Glycosyltransferases in the Golgi Membranes of Onion Stem

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Cell fractions consisting largely of Golgi membranes were prepared from the meristematic region of the onion. Several enzyme activities were found to be localized in these fractions: inosine diphosphatase, galactosyltransferases and glucosyltransferases. The fractions catalysed the transfer of [^{14}C]galactose from UDP-galactose to endogenous and cell-sap acceptors, to *N*-acetylglucosamine and to ovalbumin. In the presence of bovine α -lactalbumin, transfer to glucose (lactose synthesis) was catalysed. [^{14}C]Glucose was transferred from UDP-glucose to endogenous and cell-sap acceptors, to cellobiose and to fructose (sucrose synthesis). All these activities were latent, being potentiated by detergents (Triton X-100 or sodium deoxycholate). The characteristics of some of these enzyme activities are described and their biological significance is discussed.

The Golgi apparatus functions in the synthesis and modification of lipoproteins, glycoproteins and polysaccharides, and in the formation of secretory and intracellular vesicles (Northcote, 1971). Specific glycosyl transferases are concentrated in the Golgi-membrane fractions obtained from animal cells; in particular a galactosyltransferase (EC 2.4.1.38) which catalyses the transfer of galactose from UDP-galactose to free or protein-bound *N*-acetylglucosamine has been suggested as a marker enzyme for the organelle (Schachter *et al.*, 1970). This enzyme is present in the Golgi membrane of the mammary gland (Coffey & Reithel, 1968) and forms, during lactation, part of lactose synthase (EC 2.4.1.22). The monosaccharide-binding capacity of the galactosyltransferase is modified by the regulatory protein α -lactalbumin, so that the transfer of galactose from UDP-galactose, to glucose is catalysed at physiological concentrations (Brew *et al.*, 1973). The regulatory protein has similar effects on galactosyltransferases from rat liver (Fleischer *et al.*, 1969), chick cerebrospinal fluid and rat brain (Hill *et al.*, 1968).

Glycosyltransferases have also been detected in Golgi-rich fractions of etiolated pea shoots, although their precise specificities remain uncertain (Ray *et al.*, 1969), and the plant Golgi apparatus has been implicated, by electron-microscope radio-autographic studies, in the synthesis of oligosaccharides and polysaccharides. However, the only documented specific marker enzyme for the plant membranes is IDPase (inosine diphosphatase, EC 3.6.1.6), an enzyme of uncertain biological significance (Dauwalder *et al.*, 1969).

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During an examination of the enzyme constitution of Golgi membrane from various sources we have isolated such membranes from a plant tissue, onion stem, and find that they contain several glycosyltransferase activities, including UDP-galactose-*N*-acetylglucosamine β -4 galactosyltransferase (EC 2.4.1.38), the marker enzyme for Golgi membranes from animal sources. This galactosyltransferase, like the animal enzymes, also catalyses the synthesis of lactose in the presence of α -lactalbumin. The biological function of this and other observed glycosyltransferases is discussed.

Methods

Dextran, ovalbumin, UDP-glucose and UDP-galactose were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.; UDP-[^{14}C]glucose and UDP-[^{14}C]galactose were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Bio-Gel P2 and AG-1 (X8; 200–400 mesh) were obtained from Bio-Rad Laboratories, Richmond, Calif., U.S.A. Bovine serum albumin, Triton X-100, sugars and other reagents of AnalaR grade were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Sprouting onions were purchased locally and stored at 4°C for 2 days. Bovine α -lactalbumin was prepared by the method of Brew *et al.* (1970).

Stem explants (8g) were obtained by dissection from 3kg of onions. The Golgi membranes were prepared by a procedure similar to that of Morré & Mollenhauer (1964), all operations being conducted at 4°C. The explants were finely chopped with razor blades and gently homogenized by hand in an all-glass Potter-Elvehjem homogenizer, in 12ml of 0.5M-sucrose containing 10mM-sodium cacodylate, pH 7.0, 10mM-MgCl₂ and 1% dextran (mol.wt. 200000). The

homogenate was centrifuged at 9000g for 15 min, and the supernatant (S1) layered over 0.5 ml of 1.6 M unbuffered sucrose over 0.25 ml of 1.8 M-unbuffered sucrose and centrifuged at 37000g for 30 min in an SW 39 rotor. The clear solution above the membranes was discarded and 1 ml of 1.5 M-sucrose, then 1 ml of 1.25 M-sucrose, and 0.75 ml of 0.5 M-sucrose were layered beneath the membranes on top of the layer of 1.6 M-sucrose. Membranous layers were collected at the 0.5 M-/1.0 M-sucrose and 1.0 M-/1.25 M-sucrose interfaces after centrifugation at 95000g for 3 h. These fractions were resuspended in 50 mM-sodium cacodylate, pH 7.0, collected as pellets by centrifugation at 12000g for 30 min, and stored frozen after suspension in the same buffer at 6 mg of protein/ml. Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

IDPase activities were either measured by a procedure similar to that of Ray *et al.* (1969), by using 3 mM-IDP, 2 mM-MgCl₂ and 50 mM-sodium cacodylate, pH 7.5, or by the same method with the addition of 0.5% deoxycholate.

Galactosyltransferase activities with low-molecular-weight acceptors (monosaccharides, sucrose, cellobiose) were determined as described previously (Brew *et al.*, 1968) by using UDP-galactose (sp. radioactivity 3.8×10^5 c.p.m. of ¹⁴C/ μ mol), except that 20 mM-sodium cacodylate was substituted for Tris-HCl as the buffer. The transfer of glucose from UDP-glucose to fructose and cellobiose was measured by a similar procedure, in 100 μ l incubation mixtures containing 0.56 mM-UDP-glucose and 0.14 mM-UDP-glucose respectively (sp. radioactivity 1.75×10^5 c.p.m./ μ mol), 20 mM-sodium cacodylate, pH 7.4, 0.5% Triton X-100, 0.1% bovine serum albumin and 20 mM-acceptor.

For measuring the transfer of galactose to ovalbumin, similar incubation mixtures were prepared containing 0–1200 μ g of ovalbumin and the reactions terminated by the addition of 12% (w/v) trichloroacetic acid (1.0 ml). Ovalbumin (400 μ g) was added as carried protein, and the precipitate collected by centrifugation (3000g for 10 min) and washed three times with 10% trichloroacetic acid (1.0 ml). The final precipitate was dissolved in 5% formic acid (1.0 ml) and counted for radioactivity in 10 ml of dioxan-based scintillation fluid (Brew *et al.*, 1968). For measuring the transfer of glucose or galactose to endogenous acceptors or cell-sap 100 μ l incubation mixtures were prepared containing 20 mM-sodium cacodylate buffer, pH 7.4, 0.5% Triton X-100, 0.1% bovine serum albumin, UDP-sugar and metal ion as indicated (Tables 1 and 2). After incubation for 45 min, reactions were terminated by the addition of 0.2 M-EDTA (0.1 ml), 10% dextran (0.1 ml) and ethanol (0.7 ml). The precipitate was collected by centrifugation and washed three times with 70%

(v/v) ethanol, suspended in water (1.0 ml) and counted for radioactivity as described above.

For the identification of reaction products, assay mixtures after incubation were freeze-dried, redissolved in water (20 μ l) and applied to Whatman 3MM paper. Standards of galactose, glucose, fructose, lactose, sucrose and *N*-acetyl[¹⁴C]lactosamine [prepared by using bovine milk *N*-acetyl-lactosamine synthase (Brew *et al.*, 1968)] were applied separately to the same sheet. Chromatograms were developed in an ascending manner for 36 h with propan-1-ol-water-ethyl acetate (7:2:1, by vol.). Reducing sugars in standards were detected with AgNO₃, fructose with a urea-phosphoric acid spray (Wise *et al.*, 1955) and *N*-acetyl-lactosamine by its ¹⁴C label. The sections of the chromatograph to which incubation mixtures had been applied were cut into strips (1 cm) and counted for ¹⁴C radioactivity in 6 ml of toluene, containing 5 g of 2,5-diphenyloxazole/litre.

The Golgi fractions were examined by electron microscopy, by using negative staining with phosphotungstic acid. Alternatively, pelleted samples were embedded in Epon, stained with OsO₄ and lead acetate, and thin sections examined in the electron microscope.

Results

Characterization of membranes

Preparations of the membrane fractions that banded at densities of 1.13 and 1.15 g/ml (see Morré, 1970) were examined by electron microscopy.

Preparations of both fractions, negatively stained with phosphotungstic acid, appeared as small vesicles together with membrane fragments, which could have originated from almost any membrane system. However, Mollenhauer *et al.* (1973) have shown that this process of negative staining would cause rapid unstacking of any dictyosomes in the fractions. When the fractions prepared in the absence of fixative were embedded in Epon and stained with OsO₄-lead acetate, intact dictyosomes were observed in both fractions. In the lighter fraction (Plate 1a) visual inspection suggested that the stacked dictyosomes represented less than 5% of the membranes, whereas in the denser fraction (Plate 1b) the proportion of intact dictyosomes was 10–20% of the membranes. The denser fraction was only observed by electron microscopy with a single preparation, since there was not enough material to permit embedding and staining, in addition to biochemical characterization, in the other preparations. The relative yields of the two fractions varied twofold over four preparations and the denser fraction never contained more than one-third of the total protein in the two fractions. The lighter fraction appeared

EXPLANATION OF PLATE I

Electron micrographs of purified Golgi functions

(a) Electron micrograph showing Golgi fractions prepared in the absence of fixative (banding at a density of 1.13), embedded, and fixed with OsO_4 , stained with lead acetate. (b) Electron micrograph showing Golgi fractions banding at a density of 1.15, conditions as for (a).

by visual inspection of micrographs to be homogeneous; the appearance of both fractions in the electron microscope was similar. Our observations confirm that both fractions contain recognizable intact dictyosomes in significant numbers.

The further identification of the two membrane fractions as originating from the Golgi membranes is consequent on the high IDPase activity associated with the two fractions. Cytochemical experiments have shown that IDPase activity is located in the Golgi apparatus (dictyosomes) of most plant cells (Dauwalder *et al.*, 1969). In the absence of other marker enzymes, this specific localization of IDPase activity provides the only convenient biochemical method of assessing the purity of the two membrane fractions. Both fractions contained high IDPase activities (3 μ mol of P_i liberated/min per mg of protein), an enrichment of approx. 20-fold over the 9000g supernatant (S1) of the initial homogenate. Of the discarded fractions only the 9000g precipitate showed a higher specific activity (0.7 μ mol of P_i liberated/min per mg of the protein) than the homogenate; 40% of the IDPase activity of supernatant S1 was recovered in the combined membrane fractions. In the absence of sodium deoxycholate, the activity was latent, and became detectable only after samples had been stored at 0–4°C for 3 days. In the presence of 0.1% sodium deoxycholate the IDPase activity could be measured immediately, and was 20% higher than that determined after 3 days storage (3.6 μ mol of P_i liberated/min per mg of protein). The succinate dehydrogenase activity of the preparations was negligible, suggesting little mitochondrial contamination (Walker & Seilgman, 1963). These biochemical observations further indicate that most of the membrane material (at least 70%) in the two fractions banding at densities of 1.13 and 1.15 g/ml is derived from the Golgi apparatus.

Galactosyltransferase activities

Both fractions showed considerable activities for UDP-galactose–*N*-acetylglucosamine galactosyltransferase, of 172 nmol of [14 C]galactose incorporated/h per mg of protein, a recovery of 50% of the activity of the S1 fraction with a 25-fold increase in specific activity. The activity was negligible in the absence of Triton X-100 (or sodium deoxycholate). The properties of the enzyme in both membrane fractions were closely similar and those of the enzyme from the less-dense fraction are summarized in Table 1. The specific localization of this enzyme in the membrane fraction (referred to below as Golgi fractions) is indicated, since all discarded cell fractions had lower specific activities than fraction S1 (7.6 nmol/h per mg of protein). The transfer of galactose to *N*-acetylglucosamine is Mn^{2+} -dependent, equivalent quantities of $MgCl_2$

producing only 50% of the stimulation caused by $MnCl_2$; no activation was observed with Na^+ , K^+ , Ca^{2+} or Zn^{2+} . The transferase activities of both S1 and Golgi fractions prepared after homogenization in 1.5% (w/v) glutaraldehyde were less than 5% of the activities of the corresponding fractions prepared in the absence of fixative.

The product of the transferase assay co-chromatographed with authentic *N*-acetyl-lactosamine, and after digestion with β -galactosidase for 1.5 h 30% of the radioactivity was released as a monosaccharide with chromatographic properties similar to those of galactose. It appears therefore that at least a high proportion of the reaction product is probably *N*-acetyl-lactosamine. The dependence of the activity on *N*-acetylglucosamine concentration followed Michaelis–Menten kinetics with an apparent K_m for monosaccharide of 5.2 mM. The dependence on UDP-galactose concentration was, in contrast, sigmoidal with maximum velocity of 636 nmol/h per mg of protein, on saturation with UDP-galactose at 20 mM-*N*-acetylglucosamine and 10 mM- $MnCl_2$ (Fig. 1).

The transfer of galactose from UDP-galactose to the following acceptors was observed: xylose, ovalbumin and 70% ethanol-insoluble cell sap and endogenous components (probably oligosaccharides). Limited transfer to arabinose also occurred, but transfer to glucose and sucrose was negligible (see Table 1). Ovalbumin contains a single oligosaccharide prosthetic group which terminates in a β -linked residue of *N*-acetylglucosamine (Montgomery *et al.*, 1965) and serves as a substrate

Table 1. *Galactosyltransferase activities*

Assays were performed at 37°C for 45 min with 0.3 mM-UDP-galactose, 40 mM- $MnCl_2$, 0.5% Triton X-100, 0.1% bovine serum albumin, 20 mM-sodium cacodylate, pH 7.4, and monosaccharide (20 mM) or ovalbumin (800 μ g/ml). Activity is given as nmol of [14 C]galactose incorporated/h per mg of protein.

	Acceptor	Variable	Activity
Fraction S1	<i>N</i> -Acetylglucosamine	—	7.6
Golgi fractions	<i>N</i> -Acetylglucosamine	—	172.3
	<i>N</i> -Acetylglucosamine	–Triton	14.4
	<i>N</i> -Acetylglucosamine	– $MnCl_2$	26.6
	Xylose		50.8
	Arabinose		30.3
	Ovalbumin		69.1
	Cell sap		50.2
	Endogenous		36.2
	Glucose		11.9
	Glucose	+ α -Lactalbumin (200 μ g/ml)	87.8

Fig. 1. Dependence of galactosyltransferase activity on UDP-galactose concentration

Assay was performed by using 10mM-MnCl₂, 20mM-sodium cacodylate, pH7.4, 0.5% Triton X-100, 0.1% bovine serum albumin, 20mM-N-acetylglucosamine.

Fig. 2. Effect of ovalbumin concentration on the rate of transfer of galactose to ovalbumin

Assay was performed by using 40mM-MnCl₂, 20mM-sodium cacodylate, pH7.4, 0.5% Triton X-100, 0.1% bovine serum albumin and 0.3mM-UDP-galactose; initial velocity is given as nmol of [¹⁴C]galactose incorporated/h per mg of protein.

for mammalian galactosyltransferases (K. Brew & J. T. Powell, unpublished work). Transfer of galactose to this glycoprotein by onion stem Golgi membranes is characterized by an apparent K_m for ovalbumin of 400 μ M (Fig. 2), which is considerably lower than that for free N-acetylglucosamine.

In the presence of bovine α -lactalbumin the Golgi fraction catalysed the transfer of galactose to

Fig. 3. Dependence of N-acetyl-lactosamine synthase and lactose synthase activity on α -lactalbumin concentration

Open circles show the inhibition of galactose transfer to N-acetylglucosamine, assayed by using 40mM-MnCl₂, 20mM-sodium cacodylate, pH7.4, 0.5% Triton X-100, 0.1% bovine serum albumin, 20mM-N-acetylglucosamine and 0.3mM-UDP-galactose. Closed circles show stimulation of galactose transfer to glucose; assay conditions were as above but with 5 mM-glucose as an acceptor.

glucose. Increasing concentrations of α -lactalbumin caused progressive stimulation of transfer to glucose, whereas the transfer of galactose to N-acetylglucosamine was progressively inhibited (Fig. 3). A maximum inhibition of 50% was reached at 200 μ g of α -lactalbumin/ml; this contrasts with 80% inhibition of the bovine milk enzyme at the same concentration of α -lactalbumin (Hill *et al.*, 1968). The maximum activity for transfer to glucose (700 μ g of α -lactalbumin/ml) was 55% of that for the transfer to N-acetylglucosamine in the absence of α -lactalbumin, at the same concentrations of UDP-galactose and MnCl₂. The apparent K_m for glucose at 700 μ g of α -lactalbumin/ml was 13 mM (Fig. 4). The product of the transfer to glucose in the presence of α -lactalbumin co-chromatographed with lactose and was hydrolysed to an extent of 60% during a 1.5h incubation with β -galactosidase.

Glucosyltransferase activities

UDP-glucose-fructose glucosyltransferase activity was also present in Golgi-membrane fractions with a specific activity of 138 nmol of [¹⁴C]glucose transferred/h per mg of protein (0.56mM-UDP-glucose, 20mM-fructose). No activity was observed in the absence of Triton X-100, and the enzyme did not exhibit activation by metal ions, 20mM-MgCl₂ causing 50% inhibition. The activity of the S1 homogenate was only fractionally less (95 nmol/h per mg of protein) but this activity was not

Fig. 4. Effect of glucose concentration on the rate of lactose synthesis, at different fixed concentrations of bovine α -lactalbumin

Concn. of α -lactalbumin: $\circ$, 62.5 $\mu\text{g/ml}$; $\bullet$, 83.3 $\mu\text{g/ml}$; $\square$, 125 $\mu\text{g/ml}$; $\blacksquare$, 250 $\mu\text{g/ml}$. Other assay conditions were as for Fig. 3. Initial velocity is given as nmol of [^{14}C]-galactose incorporated/h per mg of protein.

Table 2. Glucosyltransferase activities

Assays were performed at 37°C for 45 min, with 20 mM-sodium cacodylate, pH 7.4, 0.1% bovine serum albumin, 0.5% Triton X-100. For measuring glucosyl transfer to fructose (20 mM), UDP-glucose was used at 0.56 mM, whereas for transfer to cellobiose (20 mM), cell sap and endogenous acceptor the concentration was 0.14 mM. Activity is given as nmol of [^{14}C]glucose incorporated/h per mg of protein.

	Acceptor	Variable	Activity
Fraction S1	Fructose		95
	Fructose	-Triton	89
	Cellobiose		39
Golgi fractions	Fructose		138
	Fructose	-Triton	43
	Fructose	+MgCl ₂ (20 mM)	81
	Cellobiose	+MgCl ₂ (20 mM)	132
	Cell Sap	+MgCl ₂ (20 mM)	150
	Endogenous	+MgCl ₂ (20 mM)	106
	Endogenous		34

potentiated by Triton X-100. The product of the reaction co-chromatographed with sucrose and was 90% hydrolysed to monosaccharides by 1.5 h incubation with invertase. The observed reaction can therefore be ascribed to sucrose synthase activity (UDP-glucose-D-fructose α -1-glucosyltransferase, EC 2.4.1.13).

Similar activities for the transfer of glucose to cellobiose, (70% ethanol-insoluble) cell sap and

endogenous acceptors were observed which, in contrast with sucrose synthase, is activated by Mg^{2+} ions, fourfold stimulation being observed with 20 mM-MgCl₂ (Table 2).

Discussion

The membrane fractions prepared by our procedure are identified as being derived from the Golgi apparatus by isopycnic density, morphology and enzymic criteria. The densities of the two fractions are consistent with those reported for the Golgi membranes of other plant tissues (Morré, 1970). Electron micrographs show the presence of some intact dictyosomes, together with a large amount of vesicular material, as expected for fractions prepared in the absence of fixative. IDPase activity is generally accepted as a specific marker enzyme for plant Golgi membranes, on the basis of both cytochemical studies (Dauwalder *et al.*, 1969) and assay with purified Golgi membranes from plant sources (Ray *et al.*, 1969). The Golgi fractions show a 22-fold enrichment of IDPase activity, compared with the S1 supernatant, which represents a yield of 40% of the activity based on the S1 supernatant.

The specific localization of UDP-galactose-*N*-acetylglucosamine galactosyltransferase activity in the onion stem Golgi membranes is strongly suggested by the very similar increase in the specific activity of this enzyme in the purified Golgi fractions (22–25-fold) when compared with IDPase. This same galactosyltransferase activity has been identified in the Golgi membranes from various animal tissues (Morré *et al.*, 1969; Schachter *et al.*, 1970; Cunningham *et al.*, 1971). The enzyme located in onion stem Golgi fractions shows many similarities with the animal enzyme; it is Mn^{2+} -dependent; the product with *N*-acetylglucosamine as substrate appears to be *N*-acetyl-lactosamine (although our evidence does not rigidly exclude isomeric β -linked 3 or 6 disaccharides); α -lactalbumin causes inhibition of galactose transfer to *N*-acetylglucosamine, and the enzyme will transfer galactose to ovalbumin, a glycoprotein with a terminal *N*-acetylglucosamine moiety. The apparent K_m for *N*-acetylglucosamine (5.2 mM) is similar to that for the corresponding enzyme from bovine milk (Morrison & Ebner, 1971), but much higher than that for ovalbumin (400 μM) (Fig. 2).

The similarity with animal galactosyltransferases extends unexpectedly to the activation of lactose synthesis in the presence of bovine α -lactalbumin. In the Golgi membranes of the lactating mammary gland *N*-acetyl-lactosamine synthase is the catalytic component of lactose synthase; α -lactalbumin is the regulatory protein which promotes the binding of glucose to galactosyltransferase, permitting the

synthesis of lactose at physiological concentrations of glucose (Brew *et al.*, 1973). Lactose synthesis by the galactosyltransferases of rat liver, chicken brain and cerebrospinal fluid are also activated by α -lactalbumin (Hill *et al.*, 1968).

This evidence supports the identification of the onion stem enzyme as a β -4 galactosyltransferase. The recognized biological function of the animal UDP-galactose β -4 galactosyltransferase, apart from its role in lactose synthesis in mammals, is in attaching galactose through β -1-4 linkage to *N*-acetylglucosamine moieties in exported glycoproteins and oligosaccharides. It would appear likely that a similar function can be attributed to the enzyme in the Golgi membranes from onion stem. The similarity of the carbohydrate composition of many plant and animal glycoproteins has been noted (Sharon & Lis, 1972). Both *N*-acetylglucosamine and galactose are frequent constituents, but no monosaccharide sequences have been reported (Shannon *et al.*, 1966; Ericson & Chrispeels, 1973). The evidence that sugar incorporation into the glycoproteins of mammals occurs in the Golgi apparatus is quite conclusive (Neutra & Leblond, 1966), but it is less so in plants, since most work has concentrated on the sites of polysaccharide synthesis. However, in algae, glycoproteins are secreted and synthesized in the Golgi apparatus (Herth *et al.*, 1972; Callow & Evans, 1974). An alternative, but less likely, role of the galactosyltransferase from the Golgi membranes of onion stem could be a step in the synthesis of oligosaccharides, which are subsequently linked by transglycosylation to lipids, a mechanism that is well characterized for bacteria (Robbins *et al.*, 1967). In plants galactolipid synthesis, involving UDP-galactose, occurs in particulate fractions of non-chlorophyllous tissue, as well as in the chloroplast (Ongun & Mudd, 1968). The absence of activity with sucrose as acceptor excludes the possibility that the enzyme could be associated with raffinose synthesis.

The sigmoidal saturation curve for UDP-galactose, with galactosyl transferase, may be a result of phenomena other than co-operative binding of the substrate. For example, at low concentrations UDP-galactose may be preferentially binding to other components of the membrane (compare the case for UDP-glucose; Tsai & Hassid, 1971), or the transfer of galactose could be mediated by the formation of an intermediate compound, as has been found for manose-transfer reactions which involve polyisoprenoid phosphate intermediates in both animals and plants (Baynes *et al.*, 1973; Alam & Hemming, 1973), although there is no evidence for this mode of galactose transfer (Jankowski & Chojnacki, 1972).

The incorporation of glucose and galactose, from UDP-glucose and UDP-galactose, into endogenous acceptors is similar in ratio (3:1) to the activities

observed in Golgi fractions from etiolated shoots (Ray *et al.*, 1969). Comparison with the properties of β -glucan synthase activities in particulate fractions from oat coleoptiles (Tsai & Hassid, 1971) and mung bean (Villemez *et al.*, 1967) leads us to suggest that the activity in the onion stem preparations represents a β -1-4 synthase activity, since we observe Mg^{2+} -dependent transfer of glucose to cellobiose and endogenous acceptors at low concentrations of UDP-glucose.

Sucrose synthase activity has generally been found in soluble cytoplasmic fractions from plant sources (Cardini *et al.*, 1955; Avigad, 1964; Delmer, 1972), and is not dependent on the presence of detergents. Only a small fraction of the sucrose synthase activity in the S1 supernatant was dependent on Triton X-100. In contrast, the sucrose activity associated with the purified membrane fraction is largely dependent on the presence of Triton X-100. It is reasonable to suggest that this latter sucrose synthase activity may function in the synthesis of sucrose as a primer for inulin biosynthesis (Edelman & Jefford, 1968). Inulin biosynthesis occurs in the vacuoles, which are probably derived from the Golgi apparatus (Gordon *et al.*, 1965; Coulomb, 1969).

The meristematic regions of plants contain cells with a relatively large amount of Golgi apparatus. These organelles have been implicated in the assembly of glycoproteins (Herth *et al.*, 1972; Callow & Evans, 1974) and in the modification of lipids and turnover of membranes (Morré *et al.*, 1970; Morré & Mollenhauer, 1973). They have also been shown to be active in the synthesis of pectins for primary cell-wall growth (Northcote & Pickett-Heaps, 1966), and the hemicelluloses of secondary cell-wall growth are deposited from the Golgi vesicles (Wooding & Northcote, 1964). The concentration of the various glycosyltransferase activities that we have observed in the Golgi membranes of onion stem are entirely compatible with these functions and appear to reflect the synthesis of cell constituents containing specific monosaccharide sequences (*N*-acetyl-lactosamine synthase) as well as homopolymer synthesis and priming reactions for homopolymer synthesis. The enhancement of these activities with Triton X-100 is consistent with the view that these activities are located within the cisternae of the Golgi dictyosomes or vesicles.

Mollenhauer *et al.* (1967) have commented on the striking homology of form between plant and animal Golgi apparatus. It is becoming clear that this homology extends to some of the functions, and therefore enzymes, of the Golgi membranes from plants and animals. In particular the presence of UDP-galactose-*N*-acetylglucosamine β -4-galactosyltransferase in Golgi membranes from plant and animal sources confirms the use of this enzyme as a unique biochemical marker for Golgi-derived membranes.

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