg^{2+} in the pea cotyledons when these experiments were performed. This result emphasizes the need to assay for marker enzyme activities instead of taking reported densities to indicate the location of subcellular membranes and organelles in sucrose density gradients.

When the distribution of UDP-GlcNAc transferase in sucrose gradients was determined with Mg^{2+} in the assay, activity was located at a density (1.145 g/cc) coincident with the maximum absorbance at 280 nm and NADH-Cyt *c* reductase activity (Fig. 4A). Association of UDP-GlcNAc transferase activity with the RER confirmed our earlier report (17). When UDP-GlcNAc transferase was assayed in the presence of Mn^{2+}, two differences were observed. The activity of the transferase at 1.145 g/cc was greater in magnitude compared to the Mg^{2+}-stimulated activity at the same peak (Fig. 4A) and a second area of activity at 1.123 g/cc, coincident with IDPase activity, was also observed (Fig. 4A).

When the 13,000 to 40,000*g* pellet was washed in 5 mM EDTA prior to sucrose density centrifugation, the RER was stripped of

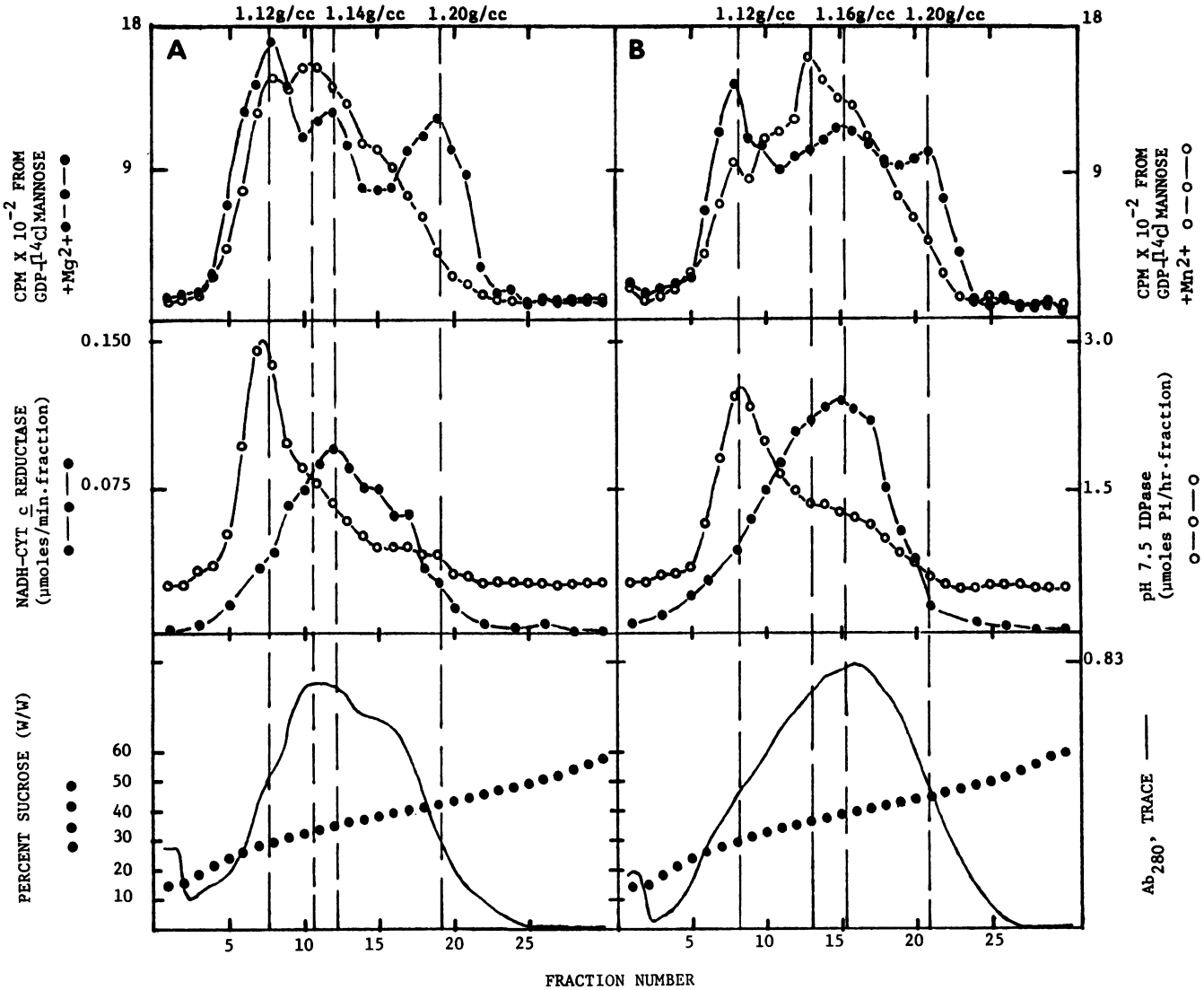

FIG. 3. Sucrose density gradient analyses of RER-associated NADH-Cyt *c* reductase activity isolated from pea cotyledons and sedimenting between 13,000 and 40,000*g*. A: a 2-ml sample of a normal washed and suspended pellet was layered over a 36-ml sucrose gradient (15–60% sucrose, w/w). Centrifugation was for 15 hr at 82,500*g*. Fraction size was 1.2 ml. GDP-Man transferase was assayed in the presence of 10 mM $MgCl_2$ or 10 mM $MnCl_2$. B: grinding mix contained 5 mM $MgCl_2$. The crude 13,000 to 40,000*g* pellet was washed in the same grinding mix and pelleted at the initial force. All further treatments were identical to A.

ribosomes, and the denuded membrane was located at a lighter density (1.102 g/cc) in the gradient (17). The denuded ER had an associated UDP-GlcNAc transferase activity detected in the presence of Mg^{2+} or Mn^{2+}. Mn^{2+} was the more effective divalent cation, and this was consistent with the results of the "normal washed" control (Fig. 4A). The IDPase activity and coincident Mn^{2+}-stimulated UDP-GlcNAc transferase at a density of 1.123 g/cc did not shift after EDTA treatment (Fig. 4, A and B).

When the distribution of Mg^{2+}-stimulated GDP-Man transferases was determined in a sucrose gradient, three peaks of activity (17) were observed (Fig. 5A). When the GDP-Man transferase was assayed in the presence of Mn^{2+}, only two major areas of activity were detected (Fig. 5A). One peak coincided with IDPase activity (1.123 g/cc), although the transferase activity at this density was greater with Mg^{2+} than Mn^{2+} (Fig. 5A). A second area of Mn^{2+}-stimulated GDP-Man transferase activity was observed in the region of the RER marker; however, the peak activities of Mg^{2+}-stimulated activity and Mn^{2+}-stimulated activity were not coincident (Figs. 3A and 5A).

After EDTA treatment, the Mg^{2+}- and Mn^{2+}-stimulated GDP-Man transferases found in the center of the gradient were shifted to a lighter density of 1.102 g/cc, coincident with the relocated RER marker (Fig. 5B). The locations of GDP-Man transferases at 1.123 g/cc and 1.201 g/cc were not changed by EDTA treatment, and the preference for Mg^{2+} by these transferases was apparent in both the control (Fig. 5A) and the EDTA-treated preparation (Fig. 5B). The membranes at a density of 1.201 g/cc contained a Mg^{2+}-stimulated GDP-Man transferase; however, these membranes have not been identified.

After EDTA treatment, no peaks of Mg^{2+} (17) or Mn^{2+}-stimulated glycosyl transferase activity remained in the center of the gradient (Figs. 4B and 5B). The density of a marker for plasma membrane, K^+-stimulated ATPase activity at pH 6.5, was not shifted by EDTA treatment (17) and these results indicated that GDP-Man and UDP-GlcNAc transferase activities, assayed under our conditions, were not associated with plasma membrane.

Linear Sucrose Gradient Analysis of 250 to 13,000*g* Pellet. Glycoprotein glycosyltransferases have been reported in mitochondria isolated from animal cells. These transferases, located on the inner mitochondrial membrane, were stimulated by Mn^{2+} (3). In our previous study, no Mg^{2+}-stimulated GDP-Man or UDP-GlcNAc transferase activities were associated with mitochondria

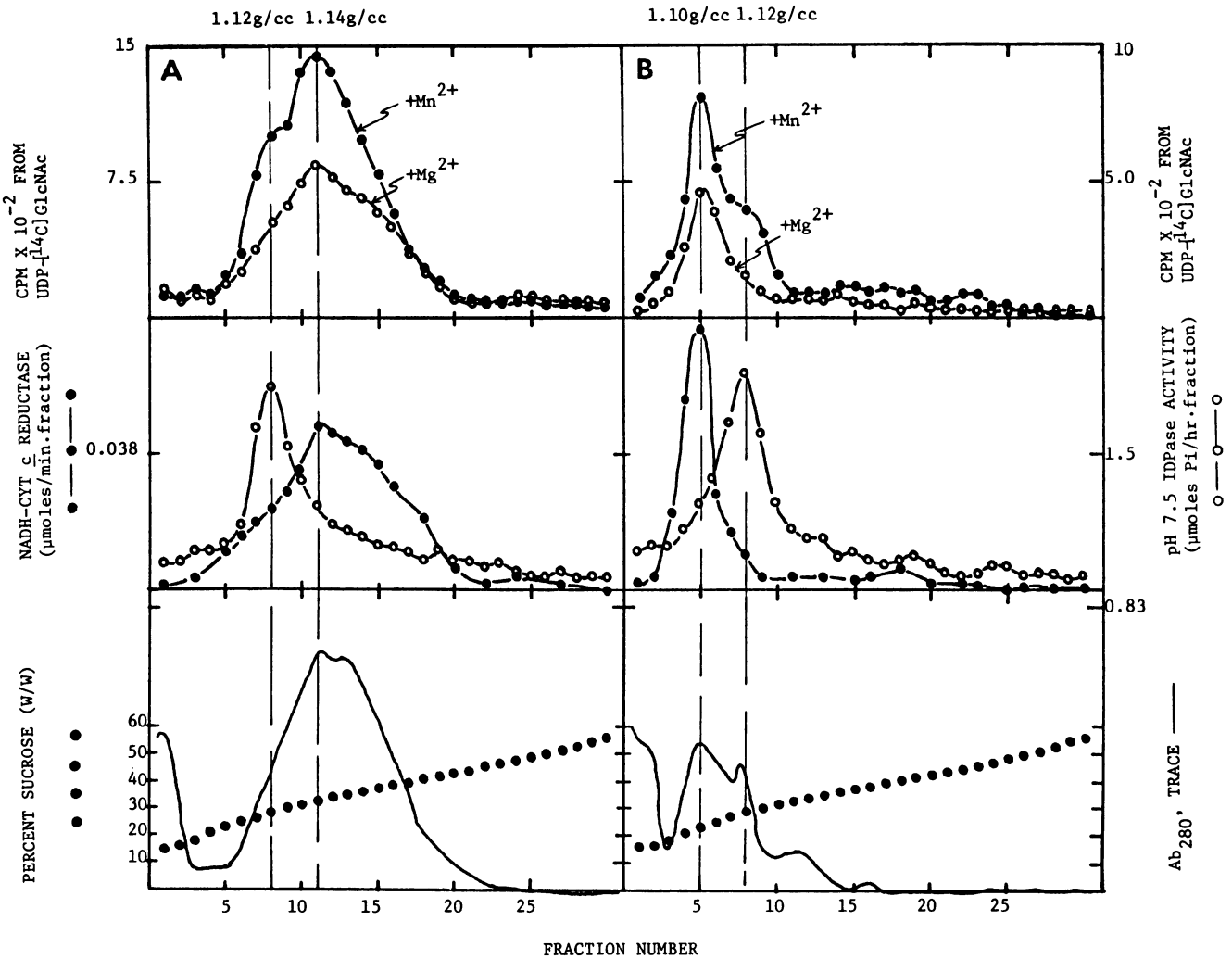

FIG. 4. Sucrose density gradient analyses of membrane-associated UDP-GlcNAc transferase activities isolated from pea cotyledons and sedimented between 13,000 and 40,000*g*. A: preparation of gradients was identical to Figure 3A. UDP-GlcNAc transferase activity was assayed in the presence of 10 mM divalent cation. B: isolated crude pellet was washed in 5 mM EDTA and pelleted at the initial force. All further manipulations were as described in Figure 3A. Transferase assays were performed in the presence of 10 mM divalent cation.

(17). When these transferases were assayed in the presence of Mn^{2+}, no peaks of activity were associated with mitochondria (Fig. 6). With Mg^{2+} or Mn^{2+}, Triton X-100 (0.1–1.0%) failed to stimulate transferase activity. Mitochondria were identified morphologically (17) and enzymically by Cyt *c* oxidase activity at a density of 1.185 g/cc (Fig. 6).

No peaks of UDP-GlcNAc or GDP-Man transferase activities were associated with protein bodies when assayed in the presence of Mg^{2+} (17) or Mn^{2+} (Fig. 6). Protein bodies had a density of 1.25 g/cc and were identified morphologically (17). The lack of transferase activity indicates that the storage proteins are glycosylated before secretion into protein bodies.

Mn^{2+}-stimulated GDP-Man transferase activities were observed at 1.123 g/cc and 1.145 g/cc. A peak of Mn^{2+}-stimulated UDP-GlcNAc transferase was also present at a density of 1.123 g/cc. The subcellular localization of these transferases agrees with results shown in Figures 4 and 5 and was consistent with previous results indicating the 250 to 13,000*g* pellet was contaminated by RER membranes and by IDPase-associated membranes (17).

Characterization of Glycosyl Transferase Products. Measuring Mg^{2+}- or Mn^{2+}-stimulated glycosyl transferase activities with trichloroacetic acid precipitation did not characterize the products of transferase reactions. To characterize products, pooled membrane fractions with average densities of 1.12 g/cc, 1.16 g/cc and 1.20 g/cc were collected from duplicate sucrose gradients centrifuged with a 13,000 to 40,000*g* pelleted overlay (Fig. 7). For the following experiments (Fig. 7, Table I and II) the density of RER was 1.165 g/cc in agreement with our earlier report (17).

Membranes with associated IDPase activity (1.12 g/cc) contained a GDP-Man transferase that was preferentially stimulated by Mg^{2+} as shown by the trichloroacetic acid-precipitable counts (Fig. 5 and Table I). However, more radioactivity was transferred to $CHCl_3$-CH_3OH (2:1)- and $CHCl_3$-CH_3OH-H_2O (1:1:0.3)-soluble components in the presence of Mn^{2+}, while the transfer to the lipid-free residue was greater with Mg^{2+} (Table I).

RER (1.16 g/cc)-associated GDP-Man transferase also transferred more radioactivity to components soluble in $CHCl_3$-CH_3OH (2:1) in the presence of Mn^{2+} compared to Mg^{2+} (Table I). RER membranes also had the most extensively labeled lipid oligosaccharide ($CHCl_3$-CH_3OH-H_2O, 1:1:0.3). Transfer to lipid-free residue was greater with Mg^{2+}.

The unidentified membrane fraction (1.20 g/cc) contained a GDP-Man transferase which was preferentially stimulated by MG^{2+} (Figs. 3 and 5 and Table I). Only low levels of radioactivity were associated with material soluble in lipid solvents and lipid-free residue was most extensively labeled.

The greatest transfer of radioactivity from UDP-[^{14}C]GlcNAc occurred in RER membranes. In the presence of Mg^{2+}, the $CHCl_3$-CH_3OH (2:1)-soluble component became extensively labeled; but there was only a low transfer of radioactivity to the lipid-free

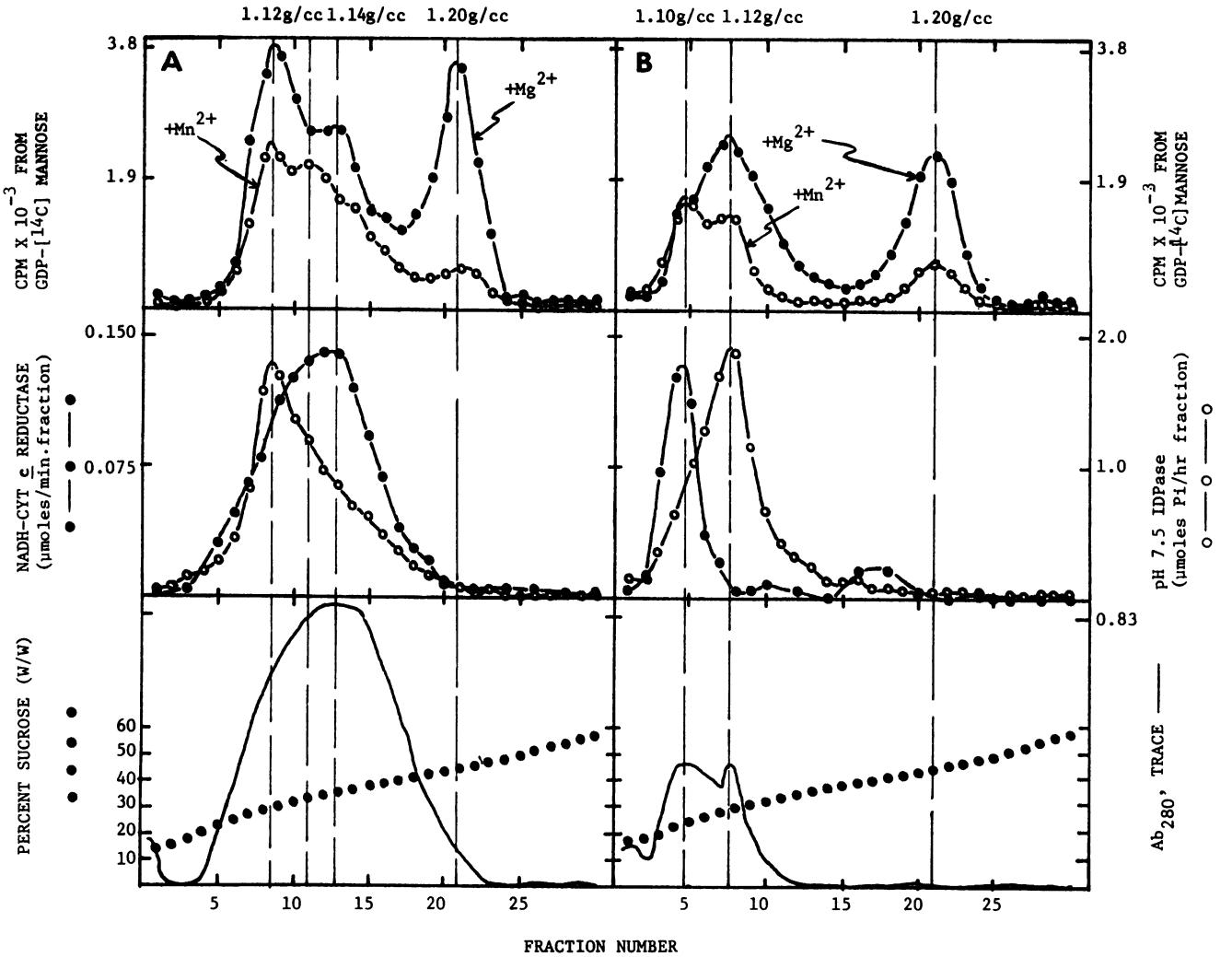

FIG. 5. Sucrose density gradient analyses of membrane-associated GDP-Man transferase activities isolated from pea cotyledons and sedimented between 13,000 and 40,000g. A: preparation of gradient was identical to Figure 3A. GDP-Man transferase was assayed in the presence of 10 mM divalent cation. B: preparation was the same as in Figure 4B. Transferase assays were performed in the presence of 10 mM divalent cation.

residue (Table I). More radioactivity was transferred to the lipid-free residue in the presence of Mn^{2+}. The membrane fraction, collected at a density of 1.12 g/cc, incorporated label from UDP-[^{14}C]GlcNAc into lipid oligosaccharide and lipid-free residue (Table I) which was consistent with the presence of a Mn^{2+}-stimulated UDP-GlcNAc transferase associated with these membranes (Fig. 6).

Isolated membrane fractions were incubated with GDP-[^{14}C]-Man in the presence of Mg^{2+} or Mn^{2+}, lipid-extracted, and the lipid-free residues were treated with protease. Only a low percentage of counts was released by protease treatment of the labeled lipid-free residue isolated from RER membranes (Table II). Radioactivity in lipid-free residues, obtained from membranes with densities of 1.12 g/cc and 1.20 g/cc, was not released by protease digestion (Table II) or SDS solubilization (17). These results were consistent with the low percentage of counts released by protease digestion and SDS treatment of a lipid-free residue obtained from a crude particulate fraction isolated from cotton fibers (8).

When isolated membranes were incubated with UDP-[^{14}C]-GlcNAc plus Mg^{2+} or Mn^{2+}, little radioactivity was solubilized by protease digestion of lipid-free residues prepared from the unidentified membranes (1.20 g/cc). When membranes at 1.12 g/cc were assayed in the presence of Mg^{2+}, the lipid-free residue only released 25% of the counts upon protease treatment while 45% of the counts were released after the transfer to the residue was performed with Mn^{2+}. The lipid-free residue, produced when RER membranes were incubated with labeled UDP-GlcNAc in the presence of Mg^{2+}, released 53% of the counts after protease treatment; and when the transferase assay contained Mn^{2+}, 74% of the radioactivity was released by protease digestion.

CONCLUSIONS AND GENERAL DISCUSSION

The most striking results of the studies with crude particulate fractions are that the transfer of label from GDP-[^{14}C]Man to lipid-extracted residue is preferentially stimulated by Mg^{2+} over Mn^{2+} while the reverse preference is observed for transfer of UDP-[^{14}C]GlcNAc to residue. The differential response to the cations can be related to differential effects of Mg^{2+} and Mn^{2+} on transferases associated with subcellular membranes.

UDP-GlcNAc transferase activity associated with the RER is involved in glycolipid and glycoprotein formation. Although these membrane-associated transferases are functional in the presence of both Mg^{2+} and Mn^{2+}, it appears that the formation of lipid-linked GlcNAc is preferentially stimulated by Mg^{2+} while transfer of label into glycoprotein is favored by Mn^{2+} (Tables I and II). Alternatively, both Mg^{2+} and Mn^{2+} may stimulate transfer of label to the $CHCl_3$-CH_3OH (2:1)- and $CHCl_3$-CH_3OH-H_2O (1:1:0.3)-soluble components but the transfer of label to lipid-extracted residue prefers Mn^{2+}. Thus, the GlcNAc "lipid intermediates"

may be turning over rapidly in the presence of Mn^{2+} and hence the level of associated radioactivity is not as high as in the presence of Mg^{2+} (Table I). This is consistent with the higher level of counts transferred to residue in the presence of Mn^{2+} (Tables I and II) and higher percentage of protease-releasable counts in the presence of Mn^{2+} (Table II). The specific requirements for the various glycosyl transferase reactions will not be resolved until each enzyme is purified and characterized.

The UDP-GlcNAc transferase activity associated with membranes at a density of 1.12 g/cc is stimulated by Mn^{2+} (Fig. 4). The transfer to lipid-free residue prefers Mn^{2+} (Table I) and radioactivity is released from the residue by protease digestion (Table II). The role of the UDP-GlcNAc transferase in membranes with associated IDPase activity (1.12 g/cc) is unclear, but may be involved in attaching glycosyl residues to the core oligosaccharide of the glycoprotein which is initially synthesized and linked to the peptide in the RER (16, 19).

The preference for Mg^{2+} in the transfer of radioactivity from GDP-[^{14}C]Man in a crude preparation to the lipid-free residue can be related to the activity of transferases associated with membranes at a density of 1.12 g/cc, 1.165 g/cc, and 1.20 g/cc. All three membrane fractions show a reduction in the transfer of label to lipid-free residue when Mn^{2+} replaces Mg^{2+} in the assay, and the largest reduction is observed in membranes of unknown origin

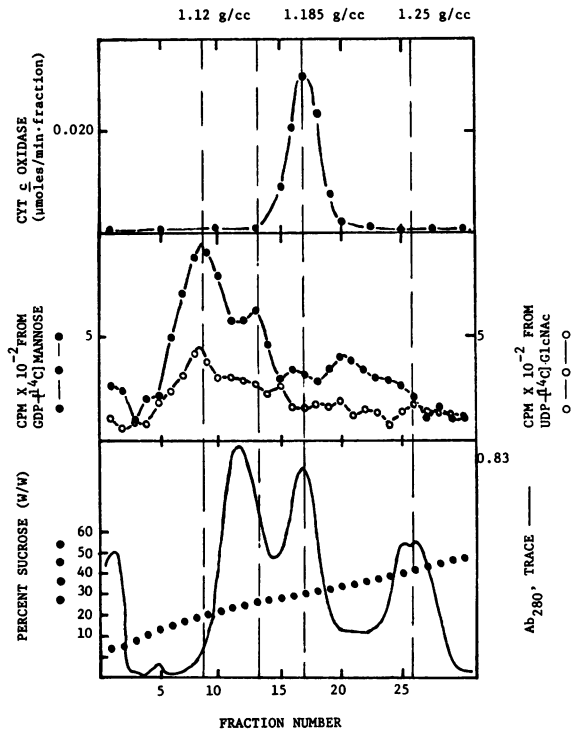

FIG. 6. Sucrose density gradient analyses of membrane-associated glycosyl transferase activities isolated from pea cotyledons and sedimented between 250 and 13,000g. Crude pellet was washed in normal grinding mix and pelleted at the initial force. Gradient manipulations were identical to Figure 3A. Transferases were assayed in the presence of 10 mM $MnCl_2$.

Table I. Influence of Mg^{2+} or Mn^{2+} on glycosyl transferase activities of various membrane fractions from pea cotyledons. Membrane fractions were collected after linear sucrose gradient centrifugation of the 13,000 to 40,000 g pellet. Aliquots (0.3 ml) from isolated fractions were incubated with UDP-[^{14}C] GlcNAc or GDP-[^{14}C] Man in the presence of 10 mM, $MgCl_2$ or $MnCl_2$ and were either precipitated with trichloroacetic acid or extracted with lipid solvents. Figures in parentheses represent range between duplicate samples.

DENSITY	$CHCl_3$-CH_3OH (2:1)	$CHCl_3$-CH_3OH-HOH (1:1:0.3)	RESIDUE	TCA PPT
CPM from GDP-[^{14}C] MANNOSE				
		+Mg^{2+}		
1.12g/cc	839(± 34)	185(± 10)	401(± 39)	2918(± 50)
1.16g/cc	1686(± 130)	574(± 19)	322(± 65)	1830(± 15)
1.20g/cc	181(± 18)	75(± 2)	1475(± 75)	1228(± 10)
		+Mn^{2+}		
1.12g/cc	1046(± 12)	448(± 12)	311(± 28)	2462(± 22)
1.16g/cc	2209(± 95)	643(± 58)	170(± 12)	3226(± 120)
1.20g/cc	232(± 30)	87(± 1)	167(± 10)	690(± 7)
CPM from UDP-[^{14}C] N-Acetylglucosamine				
		+Mg^{2+}		
1.12g/cc	308(± 22)	121(± 5)	65(± 4)	302(± 11)
1.16g/cc	1053(± 25)	367(± 10)	43(± 10)	910(± 13)
1.20g/cc	196(± 34)	87(± 10)	7(± 3)	262(± 7)
		+Mn^{2+}		
1.12g/cc	93(± 10)	217(± 2)	164(± 9)	804(± 8)
1.16g/cc	656(± 16)	434(± 28)	153(± 3)	1470(± 20)
1.20g/cc	94(± 0)	52(± 4)	60(± 13)	250(± 10)

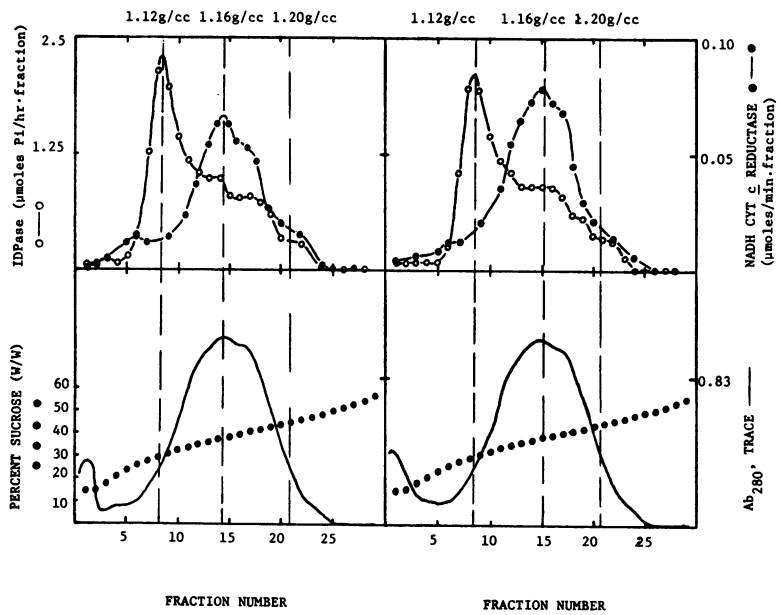

FIG. 7. Sucrose density gradient analyses of a 13,000 to 14,000g pellet isolated from pea cotyledons. Two identical gradients show location of the three membrane fractions (1.12 g/cc, 1.16 g/cc, and 1.20 g/cc) used for the analyses in Tables I and II.

Table II. Protease digestion of labeled lipid-free residue. Subcellular membrane fractions from pea cotyledons were collected from a linear sucrose gradient centrifuged with a 13,000 to 40,000 g pellet. Aliquots from isolated fractions were incubated with UDP-[^{14}C] GlcNAc or GDP-[^{14}C] Man in the presence of 10 mM $MgCl_2$ or $MnCl_2$, lipid-extracted, and lipid-free residues were treated with protease. Figures in parentheses represent range between duplicate samples.

	CPM from GDP-[^{14}C] MANNOSE					
	+Mg^{2+}			+Mn^{2+}		
DENSITY	CONTROL	+PROTEASE	% RETAINED ON FILTER	CONTROL	+PROTEASE	% RETAINED ON FILTER
1.12g/cc	8079(± 150)	8110(± 133)	100	435(± 24)	414(± 3)	95
1.16g/cc	3076(± 58)	2678(± 73)	87	212(± 12)	180(± 2)	85
1.20g/cc	1530(± 68)	1613(± 30)	100	210(± 5)	195(± 5)	93
	CPM from UDP-[^{14}C] N-ACETYLGLUCOSAMINE					
	+Mg^{2+}			+Mn^{2+}		
1.12g/cc	138(± 4)	104(± 2)	75	168(± 5)	92(± 2)	55
1.16g/cc	270(± 14)	126(± 0)	47	420(± 7)	108(± 8)	26
1.20g/cc	98(± 4)	90(± 5)	92	94(± 2)	92(± 4)	98

(1.20 g/cc). The GDP-Man transferases associated with membranes at a density of 1.12 g/cc and 1.20 g/cc produce lipid-free residues which are resistant to protease digestion. The labeled products in the lipid-free residues could represent polysaccharide complexes (7) or polymannans which have been reported to be synthesized in sycamore cells (20).

The Mg^{2+}-stimulated GDP-Man transferase complex and the Mn^{2+}-stimulated GDP-Man transferase complex in the center of the sucrose gradient (Figs. 3 and 5A) have different peaks of activity. Upon EDTA treatment, both peaks of transferase activities are shifted to a lighter density (1.10 g/cc) concomitant with the RER marker (Fig. 5B). This indicates that the two groups of GDP-Man transferases are most likely localized on different regions of RER membrane. The physiological function of the two transferase complexes is not clear and their close proximity in a sucrose gradient has so far prevented further characterization.

The results of this investigation support the use of isolated membrane fractions in experiments designed to study glycoprotein biosynthesis from UDP-GlcNAc and GDP-Man in plants. If crude particulate preparations are used, it would be beneficial to perform transferase reactions in the presence of Mn^{2+} rather than Mg^{2+}. This precaution will restrict the incorporation of radioactivity from GDP-[^{14}C]Man into nonproteinaceous components of the lipid-extracted residue.

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