Glucan Biosynthesis in Red Beet Root Microsomes and Tissue Slices

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As the first step in determining whether polysaccharide biosynthetic enzymes can be utilized to improve textural properties in cut vegetables, the glucan synthase II of red beet root microsomes and tissue slices was characterized. In microsomes, maximal rates of glucose incorporation using uridine diphosphate glucose (UDPG) were obtained at pH 7.0 with cellobiose and 5 mM Mg^{2+}. Approximately 30% of the substrate was incorporated into products. Products were partially solubilized by treatment with $\beta(1\rightarrow3)$- and $\beta(1\rightarrow4)$-glucanases. Subcellular localization experiments demonstrated that the enzyme is located predominantly in the plasma membrane, with lesser levels associated with Golgi-derived vesicles. UDPG was readily incorporated into tissue disks, with the bulk of [^{14}C]glucose associated with the 5000g fraction.

It is widely recognized that cell wall glucans play an important role in determining the texture of fruits and vegetables. The cell walls of crisp, unripened vegetables contain high levels of the crystalline β-(1→4)-linked polysaccharide cellulose (Herranz et al., 1981, 1983). In addition, callose, a β-(1→3, 1→4)-linked amorphous glucan, may also be present. Callose formation and lignification often occur as the result of wounding or other trauma (DeKazos, 1972; Tighe and Heath, 1982).

The enzyme considered responsible for the formation of cellulose and callose in higher plants is a uridine diphosphate glucose—glucosyltransferase (glucan synthase). Two forms of the enzyme are thought to exist. Glucan synthase I, believed to be located on the Golgi body membrane, is active at micromolar concentrations. The other form, glucan synthase II, is found primarily on the plasma membrane and utilizes substrate at millimolar concentrations. Wide variations of the enzymes have been observed from plant to plant. In onion stem, the two activities could be distinguished by discontinuous sucrose gradient centrifugation (Van Der Woude et al., 1974), in ryegrass endosperm protoplasts, all activity was associated with intracellular membranes (Henry et al., 1983), and in sugarcane suspension cultures, the two activities were found in both the Golgi and plasma membrane fractions (Robinson and Glas, 1983) and could not be separated from each other. Most workers have found that polysaccharides with mixed β-(1→3) and β-(1→4) linkages, rather than true cellulose, are obtained upon incubation of particulate fractions with uridine diphosphate glucose (UDPG) in vitro.

If the exposed surfaces of cut vegetables contain active glucan synthase, it would be possible to synthesize surface polysaccharides by dipping tissue slices in a solution containing substrate and appropriate cofactors. Such a technique has been demonstrated in young pea stem slices (Anderson and Ray, 1978; Raymond et al., 1978; Mueller and Maclachlan, 1983) and may be a useful means of introducing desirable textural modifications in cut vegetables.

This paper reports the characterization of glucan synthase II in red beet root. This study represents the first step of determining the feasibility of whether glucan synthases can be used to introduce textural modifications at cut vegetable surfaces.

MATERIALS AND METHODS

Materials. Red beets (*Beta vulgaris* L., cv. Detroit Dark Red) were grown outside (May–October) or in a greenhouse (October–April) in 13-cm corrugated plastic pots containing a 1:1 peat moss–vermiculite, pH 6.5, mixture. Upon reaching a height of 8–10 cm, growing plants were fertilized biweekly with a mixture containing 79 g/L nitrogen, potassium, and potash and approximately 0.3 g/L of a soluble trace element mixture (Peters Fertilizer Co.). Beet roots were harvested between 2 and 4 months and used immediately or stored at 4 °C in moist vermiculite. Comparisons with beets obtained from local markets showed no differences in glucan synthase activity.

UDP-[U-^{14}C]glucose (sp act. 340 mCi/mmol) was purchased from New England Nuclear. UDPG, ATP, and IDP were obtained from Sigma Chemical Co. ATP was converted to the Tris salt as described by Hodges and Leonard (1974). All solutions were prepared with sterile distilled–deionized water. Soy β-(1→3)-endoglucanase (Keen and Yoshikawa, 1983) was generously contributed by Dr. N. T. Keen (University of California, Riverside). The *Trichoderma reesei* β-(1→4)-endoglucanase and exocellobiohydrolase were kindly provided by Dr. D. Eveleigh and M. Frain (Rutgers University).

Isolation of Microsomal Membranes. Microsomal fractions were isolated by differential centrifugation as described by Briskin and Poole (1983). The microsomal pellet was resuspended in 250 mM sucrose, 1 mM tris(hydroxymethyl)aminomethane–2-(*N*-morpholino)ethanesulfonic acid (Tris–Mes), pH 7.2, and 1 mM dithioerythritol (suspension buffer) and centrifuged a second time. The washed microsomal pellet was resuspended at a concentration of 1–2 mg/mL protein in suspension buffer containing 10% (w/v) glycerol. One hundred grams of beet tissue will generally yield 10 mg of microsomal protein. Microsomes were divided into 1.5-mL microcentrifuge tubes and stored at −80 °C until use. Under these conditions, over 80% enzyme activity was retained for at least 6 months.

Glucan Synthase Assay of Microsomal Membranes. Glucan synthase reactions were routinely conducted in 100-μL assay mixtures containing 1 mM UDP-[^{14}C]glucose (0.1 μCi/μmole), 5 mM $MgCl_2$, 20 mM cellobiose, 50 mM Tris-HCl, pH 7.0, and microsomes to a final protein concentration of 1.0 mg/mL. Unless otherwise indicated, mixtures were incubated at 30 °C for 5 min and the reactions then terminated by heating at 90–100 °C for 10 min. Glucose incorporation was measured by the glass fiber filter method of Smith and Stone (1973). Eighty microliters of each reaction mixture was spotted on a Whatman GF/A filter disk (2.4-cm diameter), dried, and placed in an 18 × 150 mm culture tube. The tubes were placed on ice and washed successively with 10 mL of ice-cold 66% (w/v) ethanol containing 0.85 mM EDTA, 10 mL of 66% (w/v) ethanol, and 10 mL of 70% (w/v) ethanol. Filters were rinsed with acetone and air-dried. The disks

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were then placed in scintillation vials, 10 mL of Instagel (Packard Instrument Co., Downers Grove, Il) was added, and the vials were counted on a Packard 3255 Tri-Carb liquid scintillation spectrometer. Quenching was corrected by counting known amounts of UDP-[^{14}C]glucose that had been spotted on filters.

Alkali and chloroform–methanol insoluble glucan formation was measured by the method described by Aloni et al. (1982), with several modifications. Reactions were terminated by heating, and 0.5 mL of a solution containing 0.5 M NaOH and 0.5 M $NaBH_4$ was added. Approximately 20 mg of carrier cellulose was also added. The mixtures were incubated at 65 °C for 18 h and filtered onto Whatman GF/A glass fiber filters. The filters were washed 6 times with 4 mL of water and once with a 2:1 mixture of chloroform–methanol. The filters were then dried and counted as described above.

The crystalline cellulose content of the microsomal product was determined as follows: Glucan synthase assays were conducted and terminated as described above. Carrier cellulose (10 mg) and 450 μL of the acetic–nitric reagent of Updegraff (1969) were then added to each sample. The tubes were boiled for 60 min and the residue then passed through a GF/A filter. The filters were washed 6 times with 4 mL of H_2O and once with 4 mL of methanol. The filters were then dried and counted.

Sucrose Density Gradient Centrifugation. Freshly isolated microsomes (2 mL) containing 1.5 mg of protein were layered on a 14-mL 20–44% (w/w) continuous sucrose gradient containing 1 mM Tris–Mes, pH 7.2, and 1 mM dithioerythritol. The gradients were centrifuged for 2 h at 100000*g* in a Beckman SW 28.1 rotor. The gradient was fractionated by plunging a glass capillary to the bottom of the tube and pumping out 21 fractions of 0.8 mL each. Sucrose concentration was measured by refractometry.

Marker Enzyme Assays. ATPase activity was assayed as described by Briskin and Poole (1983). In assays containing sodium orthovanadate, its concentration was 50 μM. IDPase activity was measured after the fractions were stored 3 days at 4 °C, in 1.0-mL reaction volumes containing 3 mM $MgSO_4$, 50 mM KCl, 2.4 mM IDP, and 30 mM Tris–Mes, pH 7.5 (Ray et al., 1969). Phosphate release was assayed by the method of Ames (1966). NADPH-dependent cytochrome *c* reductase and cytochrome *c* oxidase were assayed as described by Hodges and Leonard (1974).

Protein. Samples were precipitated with TCA and assayed by the method of Peterson (1977).

Tissue Disk Studies. Beet roots were surface sterilized by a 10-min immersion in 10% (v/v) bleach and 1% (v/v) Triton X-100. The beets were then washed with sterile water, and cores 2 mm in diameter were taken with a no. 1 cork borer. The cores were then sectioned into slices 1 mm thick with a razor.

One gram of disks was weighed into a 7-mL Dounce homogenizer, and 1 mL of substrate solution containing 1 mM UDP-[^{14}C]glucose (0.1 μCi/μmol), 5 mM $MgCl_2$, 20 mM cellobiose, and 50 mM Tris-HCl, pH 7.0, was added. The disks were incubated for 1 h at 30 °C in a shaking water bath. The reaction was terminated by aspiration of the substrate solution with a Pasteur pipet and washing 3 times with a solution containing 250 mM sucrose and 1 mM Tris–Mes, pH 7.2.

Subcellular fractionation studies to determine the location of incorporated label were conducted by homogenization and a series of differential centrifugations as follows: One milliliter of a homogenization buffer [250 mM sucrose, 3 mM EDTA, 0.5% (w/v) polyvinylpyrrolidone, 70 mM Tris-HCl, pH 8, and 4 mM dithioerythitol] was added to the Dounce homogenizer, and the disks were then ground until a uniform homogenate was obtained. The homogenate was brought to a volume of 3 mL in homogenization buffer and transferred to a 13 × 100 mm culture tube. Residual material was transferred from the Dounce homogenizer to the culture tube by washing with an additional 2 mL of homogenization buffer. The homogenate was then centrifuged at 1000*g* for 10 min in a Beckman Model TJ-6 centrifuge. The supernatant was transferred to a 50-mL preparative centrifuge tube, and the 1000*g* pellet was resuspended in 5 mL of homogenization buffer and spun again at 1000*g*. The pellet was resuspended in 2 mL of homogenization buffer and a 1.25-mL aliquot was counted for radioactivity in 3.75 mL of Instagel. The supernatants were combined and centrifuged at 5000*g* in a Sorvall preparative centrifuge. The pellet was resuspended in 2 mL of homogenization buffer, and an aliquot was counted as above. The 5000*g* supernatant was centrifuged for 10 min at 13000*g*, and the 13000*g* supernatant was centrifuged at 100000*g* for 30 min. The pellets were resuspended in 2 mL of homogenization buffer and counted as described above.

Purified Glucanase Digestion of Microsomal Products. Glucan synthase reactions were conducted according to the standard procedure with the exception that the specific activity of UDP-[^{14}C]glucose added to the incubation mixture was increased to 0.3 μCi/μmol. Mixtures were incubated at 30 °C for 45 min to maximize synthesis of glucan. Reactions were terminated by heating at 90–100 °C for 10 minutes.

The incubation mixtures were spotted on Whatman GF/A glass fiber filters that were placed in a Millipore filter apparatus and washed with 1 mL of chloroform–methanol (2:1), 0.85 mM EDTA in 66% (v/v) ethanol, 66% ethanol, and 70% ethanol, respectively. Filters were then placed in vials and suspended in 0.1 M sodium acetate buffer, pH 4.5, containing the specified enzymes. Incubation was conducted for 24 h at 50 °C in a shaking water bath, after which the supernatants were decanted and the filters washed with 0.1 M sodium acetate buffer. The dried filters were placed in scintillation vials with 10 mL of Instagel and were counted to determine the amount of filter-bound nonsolubilized glucan.

Purified Glucanase Digestion of Cell Wall Bound Glucan. Aliquots containing 2000 cpm of the 5000*g* fraction, isolated from tissue disks as described above, were suspended in 400 μL of homogenization buffer in a 1.5-mL microcentrifuge tube. The suspension was centrifuged at 13000*g*, and the pellets were resuspended in 100 μL of 0.1 M sodium acetate buffer, pH 4.5, containing the specified enzymes. Incubation was conducted at 50 °C for 24 h in a shaking water bath. After incubation, 5 mg of carrier cellulose (Sigmacell, Type 100; Sigma Chemical Co., St. Louis MO) was added. The cell wall bound undigested glucan was removed by centrifugation at 13000*g*. A 90-μL aliquot of the supernatant was then applied to a 1.0 × 43 cm gel filtration column containing Bio-Gel P-2. The column was eluted with water, and 1.0-mL fractions were collected. Aliquots were transferred to scintillation vials and counted.

RESULTS

Kinetics of Glucose Incorporation. In initial experiments, microsomal fractions were screened to establish optimal conditions for glucose incorporation. Linearity for a 5-min reaction was observed up to a microsomal protein content of 50 μg/assay. Specific activities of various membrane preparations ranged between 50 and

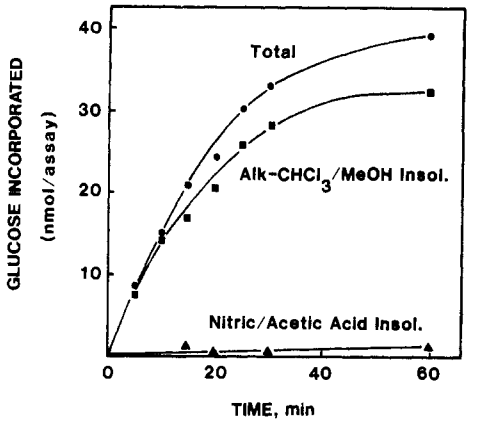

Figure 1. Time course of glucose incorporation into ethanol-insoluble (●), alkali–chloroform–methanol-insoluble (■), and acetic–nitric acid insoluble (▲) products. Assays were conducted as described under Materials and Methods. Each assay contained 35 μg of protein.

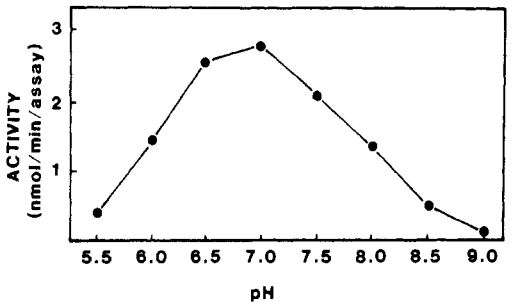

Figure 2. Effect of pH on glucose incorporation. Each assay contained 22 μg of protein.

200 nmol of glucose incorporated min^{-1} (mg of protein)$^{-1}$. The addition of 1 mM UDP to reaction mixtures resulted in a 42% reduction in the amount of glucose incorporated after 5 min. The microsomal enzyme had an apparent K_m of 1.5 ± 0.4 mM.

The time course of glucose incorporation at a protein level of 35 μg is shown in Figure 1. Assays for both total alcohol-insoluble product and alkali-insoluble–chloroform–methanol-insoluble product were performed. Both assay methods showed that glucose incorporation into the membranes was close to linear during the first 10 min of the reaction. After 10 min the rate of glucose incorporation began to decrease. Incorporation leveled off after 30 min. Total incorporation of glucose into alkali- and chloroform-insoluble product was 30 nmol (33% conversion). The addition of UDP-[^{14}C]glucose to reaction mixtures at 20 min did not result in a stimulation of the rate of incorporation. In contrast to the yeast enzyme (Shematek et al., 1980), 1 mM ATP had no stimulatory effect on the rate of glucan formation nor was [^{14}C]glucose incorporated into alcohol-insoluble product.

Approximately 85% of the total ethanol-insoluble products were alkali and chloroform–methanol insoluble. This value is high in comparison to some of the other plant particulate glucan synthase II systems that have been studied (Smith and Stone, 1973; Helsper et al., 1977) where greater levels of glucose incorporation into lipid soluble fractions were observed.

Since most of the glucose was incorporated into the polysaccharide fraction, the overnight alkali–borohydride extraction was omitted for most subsequent assays. Unless otherwise indicated, assay mixtures were spotted on glass fiber filters and extracted with ethanol as described under Materials and Methods.

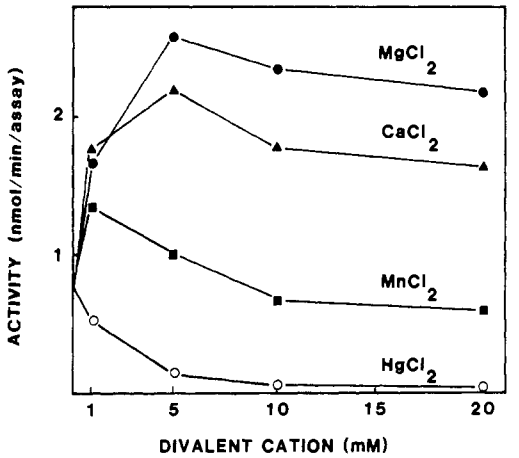

Figure 3. Effect of divalent cations on glucan synthase activity. Each assay contained 22 μg of protein.

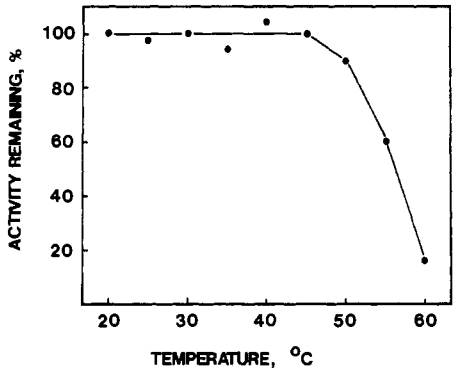

Figure 4. Thermal inactivation of glucan synthase activity. Microsomes were held 10 min at the indicated temperature. The tubes were placed in an ice bath, and 20-μL aliquots were assayed at 30 °C by using the standard method. Each assay contained 50 μg of protein.

Effect of pH. Optimal rates of glucose incorporation were achieved between pH values of 6.5 and 7.0 (Figure 2). This correlates with the pH optimum of the carrot enzyme (Boss and Ruesink, 1979). Between pH 7.4 and pH 8.5, the pH range where plant glucan synthases are most typically assayed (Anderson and Ray, 1978; Ephritikhine et al., 1980; Helsper et al., 1977; Henry et al., 1983; Henry and Stone, 1982; Ray et al., 1969; Van der Woude et al., 1974), activity declined, with approximately 50% of the maximal activity retained at pH 8.0.

Effect of Divalent Cations. Exogenous metals were not essential for activity; however, [^{14}C]glucose incorporation from UDP-[^{14}C]glucose was stimulated 2–3-fold by the presence of 5 mM Mg^{2+} or Ca^{2+} (Figure 3). These results correlate with the findings of Anderson and Ray (1978). Mn^{2+} exhibited a slight stimulatory effect at levels of 1 and 5 mM but did not stimulate activity at higher concentrations. Activity was inhibited by Hg^{2+}, with total loss of activity at 10 mM (Figure 3).

Effect of Cellobiose. Cellobiose is a common activator of glucan synthase (Feingold et al., 1958; Delmer et al., 1977; Callaghan and Benziman, 1984) and was routinely added to assay mixtures at a concentration of 20 mM. Its exclusion from assay mixtures led to a 50% decline in activity.

Temperature Effects. Figure 4 shows that when microsomes were preincubated for 10 min and then assayed at 30 °C, full activity was retained until a temperature of 45 °C was reached. On the other hand, when assay temperature was varied (Figure 5), activity losses became apparent at 35 °C.

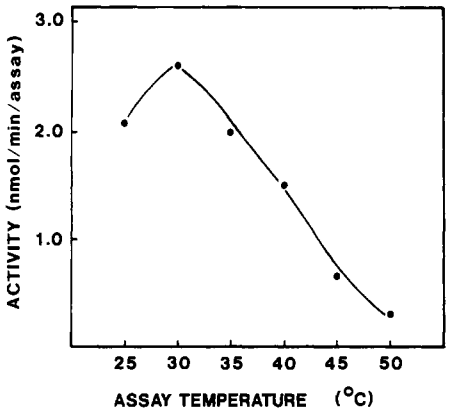

Figure 5. Effect of assay temperature on glucose incorporation. Each assay contained 22 μg of protein.

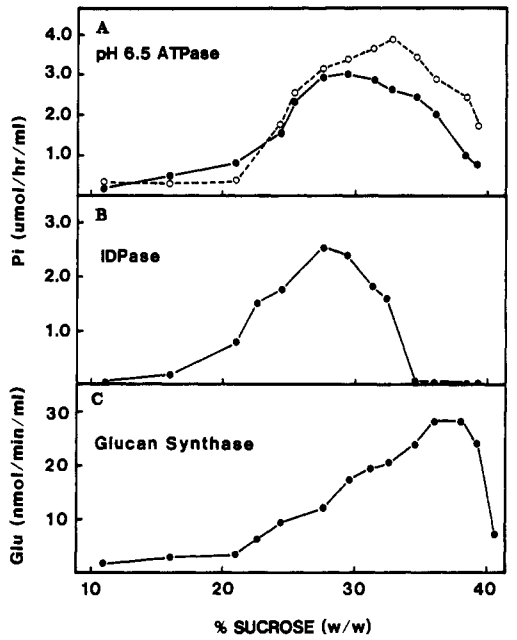

Figure 6. Subcellular fractionation of microsomal membranes on a 20–44% (w/w) continuous sucrose gradient. Microsomes were prepared and fractionated as described under Materials and Methods. The pH 6.5 K^+,Mg^{2+}-ATPase was assayed in the absence (○) and presence (●) of 50 μM vanadate.

Subcellular Localization. In higher plants, glucan synthase II activity has been found in the plasma membrane (Boss and Ruesink, 1979) and Golgi (Henry et al., 1983). The relative distribution of the enzyme between the two membrane types appears to vary from source to source.

To localize the red beet root enzyme, microsomes were centrifuged on a continuous sucrose gradient. The gradients were fractionated and assayed for NADPH cytochrome c reductase (endoplasmic reticulum), latent IDPase (Golgi vesicles), and pH 6.5 vanadate-sensitive, K^+-stimulated Mg^{2+}-ATPase (plasma membrane). The distributions of the ATPase, IDPase, and the glucan synthase are shown in Figure 6. Total pH 6.5 K^+,Mg^{2+}-ATPase activity sedimented between 25 and 40% (w/w) sucrose. However, vanadate-sensitive activity, a more specific marker of the plasma membrane of red beet (Briskin and Poole, 1983; Poole et al., 1984) and other sources (Gallagher and Leonard, 1982; Scalla et al., 1983) was found only between 30 and 40% sucrose (Figure 6A). This falls within the range of generally accepted plasma membrane peak equilibrium densities (Leonard and Hodges, 1980) and is in agreement with the results of Poole et al. (1984) and Bennett et al. (1984). It can therefore be inferred that membrane vesicles in the 32–38% (w/w) sucrose range are plasma membrane enriched.

Table I. Subcellular Distribution of [^{14}C]Glucose Incorporated into Tissue Slices[a]

	[^{14}C]UDPG-intact slices		[^{14}C]UDPG-heated slice[b]		[^{14}C]glucose-intact slices[c]	
	cpm	% recovery	cpm	% recovery	cpm	% recovery
radioactivity added	1.6×10^5		2.5×10^5		1.6×10^6	
0–1000*g*	10317	6.4	1334	0.5	1096	0.07
1000–5000*g*	32678	20.4	697	0.3	1072	0.07
5000–13000*g*	4845	3.0	572	0.2	637	0.04
13000–100000*g*	3680	2.3	465	0.2	746	0.04

[a] Tissue disks were fractionated as described under Materials and Methods. [b] Tissue slices were heated at 60 °C for 1 h in 250 mM sucrose and 1 mM Tris-HCl, and pH 8.0, and washed 3 times with 250 mM sucrose and 1 mM Tris-HCl, pH 8.0, prior to incubation with substrate. [c] [^{14}C]Glucose in 1 mM [^{12}C]glucose was used in place of UDPG.

IDPase activity (Figure 6B) gave a broad peak with maximal activity at 28–30% (w/w) sucrose. Although there was some overlap with vanadate-sensitive ATPase activity, IDPase activity decreased at densities greater than 30% (w/w) sucrose. The distribution of the plasma membrane and Golgi markers obtained in the experiment is in agreement with the results of Poole et al. (1984).

Glucan synthase activity was observed throughout the gradient, with the peak of activity between 32 and 38% (w/w) sucrose (Figure 6C). This coincides with vanadate-sensitive ATPase activity. Smaller levels of activity were found between 22 and 32% (w/w) sucrose with a small, but reproducible, shoulder occurring at 30% (w/w) sucrose. This suggests that glucan synthase activity may also be present in Golgi-derived vesicles.

The endoplasmic reticulum marker was also assayed. A peak of NADPH cytochrome c reductase activity was found at approximately 20% (w/w) sucrose, in good agreement with the results obtained by Briskin and Poole (1983) on discontinuous sucrose gradients. Bennett et al. (1984) have shown that mitochondria do not co-sediment with glucan synthase activity.

Incorporation of [^{14}C]-Glucose into Tissue Slices. To determine whether polysaccharides could be synthesized at cut surfaces in red beets, disks were incubated with UDP-[^{14}C]glucose. After a 2-h incubation, approximately 33% of the added label was incorporated. Table I shows that most of the recovered label was in the 5000*g* pellet. Only 2.3% was recovered in the microsomal fraction. Thus, most of the radioactivity appears to have been associated with the cell wall material.

With preheated tissue slices (Table I), less than 2% of the added radioactivity was incorporated. This demonstrates that incorporation was due to the enzymatic incorporation of UDPG rather than its binding to the disks. In another experiments, disks were incubated with [^{14}C]-glucose (Table I). The failure of glucose to become incorporated indicates that the initial step in glucose incorporation from UDPG is not the breakdown of UDPG to UDP and glucose.

Glucanase Analysis of Reaction Products. To identify linkage types within the microsomal glucan products, filter-bound microsomes were incubated at 50 °C with purified linkage-specific glucanases. Percent solubilization was calculated by comparing the amount of radioactivity retained on enzyme-treated filters to the amount retained on control filters. The results (Table II)

Table II. Solubilization of Microsomal Products by Purified Glucanases[a]

enzyme	product recovered on filter, cpm	solubilization, %
control	5700	0
β-(1→3)-endoglucanase	4272	25
cellobiohydrolase and β-(1-4)endoglucanase	2908	49
β-(1-3)-endoglucanse, cellobiohydrolase, and β-(1→4)-endoglucanase	2168	62

[a] Data are the average of duplicate digestions. Enzyme concentrations were for cellobiohydrolase 30 μg/mL, for β-(1→4)endoglucanase 30 μg/mL, and for β-(1→3)-endoglucanse 100 μg/mL.

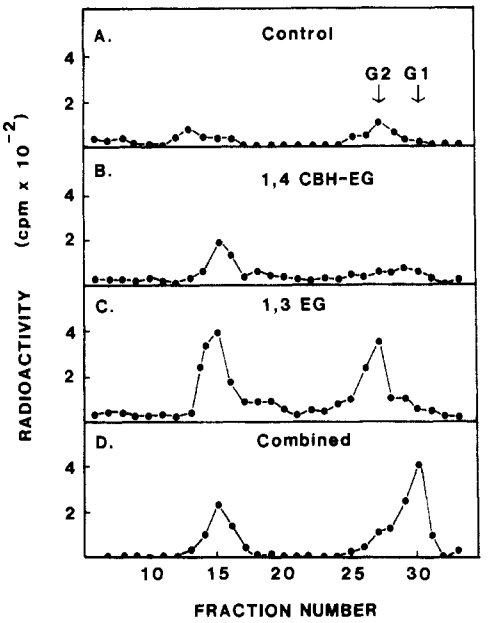

Figure 7. Bio-Gel P-2 chromatography of the enzymatic digestion products of the 5000*g* particulate fraction. (A) Incubated without enzymes; (B) β-(1→4)-cellobiohydrolase (30 μg/mL) and β-(1→4)-endoglucanase (30 μg/mL); (C) β-(1→3)-endoglucanase (100 μg/mL); (D) mixture containing the three enzymes.

indicate that the microsomal products could be partially solubilized by digestion with enzymes which cleave both β-(1→3) and β-(1→4) linkages. The most effective treatment was the three enzymes combined, which resulted in release of over 60% of the counts. These data suggest that the microsomal products contain β-(1→3) linkages. Since treatment with these enzymes did not result in complete solubilization, it is very likely that additional linkage types are contained as well.

The 5000*g* tissue disk fraction was analyzed by glucanase digestion followed by gel filtration chromatography of solubilized products (Figure 7). Figure 7A shows that in the controls, low levels of high molecular weight material (fractions 12–16) and glucose dimer (fraction 27) were released. Higher levels were solubilized by treatment with both the individual and combined β-(1→3)- and β-(1→4)-glucanases. The β-(1→4)-endoglucanase and cellobiohydrolase treatment (Figure 7B) resulted in the solubilization of a high molecular weight product but was ineffective in hydrolyzing the polysaccharide into mono- or disaccharide. In contrast, a significant proportion of the product cleaved by the β-(1→3)-glucanase was glucose dimer (Figure 7C, fraction 27). Thus, β-(1→3) linkages appear to be present in the glucan product. The combined β-(1→3)- and β-(1→4)-glucanase treatments were most effective in hydrolyzing the product into glucose (Figure 7D). The predominance of glucose in the hydrolysis products of the combined glucanase treatments indicates the presence of β-glucosidase activity in one of the enzyme preparations.

DISCUSSION

These results demonstrate that the red beet root contains a highly active glucan synthase II system. Specific activities of the various red beet microsomal enzyme preparations were between 4 and 8 times greater than values reported for the potato tuber enzyme (Heiniger, 1983), approximately twice as high as a plasma membrane enriched fraction from carrot suspension cells (Boss and Ruesink, 1979), and equivalent to a highly active crude membrane preparation specific for the synthesis of β-(1→4) linkages from mung bean (Callaghan and Benziman, 1984). The pH optimum of the beet enzyme was 7.0 with 50% maximal activity was observed at pH 6 and at pH 8. The glucan synthase from carrot showed a similar pH dependence (Boss and Ruesink, 1979). The red beet enzyme, like most glucan synthases, is activated by divalent cations. Maximal activity was obtained in the presence of 5 mM Mg^{2+}, with inhibition at higher concentrations. The enzyme from detached cotton fibers also exhibited maximal activity at 5 mM Mg^{2+} (Delmer et al., 1977). Cellobiose also stimulated activity. Under these conditions microsomes incorporated over 30% of the added substrate into alkali and chloroform–methanol insoluble products when reactions were continued to completion.

The high degree of overlap observed between the plasma membrane and Golgi marker activities on sucrose gradients (Figure 6) with red beet membranes was consistent with results obtained in a previous study (Poole et al., 1984). It was therefore difficult to definitively quantitate the relative distribution of activity between the two fractions. The data presented in Figure 6 suggest that glucan synthase activity is present predominantly in the plasma membrane with lesser amounts associated with the Golgi. This interpretation is supported by the observation that plasma membranes are derived from Golgi vesicles and continuity between the plasma membrane and Golgi has been observed (Mollenhauer and Morré, 1980). In addition, Mueller and Maclachlan (1983) have shown, using radioautography, that incorporation of [^{14}C]glucose from UDPG occurs at the plasma membrane surface.

In tissue slices, incorporation was also high with over 30% of the UDPG converted to product. Six percent of the radioactivity was recovered in the 1000*g* cell wall fraction, and 20% was found in the 1000–5000*g* pellet, which contained finely ground cell wall fragments. Cytochrome *c* oxidase assays (data not shown) showed that this pellet also contains mitochondria. The fact that much smaller amounts of radioactivity were incorporated into the 13000*g* fraction (Table I), but that the level of cytochrome *c* oxidase activity found in the 13000*g* fraction was equivalent to the 5000*g* fraction, suggests that the reaction products are associated with cell wall material or plasma membrane fragments containing nascent polysaccharide chains rather than with mitochondria. These findings bear a similarity to the study by Anderson and Ray (1978).

Structurally, both microsomal and tissue slice products were found to contain β-(1→3) and β-(1→4) linkages. At no time during enzymatic product characterization was digestion complete. This suggests that other linkage types may be present.

The production of a mixed-linkage glucan in beet, rather than true cellulose, is consistent with the kinds of products obtained with particulate fractions from onion stem (Van Der Woude et al., 1974), pea stem (Raymond et al., 1978; Anderson and Ray, 1978), ryegrass (Henry and Stone,

1982), and suspension-cultured sugarcane cells (Robinson and Glas, 1983). In these studies, incubations were performed at both micromolar and millimolar levels of UDPG. With pea and onion stem, the ratio of β-(1→4) to β-(1→3) linkages found in glucan products was highest at micromolar levels of UDPG.

From the standpoint of texture modification, it would be desirable to selectively introduce specific linkage types. As of now, in vitro cellulose biosynthesis has not yet been demonstrated; it is only possible to synthesize callose in situ. It has been suggested that callose is synthesized as a cellulose precursor (Meier et al., 1981); however, direct proof is lacking. There has been speculation that cytoskeletal interactions or that the presence of fibrils adjacent to the external face of the plasma membrane influences product structure. Understanding the enzymatic regulation of cellulose vs. callose biosynthesis would constitute a major step toward the development of biotechnological techniques for achieving selective textural modifications in vegetable tissue.

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Registry No. Mg, 7439-95-4; Ca, 7440-70-2; Hg, 7439-97-6; glucan synthase, 9032-91-1; cellobiose, 528-50-7; cellobiohydrolase, 37329-65-0; B-(1→3)endoglucanase, 9025-37-0; B-(1→4)endoglucanase, 9012-54-8.

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