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SYNTHESIS OF WALL GLUCAN FROM SUCROSE BY ENZYME PREPARATIONS FROM *PISUM SATIVUM*

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Abstract—Radioactive sucrose, supplied through the cut base to *Pisum sativum* epicotyls, was transported to the growing apex (plumule and hook) and used there for the synthesis mainly of uridine diphosphoglucose (UDP-glucose), fructose and cell wall glucan. Enzyme extracts of the apical tissue contained sucrose synthetase activity which was freely reversible, i.e. formed UDP-glucose and fructose from sucrose (pH optimum = 6·6 for the cleavage reaction, K_m for sucrose = 63 mM). Particulate fractions of the same tissue contained a β-glucan synthetase which utilized UDP-glucose for formation of alkali-soluble and -insoluble products (pH optimum = 8·4, K_m for UDP-glucose = 1·9 mM). Values for V_{max} and yields of these two synthetase activities were sufficient to account for observed rates of cellulose deposition during epicotyl growth (15–25 μg/hr/epicotyl). When soluble pea enzyme was supplied with sucrose and UDP at pH 6·6 and then the preparation was supplemented with particles bearing β-glucan synthetase at pH 8·4, the glucose moiety of sucrose was converted to glucan *in vitro*. The results indicate that it is feasible for these synthetases to co-operate *in vivo* to generate β-glucan for expanding cell walls.

INTRODUCTION

UDP-GLUCOSE is used as a glucosyl donor for formation of sucrose and cell wall β-glucan by soluble and particulate enzyme preparations respectively from many higher plant tissues, including pea epicotyls.[1–3] In apical growing regions of epicotyls, deposition of wall-glucan (e.g. cellulose) is well known to take place[4–6] as a normal, and probably essential, part of the process of cell enlargement. Sucrose can also be synthesized from hexoses during epicotyl growth, however, this reaction may not be important in the intact seedling where most of the sucrose probably translocates to growing regions from cotyledons. Since sucrose synthetase (E.C. 2.4.1.13) is present in the epicotyl,[1] and this enzyme from other plants has been found to catalyze sucrose cleavage,[7–9] the following sequence of reactions may take place:

$$\text{sucrose} + \text{UDP} \underset{\text{synthetase}}{\overset{\text{sucrose}}{\longleftrightarrow}} \text{UDP-glucose} + \text{fructose}$$

$$\text{glucan}_n + \text{UDP-glucose} \xrightarrow[\text{synthetase}]{\text{glucan}} \text{glucan}_{n+1} + \text{UDP}.$$

[1] MACLACHLAN, G. A., DATKO, A. H., ROLLIT, J. and STOKES, E. (1970) *Phytochemistry* **9**, 1023.
[2] SPENCER, F. S., ZIOLA, B. and MACLACHLAN, G. A. (1971) *Can. J. Biochem.* **49**, 1326.
[3] RAY, P. M. (1973) *Plant Physiol.* **51**, 601.
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[7] AVIGAD, G. and MILNER, Y. (1966) in *Methods in Enzymology* (NEUFELD, E. F. and GINSBURG, V., eds.), Vol. 8, pp. 341–345, Academic Press, New York.
[8] PRESSEY, R. (1969) *Plant Physiol.* **44**, 759.
[9] GRIMES, W. J., JONES, B. L. and ALBERSHEIM, P. (1970) *J. Biol. Chem.* **245**, 188.

Such a pathway for wall synthesis from sucrose is especially attractive because it is energetically conservative compared to alternative routes which utilize invertase (E.C. 3.2.1.26) and UDP-glucose pyrophosphorylase (E.C. 2.7.7.9). Certainly sucrose and starch synthetases (e.g. from rice[10] or corn[11]) can be combined *in vitro* and made to co-operatively synthesize α-1,4-glucan from sucrose as in the above reactions. Also, developmental studies (using potato[8] and mung bean[12]) have demonstrated relatively high activity levels of sucrose synthetase in non-photosynthetic and immature tissue, where a clear role for the enzyme in starch synthesis can be envisioned. In the pea epicotyl there is almost no starch,[4,5] nevertheless in meristematic regions where wall glucans are rapidly formed there is a large excess of free fructose over glucose and very low invertase activity.[1] This pea system was therefore employed for tests both *in vivo* and *in vitro* of the feasibility and likelihood that the glucose moiety of sucrose could be transferred to wall glucan by such coupled reactions.

RESULTS AND DISCUSSION

Sucrose metabolism in vivo

Table 1 records the main products which were formed from labelled sucrose in various sections of the third internode of pea epicotyls when the sugar was supplied through cut bases. In the brief time of this experiment (60 min), the only labelled products detected by chromatography and radioautography were free sugars, phosphorylated glucose derivatives including UDP-glucose (origin material) and wall polysaccharides. Total label at any one time decreased from base to apex, and uptake continued for at least 3 hr. Sucrose was the major labelled component in every section of the epicotyl except in the elongating region (0–10 mm), where its inversion to equal concentrations of glucose plus fructose was most pronounced. This is the region of highest invertase activity.[1] In the most apical section (plumule plus hook, P + H), where invertase activity is relatively very low, labelled fructose became much more concentrated than labelled glucose. At the same time, the proportions of label found in wall materials and at the origin of chromatographs was highest in this apical region.

TABLE 1. PRODUCTS DERIVED FROM ^{14}C-SUCROSE SUPPLIED THROUGH THE BASE OF EXCISED *Pisum sativum* EPICOTYLS*

Epicotyl section	Total cpm incorporated	% total cpm in: Origin	GLC-1-P	Sucrose	Glucose	Fructose	Wall
P + H	6620	18·7	8·0	32·8	7·6	16·3	16·6
0–10 mm	7590	6·3	3·0	15·2	33·9	36·2	5·5
10–20 mm	13900	4·4	1·8	41·0	25·9	22·0	4·9
20–30 mm	24900	3·3	0·9	62·8	13·7	14·0	5·4

* 10 epicotyls were excised 45 mm below the plumule, their bases were immersed in 1·5 ml U-^{14}C-sucrose (100 μCi, 186 × 10^6 cpm, 7·3 mg) and they were incubated at 25° in darkness in a closed humid chamber. At 60 min, epicotyls were removed and separated sections thoroughly extracted with 70% EtOH. Radioactivity in soluble components was assayed following chromatography and radioautography (as in enzyme assays, see Experimental). The EtOH-insoluble residue was dried and counted by scintillation: it is referred to here as wall since all detectable ^{14}C was present in polysaccharide. Similar patterns of label distribution were observed in shorter (15 or 30 min) and longer (120–180 min) experiments. P + H is plumule plus hook.

[10] MURATA, T., SUGIYAMA, T. and AKAZAWA, T. (1964) *Arch. Biochem. Biophys.* **107**, 92.
[11] DEFEKETE, M. A. R. and CARDINI, C. E. (1964) *Arch. Biochem. Biophys.* **104**, 173.
[12] DELMER, D. P. and ALBERSHEIM, P. (1970) *Plant Physiol.* **45**, 782.

These observations are consistent with the translocation of sucrose intact into apical regions of the epicotyl, followed by its cleavage there by sucrose synthetase to form fructose plus UDP-glucose, with the latter then available for wall synthesis. The only other major labelled product detected in these experiments was glucose-1-phosphate, and the fact that it was present in every section at all times at close to half the concentrations of UDP-glucose (Table 1) suggests that an equilibrium existed between these components catalysed by UDP-glucose pyrophosphorylase.[13,14] It is, of course, via such an interconversion that any hexoses derived from sucrose by invertase activity could be activated for wall synthesis, albeit with relative energetic inefficiency.

TABLE 2. FRACTIONATION OF SYNTHETASE ACTIVITIES*

Enzyme	Substrate	nmoles glucose transferred/hr by enzyme from 1 section 750–20 000 *g* pellet	20 000–140 000 *g* pellet	140 000 *g* supernatant
Sucrose synthetase	UDPG	9·5	4·5	120·4
Glucan synthetase				
Alkali-soluble product	UDPG	15·1	1·2	0·7
	GDPG	0·19	0·48	Nil
Alkali-insoluble product	UDPG	12·3	0·02	Nil
	GDPG	0·13	0·07	Nil

* Apical regions of epicotyls (P + H plus 0–10 mm) were homogenized (see Experimental) and fractionated as above. The low- and high-speed pellets and supernatant contained 0·10, 0·03 and 0·25 mg protein/section respectively. Sucrose synthetase assay mixtures (0·1 ml) contained: supernatant enzyme (0·1–0·2 mg protein) UDP-^{14}C-glucose (10 mM, 168 000 cpm), Tris buffer, pH 8·0 (100 mM), $MgCl_2$ (5 mM), dithiothreitol (5 mM) fructose (30 mM, omitted in controls). Synthesis of fructose-dependent ^{14}C-sucrose was estimated after 60 min incubation using chromatography and radioautography (see Experimental). Glucan synthetase assay mixtures (0·1 ml) contained particulate protein (0·1–0·3 mg) and the same additives (without fructose) as above. After 15 min incubation, 70% ethanol-insoluble products were washed with buffer and extracted with hot alkali (see Experimental). Each value in this table is an average of triplicate samples which agreed within 10%.

Fractionation of synthetases

Using high concentrations (10 mM) of UDP-^{14}C-glucose as substrate, the initial rates of glucosyl transfer to sucrose or glucan was measured in assay mixtures containing soluble or particulate fractions prepared from apical regions of the pea epicotyl. The results (Table 2) show that about 90% of total sucrose synthetase activity was in the supernatant of centrifuged homogenates, whereas 90% of glucan synthetase activity sedimented in a low-speed particulate fraction. The most active fractions of each enzyme were used in later assays.

The homogenization technique in this experiment (rapid chopping with razor blades[14]) was also used in subsequent tests because conventional methods (grinding or shearing) resulted in less sharply resolved and more readily suspended glucan synthetase activity. No substantial part of sucrose synthetase activity was found associated with a particulate fraction in any of these tests, despite the possibility[9,12] that it is membrane-bound *in vivo*.

UDP-glucose was used as a substrate in preference to GDP-glucose because the latter was less effectively incorporated into glucan under standard assay conditions in this test.

[13] GUSTAFSON, G. L. and GANDER, J. E. (1972) *J. Biol. Chem.* **247**, 1387.
[14] MORRÉ, D. J. (1971) *Isolation of Golgi Apparatus*, in *Methods in Enzymology* (JACOBY, W. B., ed.), Vol. 22, pp. 130–148. Academic Press, New York.

At much lower concentrations of these substrates (5 μM), GDP-glucose was usually a more effective donor (see also Refs. 2, 3), but the absolute amount of incorporation was relatively very low (<0·1% of values for UDP-glucose in Table 2). The products formed in the present test were about half alkali-soluble and half alkali-insoluble and together these made up the bulk (>80%) of the total 70% ethanol-insoluble product.

Synthetase activities and wall deposition in vivo

Table 3 gives values for amounts of a number of components of various sections of the pea epicotyl. The meristematic (P + H) regions contained the highest levels (per section or per mg fr. wt) of total wall components, protein and DNA, as well as the greatest recoveries of sucrose and glucan synthetases. However, calculated on a DNA basis (related to cell number), all of the above components were present at their lowest levels in the meristem, and increased during development (see also Refs. 1 and 3). Similar patterns of development were observed for cellulose[5] and certain glycosidase[15] and sugar phosphatase[1] activities in the pea epicotyl (but see distribution of β-glucosidase[15] and invertase[1] activities).

TABLE 3. PROPERTIES AND COMPONENTS OF PEA EPICOTYL SECTIONS*

Epicotyl section	Fr. wt (mg)	DNA (μg)	Total protein (μg)	Wall materials (μg) 70% EtOH -insoluble	Wall materials (μg) Alkali -insoluble	Synthetase activities (μg glucose transferred/hr) Sucrose synthetase	Synthetase activities (μg glucose transferred/hr) Glucan synthetase
P + H	9·1	64·4	660	505	298	25·4	5·2
0–10 mm	19·5	8·2	338	395	249	15·5	2·7
10–20 mm	24·0	3·1	132	301	195	18·0	1·5
20–30 mm	26·6	2·7	132	300	180	9·8	1·8

* Details of assay methods for non-enzymic components are described in Experimental. Enzymic assays utilized UDP-glucose. Sucrose synthetase assay mixtures (0·1 ml) contained dialyzed supernatant enzyme (0·4–0·9 mg protein), and glucan synthetase assay mixtures (0·1 ml) contained particulate enzyme (0·1–0·3 mg protein). Other ingredients and conditions were as in Table 2.

From these data (Table 3), and the simple observation that intact pea epicotyls grow from the apex *ca.* 20 mm/day, it is possible to estimate the rates of wall deposition *in vivo*. The growth rate is responsible for adding a region of epicotyl equivalent to the 2 basal sections (10–30 mm) which were studied here, i.e. a total of about 600 μg ethanol-insoluble wall material, including 375 μg alkali-insoluble material (cellulose), deposited per day. The yield of sucrose synthetase activity from apical regions which were responsible for this wall formation could account for the required rates of glycosyl transfer (*ca* 15–25 μg/hr/epicotyl) assuming that it was freely reversible (see below). The glucan synthetase activity, although relatively low (by factors of 2–5) in relation to rates of glucan deposition *in vivo* in this particular test, was often recovered in adequate amounts in later tests (e.g. 27 μg glucan deposited/hr/P + H section, Fig. 1a).

Synthetase properties

The rate of sucrose formation from saturating concentrations of UDP-^{14}C-glucose plus fructose by soluble pea epicotyl enzyme preparations was constant for at least 1 hr at 30°, pH 8 (Fig. 1a). This pH was close to the optimum for activity in the direction of

[15] DATKO, A. H. and MACLACHLAN, G. A. (1970) *Can. J. Botany* **48**, 1165.

sucrose synthesis (Fig. 1b), and under such conditions the reaction rate was proportional to protein levels up to 10 mg/0·1 ml reaction solution. Glucan synthetase activity using UDP-glucose as substrate also had a pH optimum close to 8, but its reaction velocity was linear for only about 15 min. These time and pH limitations were observed in all subsequent assays for the two synthetase activities.

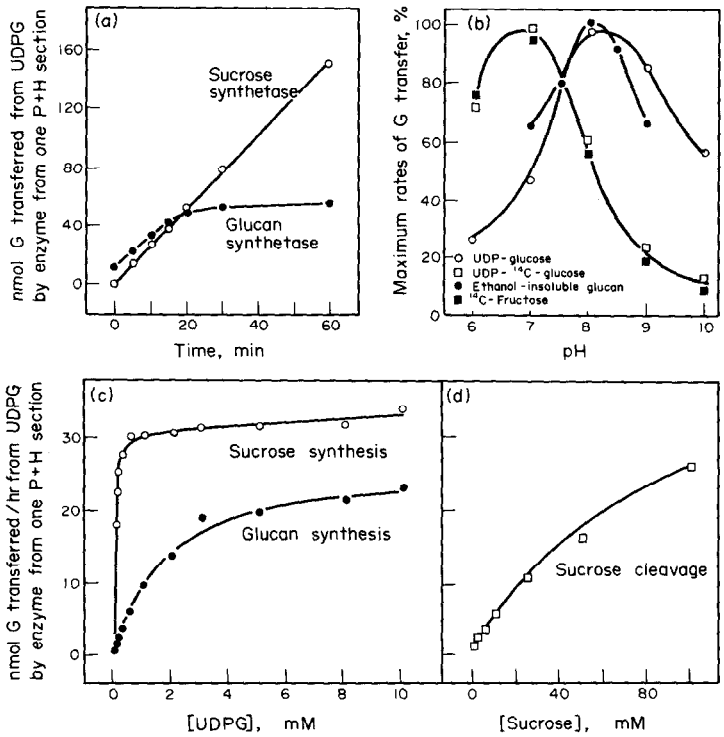

FIG. 1. CHARACTERIZATION OF PEA EPICOTYL SUCROSE AND GLUCAN SYNTHETASES.

(a) Progress of glucosyl transfer from UDP-glucose into sucrose and into 70% ethanol-insoluble wall material. Sucrose synthetase assay mixtures (0·2 ml) contained: supernatant enzyme (0·5 mg protein from 3·6 P + H sections) and other ingredients as in Table 2. Glucan synthetase assay mixtures (0·1 ml) contained the same ingredients (fructose omitted) supplied to particulate enzyme (0·2 mg protein from 3·2 P + H sections).

(b) Effect of pH on synthetase activities. Sucrose synthesis from UDP-glucose was measured as in Table 2 except that $MgCl_2$ was omitted and the buffer was citrate–phosphate–borate–veronal (each at 50 mM) adjusted to various pH values. Sucrose cleavage was measured by the amount of UDP-^{14}C-glucose and ^{14}C-fructose formed in 60 min. Assay mixtures (0·1 ml) contained: ammonium-sulfate enzyme (0·5 mg protein from 12·5 P + H sections), U-^{14}C-sucrose (100 mM, 625 000 cpm), glycine-Tris-cacodylate (each 50 mM) adjusted to various pHs, $MgCl_2$ (5 mM), dithiothreitol (5 mM), UDP (50 mM, omitted in controls). Synthesis of total ethanol-insoluble glucan was measured as described in Table 2, except that the buffer used was glycine-tris-cacodylate. At the pH optima observed in these tests, nmol glucose transferred/hr/section were 20·2 and 120 for synthesis and cleavage of sucrose respectively, and 33·2 for synthesis of glucan.

(c) Effect of UDP-glucose concentration on rates of sucrose and glucan synthesis. Assay mixtures 0·1 ml) contained supernatant or particulate enzyme plus other ingredients as in Table 2. Reciprocal plots of the data yielded straight lines and K_m of 0·08 mM for sucrose synthetase and 1·8 mM for glucan synthetase.

(d) Effect of sucrose concentration on rates of sucrose cleavage to UDP-glucose. Assay mixtures (0·1 ml) contained ^{14}C-sucrose (500 000 cpm), UDP (100 mM), and other ingredients as in Fig. 1b; $K_m = 63$ mM.

In contrast to the above reactions in the direction of synthesis, the pH optimum for UDP-dependent cleavage of sucrose to form UDP-glucose plus fructose was close to 6·6 (Fig. 1b). The maximum velocities for the synthesis and cleavage reactions were comparable at pH 8·0 and above (cf. Fig. 1c and d), but the equilibrium favoured cleavage at lower pH values. Similar relationships were observed for the enzyme extracted from sugar beet[7] and potato.[8]

TABLE 4. SYNTHESIS OF GLUCAN FROM SUCROSE *in vitro*

Pre-incubation* of ^{14}C-sucrose with sucrose synthetase (pH 6·6)		Incubation† for 15 min with glucan synthetase (pH 8·4) 70% EtOH-insoluble (cpm)	Alkali-insoluble (cpm)
−UDP	for 60 min	176	60
	0 min	131	86
	Δ	45	Nil
+UDP	for 60 min	839	421
	0 min	77	50
	Δ	762	371

* Pre-incubation mixtures (0·125 ml) contained pea sucrose synthetase (1 mg ammonium sulfate enzyme), U-^{14}C-sucrose (2 mM, 1·1 × 10^6 cpm), sodium cacodylate (20 mM, pH 6·6), $MgCl_2$ (5 mM) and dithiothreitol (5 mM) ± UDP (50 mM). After 60 min, chromatographs showed UDP-dependent synthesis of labelled origin material (18 500 cpm) and a comparable excess of free fructose over glucose (21 400 cpm).

† Incubation mixtures (0·35 ml) contained complete pre-incubation mixtures as above (±UDP ± 60 min) plus particulate glucan synthetase (3·4 mg protein from P + H region), unlabelled UDP-glucose (0·25 mM), $MgCl_2$ (5 mM), dithiothreitol (5 mM), Tris (100 mM, pH 8·4). Following 15 min reaction 70% EtOH-insoluble and alkali-insoluble products were isolated and radioactivity determined (as in Experimental).

The K_m value for UDP-glucose as substrate for sucrose synthesis (fructose saturating) was 0·06 mM (Fig. 1c). This concentration is similar to that reported for the mung bean epicotyl enzyme,[9] but much lower (i.e. 1/10) than K_m values for sucrose synthetase in starch-synthesizing tissues.[7,8,16,17] The K_m value for UDP-glucose as a substrate for pea β-glucan synthetase was 1·8 mM (Fig. 1c), which is remarkably close to reported values for starch synthetase.[11] The K_m value for sucrose in the cleavage reaction (UDP saturating, pH 8) was 63 mM (Fig. 1d), a concentration often reached in plant tissues and midway between previously reported K_m values.[8,16–18] The sucrose K_m would be expected to be lower at lower pH values (closer to the optimum). These observations indicate that it is kinetically feasible for sucrose synthetase to cleave sucrose and generate UDP-glucose which is then available for synthesis of starch or other glucans, provided the appropriate pH optima and substrate concentrations exist.

Combined synthetase activities

The K_m values observed above (Fig. 1c, d) were all measured at pH 8·0, which is close to the optimum for glucan and sucrose synthesis but much higher than the optimum for

[16] MURATA, T. (1971) *Agr. Biol. Chem.* **35**, 1441.
[17] MURATA, T. (1972) *Agr. Biol. Chem.* **36**, 1815.
[18] DELMER, D. P. (1972) *Plant Physiol.* **50**, 469.

sucrose cleavage (Fig. 1b). At this pH, the relative V_{max} and K_m values for UDP-glucose utilization favored sucrose synthesis. It was not surprising, therefore, that efforts to synthesize glucan from sucrose in a coupled reaction with pea enzymes at pH 8 were not successful. The fact that sucrose synthetase would couple to starch synthetase at high pH values[10,11] can be accounted for by the relatively high K_m value characteristic of sucrose synthetase in starch-forming tissues.

Nevertheless, it was possible to demonstrate glucan synthesis from sucrose using pea enzymes *in vitro* by pre-incubating sucrose with soluble sucrose synthetase at its pH optimum for production of UDP-glucose, followed by incubation of the whole reaction mixture with particulate glucan synthetase at its pH optimum. The results of a typical experiment are shown in Table 4. In this test, in addition to the sucrose which was inverted (*ca.* 5%), 2% of the ^{14}C supplied as sucrose was converted to UDP-glucose (and another 2% to fructose) during pre-incubation. Subsequently, in 15 min with added particulate enzyme, about 5% of this UDP-glucose was converted to glucan. Control values (minus UDP or without pre-incubation) were negligible.

Thus, the glucose moiety of sucrose can be converted to wall β-glucan via UDP-glucose and co-operative actions of sucrose and glucan synthetases. The fructose moiety of sucrose, which accumulates in meristematic regions, can also enter wall material in time;[1] presumably following phosphorylation, isomerization and the action of UDP-glucose pyrophosphorylase.[10,12] Questions of whether *in vivo* there is any physical association between these enzymes, or hormonal regulation of them, which would increase the likelihood of sucrose being utilized for the purpose of wall synthesis during growth, are currently under study in this laboratory.

EXPERIMENTAL

Plant material.[1] Seeds of *Pisum sativum* L. var. Alaska were surface-sterilized with 0·5% NaOCl and grown in vermiculite in darkness for 8 days, or until 3rd internodes of epicotyls were 30–50 mm long. The excised plumule and hook region (P + H), which is composed of small meristematic or embryonic cells, served as the enzyme source for most characterization studies. Sequential 10 mm sections below P + H were removed for analysis in developmental studies. DNA was estimated in hot $HClO_4$ extracts of these tissue sections with diphenylamine.[19] Crude insoluble wall material was collected after thoroughly homogenizing with 0·02 M sodium phosphate buffer pH 7, adding EtOH to 70% (v/v), washing with 70% EtOH, and drying. Dry weight of this fraction minus total protein (assayed with the biuret reagent[20]) is referred to as "70% ethanol-insoluble wall material". Non-cellulosic components of this fraction were extracted with N NaOH (3 × 10 min at 85°) and washing with H_2O and EtOH. The dry wt of this final residue is referred to as "alkali-insoluble wall material". Anthrone assays indicated that it was >90% hexosan (cellulose). Soluble sugars and phosphorylated derivatives in the 70% EtOH-soluble supernatants and washes of tissue homogenates were chromatographed and radioautographed as described below for enzyme assays. They were estimated after elution by the Nelson modification[21] of the Somogyi procedure.

Enzyme fractions. 3–10 g freshly harvested tissue sections were homogenized at 2° for 3 min in a motorized razor blade chopper[14] at 20–30 chops per sec using 2 vols 0·1 M Tris, pH 8, plus 5 mM dithiothreitol. The homogenate was squeezed through 2 layers of nylon and re-extracted as before. Pooled filtrates were centrifuged in the cold for 10 min at 750 *g* to remove wall debris and unbroken cells, and again for 1 hr at 20000 *g* to provide a particulate pellet. This was suspended in a small vol. of buffer and aliquots were used for all assays of glucan synthetase activity (unless otherwise noted). Sucrose synthetase activity was assayed in the 140000 *g* soluble fraction (supernatant enzyme). It was sometimes dialyzed overnight in the cold vs H_2O and then concentrated by dialysis for 1–2 hr vs polyethylene glycol (dialyzed supernatant enzyme). Alternatively, supernatant enzyme was precipitated with $(NH_4)_2SO_4$ at between 20 and 60% saturation and the precipitate resuspended in buffer and desalted on a Sephadex G 25 column (ammonium-sulfate enzyme).

[19] BURTON, K. (1966) *Biochem. J.* **62,** 315.
[20] GORNALL, G., BARDWILL, C. J. and DAVID, M. M. (1949) *J. Biol. Chem.* **177,** 751.
[21] HODGE, J. E. and HOFREITER, B. T. (1962) in *Methods in Carbohydrate Chemistry* (WHISTLER, R. L. and WOLFROM, M. L., eds.), Vol. 1, pp. 380–394, Academic Press, New York.

Enzyme assays. Sucrose synthetase assays for the formation or cleavage of sucrose were performed at 30° for 60 min unless otherwise stated. Reactions were stopped either by drying mixtures on Whatman No. 1 paper, or by adding EtOH to 70%. The 70% EtOH-insoluble precipitates were washed with 70% EtOH and pooled extracts plus washings were taken to dryness and transferred to chromatography paper. Chromatographs were run for 40 hr in *n*-PrOH–EtOAc–H_2O (7:1:2). Radioautographs were made by exposing X-ray film to the chromatographs for 2–10 days. Areas of chromatographs occupied by labelled products were cut out and counted by standard liquid scintillation techniques. Sucrose synthetase activity was determined in the direction of sucrose synthesis by measuring the difference in amounts of ^{14}C-sucrose formed from UDP-^{14}C-glucose when fructose was present in the assay medium and when it was omitted. In present tests, from 20 to 160 times more radioactivity was incorporated into sucrose when fructose was included. Sucrose cleavage was determined by measuring the difference in formation of UDP-^{14}C-glucose or ^{14}C-fructose from ^{14}C-sucrose in the presence and absence of UDP. There was no interference from invertase activity in these assays, partly because reaction conditions were not favorable (e.g. the pH optimum is 5 for pea invertase activity[1]), and also the chromatographic methods and controls took into account any sucrose inversion that might have occurred. Glucan synthetase assay media contained UDP-^{14}C-glucose (or GDP-^{14}C-glucose) in concentrations indicated in Table and Figure legends. Incubations were at 30° for 15 min unless otherwise stated. Reactions were terminated by the addition of EtOH to 70%. Newly-synthesized ^{14}C-glucan was collected by centrifugation and washed with 2 × 1 ml 70% EtOH and/or buffer followed by 3 × 1 ml N NaOH (85°, 10 min). Precipitates were washed successively with H_2O and EtOH to remove alkali. Radioactivity in dried precipitates was determined either by a Nuclear Chicago gas flow system (24·6% efficiency) or by standard liquid scintillation techniques (80% efficiency). The use of 70% EtOH as initial washing solvent for products formed in this procedure was intended to exclude low MW components, e.g. excess substrate, and to include all wall material. The subsequent wash with buffer removed any starch present and some pectin[4] but, in present tests, the fraction of total labelled product which was buffer-soluble amounted to no more than 20%. The alkali treatment was sufficient to ensure that any glucan remaining insoluble was of high-MW and β-1,4 linked (cellulose).[5] Recent studies[3] on properties of the alkali-soluble and -insoluble glucans formed from UDP-glucose by pea enzyme showed that both fractions contained β-1,4 linkages and no detectable β-1-3 linkages.

Chemicals. UDP-^{14}C-glucose (Sp. act. 203 mCi/mM), GDP-^{14}C-glucose (Sp. act. 170 mCi/mM and uniformly-labelled ^{14}C-sucrose (Sp. act. 300–500 mCi/mM) were purchased from New England Nuclear Corp. or International Chemical and Nuclear Corp.

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