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STACHYOSE SYNTHESIS IN LEAVES OF *CUCURBITA PEPO*

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Abstract—An enzyme synthesizing stachyose, galactinol-raffinose galactosyltransferase (EC 2.4.1.67), has been purified *ca* 40-fold from mature leaves of *Cucurbita pepo* using ammonium sulphate precipitation, Sephadex gel filtration and DEAE-Sephadex gel chromatography. The purified enzyme fraction was separated from all but 2% of the total α-galactosidase activity extracted from the tissue. The enzyme was optimally active at pH 6.9 and was stable for at least a month at 4° in the presence of 20 mM 2-mercaptoethanol. The enzyme displayed high specificity for the donor galactinol (K_m 7.7 mM) and the acceptor raffinose (K_m 4.6 mM) and was unable to effect synthesis of any other member of the raffinose series of galactosyl-sucrose oligosaccharides. Co^{2+}, Hg^{2+}, Mn^{2+} and Ni^{2+} ions were particularly inhibitory; no metal ion promotion was observed and 5 mM EDTA was ineffective. Myo-inositol was strongly inhibitory (K_i 2 mM), melibiose weakly so. Tris buffer (0.1 M) was also inhibitory. Galactinol hydrolysis occurred in the absence of the acceptor raffinose but there was no hydrolysis of either raffinose or stachyose in the absence of the donor galactinol. The reaction was readily reversible and exchange reactions were detected between substrates and products. It is proposed that the synthesis of stachyose in mature leaves of *C. pepo* proceeds via this galactosyltransferase and not via α-galactosidase.

INTRODUCTION

The galactosyl-sucrose oligosaccharides of the raffinose family, (*O*-α-D-galactopyranosyl-1 → 6)$_n$-sucrose, are commonly found in seeds and other storage tissues [1–3]. They also occur in the mature leaves and vascular tissues of a more restricted number of plant species where they accompany sucrose as the principal sugars translocated in the phloem [4, 5]. Senser and Kandler [6] have suggested from *in vivo* tracer studies with mature leaves, that galactinol (1L-1-*O*-α-D-galactopyranosyl-myo-inositol) in the presence of a galactosyltransferase is the most likely donor of the galactosyl moieties required for synthesis of these oligosaccharides. Additional evidence was obtained in their studies using partially purified enzyme fractions from legume seeds [7–9]. Their results indicated the probability that more than one galactosyltransferase was involved in synthesis of the series. In *Vicia faba* seeds two distinct protein fractions were obtained, one specific for raffinose synthesis and the other for both stachyose and verbascose synthesis while in *Phaseolus vulgaris* a protein fraction was obtained specific only for stachyose synthesis. We have reported the similar ability of a crude protein fraction obtained from mature leaves of *Cucurbita pepo* to synthesize stachyose by transfer of the galactosyl moiety from galactinol to raffinose [10]. We have now extended our investigation of this leaf fraction and report here the partial purification and characterization of 1-*O*-α-D-galactosyl-myo-inositol: raffinose galactosyltransferase (EC 2.4.1.67) and comment on the physiological role of this enzyme.

RESULTS

Enzyme extraction and purification

The pellet remaining after centrifugation of the leaf homogenate was unable to synthesize any detectable level of stachyose. The galactosyltransferase activity was precipitated with ammonium sulphate at between 35 and 55% saturation of the homogenate. High concentrations of ammonium sulphate appeared to have an inhibitory effect on the transferase activity when compared with the crude homogenate (Table 1); however, desalting was not investigated at this stage of the purification. For assay of the ammonium sulphate fractions the reaction products were passed through 1 ml strong anion (acetate)$^-$ exchange resin to improve their subsequent chromatographic separation.

Assaying 5-ml fractions from the Sephadex G-200 column, the galactosyltransferase emerged as a single peak of activity between fractions 20–26. α-Galactosidase activity in the eluate, assayed with *p*-nitrophenyl-α-D-galactopyranoside as substrate, began to emerge at fraction 23 with maximum activity in fractions 27 and 28 and terminating in fraction 35. Thus, considerable separation of α-galactosidase activity from the galactosyltransferase was achieved at this stage. A further separation of the two enzyme activities was achieved with the first DEAE-Sephadex column (0.1–0.5 M NaCl, pH 6.9). As indicated in Fig. 1, most of the remaining α-galactosidase activity was eluted in the salt gradient before the single peak of galactosyltransferase emerged.

Table 1. Typical purification sequence for galactosyltransferase from 50 g mature leaves

Purification step	Volume (ml)	Total enzyme activity (mU)*	Total protein (mg)	Sp. act. (mU/mg)	Recovery† (%)	Purification (fold)
Extract in phosphate buffer	177	34 500	1184	29.2	—	—
$(NH_4)_2SO_4$	12.8	6850‡	463	14.8	19.8	—
Sephadex G-200	40	25 300	77.5	326.0	73.3§	11.2§
DEAE-Sephadex (0.1–0.5 M NaCl)	5	11 000	16.5	668.0	43.6	22.9
DEAE-Sephadex (0.1–0.3 M NaCl)	5.6	9800	8.8	1110.0	88.8	38.1

* Unit of activity, 1 μmol stachyose/hr. 1 mU is equivalent to the incorporation of *ca* 600 cpm into stachyose in the standard assay system.

† Recovery is calculated relative to the previous purification step. Overall recovery: 28.4%.

‡ Strong inhibition noted.

§ Calculated from activity of phosphate buffer extract.

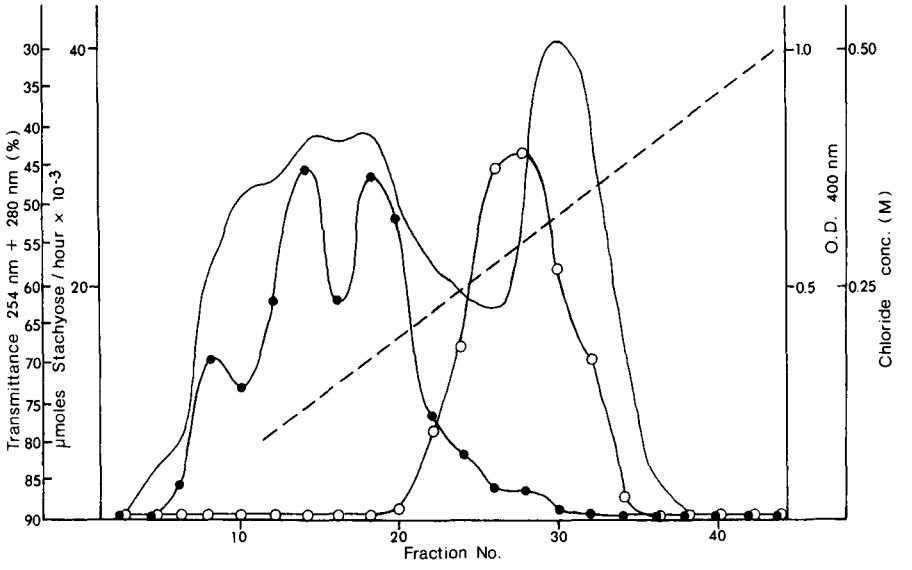

Fig. 1. Separation of galactosyltransferase from α-galactosidases on DEAE-Sephadex. ○——○, Galactosyltransferase; ●——●, α-galactosidase; ———, protein; - - - - - -, chloride ion concentration.

With the second DEAE-Sephadex column (0.1–0.3 M NaCl, pH 6.9) a single peak of galactosyltransferase activity emerged between fractions 12 and 18 which contained only 1–2% of the original total α-galactosidase activity. The stages of the purification procedure are summarized in Table 1.

To reduce the time spent on assaying fractions from the G-200 and DEAE-Sephadex columns a TLC method was devised. Good separation of the reaction products was achieved in 3 hr. Stachyose, galactinol, raffinose and galactose were detected as brown spots with R_f values of *ca* 0.2, 0.35, 0.4 and 0.7, respectively. Preliminary work comparing the TLC assay with the more time-consuming assay involving [^{14}C]galactinol, had shown that the intensity of the stachyose spot on the TLC plate was directly proportional to the amount of stachyose synthesized.

The active fractions from the second DEAE-Sephadex column were concentrated by ultrafiltration and stored at 4°. No loss of activity was observed up to 45 days of storage. Polyacrylamide gel electrophoresis (PAGE) of the galactosyltransferase preparation stained with Coomassie Blue revealed nine visible protein bands. Six bands were present in minor amounts while the remaining three were heavily stained. An attempt was made to locate the position of the galactosyltransferase by incubating 1-mm slices of the gel in the assay medium but no activity could be detected. We have found that α-galactosidase activity can be readily located in gel slices using *p*-nitrophenyl-α-D-galactopyranoside as the substrate, but we could not detect its presence in these gels. Furthermore, we could not detect any hydrolysis of either 4 mM raffinose or 4 mM stachyose when these substrates were incubated along with the galactosyltransferase under the standard assay conditions for 30 min or at pH 5.4 where α-galactosidase activity is optimally active with *p*-nitrophenyl-α-D-galactopyranoside as substrate [11].

Enzyme properties

The pH optimum for stachyose biosynthesis appeared to reach a plateau between pH 6.5 and 6.9 with *ca* 50%

reduction at pH 5.4 and 7.6. Tris buffer at 0.1 M inhibited stachyose synthesis by *ca* 50%.

Because several of the metal ions studied were precipitated in Na–Pi buffer at pH 6.9 the galactosyltransferase was transferred into MOPS buffer pH 6.9 where under otherwise standard conditions the activity was *ca* 70% that obtained in the Na–Pi buffer. Final assay concentrations of 3 mM MoO_4^{2-}, K^+, Na^+, Fe^{3+} ions and up to 5 mM EDTA had no effect on enzyme activity. Final assay concentrations of 3 mM Co^{2+}, Hg^{2+} (in the presence of 3 mM EDTA), Zn^{2+}, Mn^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}, Ag^+ and Cu^{2+} ions inhibited the reaction *ca* 78, 69, 63, 51, 48, 23, 20, 18 and 15%, respectively. Concentrations of up to 20 mM $(NH_4)_2SO_4$ were not inhibitory although, as stated above, high concentrations used in the extraction procedure were suspected to have caused considerable inhibition.

The galactosyltransferase hydrolysed galactinol when the acceptor raffinose was either absent, or present at very low concentrations. Thus during K_m determinations for raffinose concentrations at low substrate concentrations (<1 mM) free [^{14}C]galactose was released up to a maximum representing *ca* 12% hydrolysis of the [^{14}C]galactinol. This reaction was almost completely eliminated at saturation levels of raffinose (>8 mM). In the absence of raffinose a K_m of 0.33 mM for galactinol hydrolysis was obtained from a standard Lineweaver–Burk plot. As stated above, there was no detectable hydrolysis of raffinose in the absence of galactinol. For stachyose synthesis a K_m of 7.25 mM was obtained for galactinol in the presence of a saturating (10 mM) concentration of raffinose. Using the graphical procedure of Florini and Vestling [12] for determining enzyme constants for a two substrate reaction at non-saturating substrate concentrations a K_m of 7.7 mM was obtained for galactinol. Using the same procedure [12] a K_m of 4.6 mM was obtained for raffinose in the presence of non-saturating concentrations of galactinol. Both melibiose (6-*O*-α-D-galactosyl-α-D-glucose) and myo-inositol showed inhibitory effects on the synthesis of stachyose. Lineweaver–Burk plots indicated that melibiose was a non-competitive inhibitor of raffinose. At a final concentration of 8 mM melibiose in the standard assay the rate of stachyose formation decreased by *ca* 13% compared to the control. Lineweaver–Burk plots revealed myoinositol to be a competitive inhibitor. In the presence of 10 mM raffinose and 1 mM galactinol a K_i for myo-inositol of 2 mM was determined using the graphical procedure of Dixon for competitive inhibitors. Neither stachyose nor galactose were inhibitory at least up to concentrations of 40 mM in the standard assay.

The galactosyltransferase appeared to be highly specific for raffinose. However, incubation with 4 mM melibiose replacing raffinose as the galactosyl acceptor in the standard assay system resulted in the synthesis of manninotriose, α-D-galactosyl → 6-*O*-α-D-galactosyl-α-D-glucose, but at a rate of galactosyltransferase 10 times less than with 4 mM raffinose as acceptor. A K_m of 5.2 mM for melibiose was obtained under these conditions. In the presence of 4 mM galactose as acceptor two minor ^{14}C-labelled products, $R_{galactose}$ 0.33 and 0.53, respectively, were observed on the chromatogram but their identities were not investigated. No verbascose synthesis could be detected in the assay system 30 min after incubation with up to 40 mM stachyose as the acceptor molecule. In the latter assay some [^{14}C]stachyose was formed in addition to the production of free [^{14}C]galactose. The labelling of stachyose may have been caused either by a galactosyl exchange between [^{14}C]galactinol and the terminal galactosyl of stachyose or, and perhaps more likely, by the initial hydrolysis of [^{14}C]galactinol by the galactosyltransferase followed by labelling of the terminal [^{14}C]galactosyl group in stachyose through equilibration of the forward and back reactions in the synthesis of stachyose by the galactosyltransferase. The reversibility of the galactosyltransferase was readily demonstrated. A rate of 0.91 μmol [^{14}C]galactinol/hr per mg protein was observed in the presence of 8 mM stachyose and 4 mM [^{14}C]myo-inositol. Further exchange reactions occurred when the enzyme fraction was incubated in the presence of 2.1 mM galactinol and 5.7 mM [^{14}C]myo-inositol in the Na–Pi buffer at pH 6.9, producing [^{14}C]galactinol at *ca* 2.7 μmol/hr per mg protein and also when incubated in the presence of 6.2 mM [^{14}C]raffinose (galactose labelled) and 4 mM stachyose producing [^{14}C]stachyose at *ca* 0.4 μmol/hr per mg protein.

There was no detectable transfer of the galactosyl group from [^{14}C]galactinol to any of the following sugars replacing raffinose at 4 mM concentrations in otherwise standard assays: fructose, glucose, cellobiose, gentiobiose, lactose, maltose, melezitose, sucrose, trehalose, maltotriose and manninotriose. In all the above assays *ca* 10% of the galactinol was hydrolysed over 30 min resembling the results reported above for assays in the absence of the acceptor raffinose.

Neither UDP-galactose (10 mM) nor melibiose (16 mM) could replace galactinol as the galactosyl donor in the standard assay system. Incubating the enzyme with raffinose alone did not produce stachyose. However, it was found that substituting 1 mM galactinol with 4 mM *p*-nitrophenyl-α-D-galactopyranoside in the standard assay system produced a rate of stachyose synthesis *ca* 50% of that obtained with 1 mM galactinol.

DISCUSSION

As far as we are aware this is the first report of a partial purification of galactinol-raffinose galactosyltransferase from leaf tissue.

The properties of the enzyme closely resemble those of a similar enzyme obtained from seeds of *P. vulgaris*. Lehle and Tanner [9, 13] reported a pH optimum value for the formation of stachyose between 6.0 and 7.0 and K_m values for galactinol and raffinose of 7.3 and 0.84 mM, respectively. The seed enzyme also appeared to be highly specific for the donor galactinol and the acceptor raffinose and was unable to synthesize *in vitro* either raffinose or verbascose, the two homologues immediately adjacent to stachyose in the (*O*-α-D-galactopyranosyl-1 → 6)$_n$ sucrose series. We have recently obtained evidence (unpublished) of another galactosyltransferase in leaf homogenates of *C. pepo* capable of synthesizing raffinose from sucrose and galactinol, but its activity is very unstable and at present we have no knowledge of its specificity. The non-physiological substrate *p*-nitrophenyl-α-D-galactopyranoside can also act as a galactosyl donor for the synthesis of stachyose as it can for the synthesis of raffinose by a galactosyltransferase isolated from *V. faba* seeds [14]. The galactosyltransferase appears to be distinct from α-D-galactosidase galactohydrolase (EC 3.2.1.22); it separates from the bulk of the latter enzyme during fractionation and purification, it lacks an

ability to hydrolyse either raffinose or stachyose at either pH 5.4 or 6.9 and high concentrations of galactose fail to inhibit its galactosyltransferase activity at pH 6.9. A similar separation of these two enzymes was achieved from seed extracts of *V. faba* and *P. vulgaris* [9]. We propose that the hydrolysis of galactinol by the galactosyltransferase is due to a simulation of the activity of α-galactosidase galactohydrolase but only under conditions where the acceptor raffinose is absent.

The demonstration that a galactosyltransferase readily synthesizing stachyose can be separated from α-galactosidase suggests that the latter enzyme may not be involved in stachyose biosynthesis as has been proposed by several authors [15–17], at least in the leaf tissue of *C. pepo*. Furthermore, a comparison of the total activity of the galactosyltransferase (Table 1) with data previously reported for both the photoassimilation of CO_2 by mature leaves and the rate of ^{14}C incorporation into stachyose [18, 20], indicates that a more than sufficient amount of the enzyme is present in a mature leaf to account for the rate of stachyose biosynthesis. During periods of photosynthesis, mature leaves of *C. pepo* readily synthesize raffinose, stachyose and verbascose which are primarily transported along with sucrose to the immature regions of the plant where they are rapidly metabolized [4, 5]. In the immature leaves ^{14}C-tracer studies have failed to detect any synthesis of the raffinose series of oligosaccharides [18]. Their synthesis is first detectable at the semi-mature stage of leaf development [18]. We have confirmed this in so far as we have been unable to detect galactosyltransferase activity in crude extracts from immature leaves. Furthermore, we have isolated three molecular forms of α-galactosidase galactohydrolase from leaf tissues of *C. pepo* and although their respective contributions change as leaves mature the total α-galactosidase activity remains approximately constant at all stages of leaf development [19], which again suggests that α-galactosidase is not involved in stachyose biosynthesis.

EXPERIMENTAL

Plant material. Seeds of *C. pepo* L. var. *melopepo* f. *torticolis* Bailey (Early Prolific Straight-neck Squash, from W. A. Burpee, Seed Growers, Philadelphia, PA), were germinated in perlite and grown in a controlled environment cabinet [20]. Mature leaf blades from plants 3–5 weeks old were used for all enzyme extractions.

Chemicals. All sugars were purchased commercially except galactinol which was purified from mature leaves of *C. pepo* essentially according to ref. [21] and manninotriose which was prepared by mild acid hydrolysis of stachyose (0.1 N HCl; 2 hr at 45°) and isolated chromatographically pure by descending PC on Whatman No. 1, 5 × 24 hr in *n*-BuOH–pyridine–H_2O (6:4:3). Galactose-labelled [^{14}C]galactinol (1.03 μCi/μmol) was prepared by incubating a partially purified galactinol synthetase preparation from mature *C. pepo* leaves (Webb, J. A. unpublished data) with UDP-[^{14}C]galactose (New England Nuclear) and myo-inositol and isolating the products by descending PC on Whatman No. 1, 3 × 24 hr in PrOH–EtOAc–H_2O (7:1:2). [^{14}C]Raffinose was obtained from leaves exposed to $^{14}CO_2$ for 15 min. The leaves were extrd in EtOH and a neutral fraction obtained from which raffinose was isolated by descending PC on Whatman No. 1, 3 × 24 hr in PrOH–EtOAc–H_2O (7:1:2), and rerun to obtain the raffinose chromatographically pure. Over 90% of the radioactivity was located in the galactose moiety.

Extraction and enzyme fractionation. All purification steps were carried out at 0–4° using (unless otherwise stated) 100 mM Na–Pi buffer pH 6.9 containing 20 mM 2-mercaptoethanol. Leaves, *ca* 50 g fr. wt, were homogenized in a blender for 1 min with 200 ml of Na–Pi buffer. The homogenate was filtered through four layers of cheese cloth and centrifuged (20 000 g, 20 min). The pellet was discarded and the supernatant brought up to 35% satn with a satd soln of $(NH_4)_2SO_4$. The pptd protein was removed by centrifugation (10 000 g, 20 min) and the supernatant brought up to 55% satn with satd $(NH_4)_2SO_4$ soln and centrifuged (10 000 g, 20 min). The supernatant was discarded and the pptd protein redissolved in 10 ml Na–Pi buffer. The 35–55% $(NH_4)_2SO_4$ fraction was then applied to a Sephadex G-200 column (60 cm × 2.2 cm) and eluted with the Na–Pi buffer, flow rate 0.25 ml/min. Fractions of 5 ml were collected and assayed for both galactosyltransferase and α-galactosidase activity. Active galactosyltransferase fractions were pooled, made up to 0.1 M NaCl, applied to a DEAE-Sephadex A-50 column (15 cm × 1.5 cm) previously equilibrated with the Na–Pi buffer containing 0.1 M NaCl and eluted with 150 ml linear gradient 0.1–0.5 M NaCl in Na–Pi buffer, flow rate 0.22 ml/min. Fractions of 4.4 ml were collected and all even-numbered fractions assayed for both galactosyltransferase and α-galactosidase activities. The active fractions for the galactosyltransferase were pooled and the vol. reduced to *ca* 5 ml by ultrafiltration under N_2 (Amicon PM 10 filter); 20 ml Na–Pi buffer was then added and the vol. again reduced to *ca* 5 ml. This procedure was repeated twice more. The concd fractions were made up to 0.1 M NaCl and applied to a DEAE Sephadex A-50 column (15 cm × 1.5 cm) previously equilibrated with Na–Pi buffer containing 0.1 M NaCl. The sample was eluted with a 150-ml linear gradient 0.1–0.3 M NaCl in Na–Pi buffer, flow rate 0.18 ml/min. Fractions of 3.6 ml were collected and all even-numbered fractions assayed for both galactosyltransferase and α-galactosidase activities. Active fractions for the galactosyltransferase were pooled and concd by ultrafiltration as described above. The enzyme was stored at 4° in 0.1 M Na–Pi buffer pH 6.9 containing 20 mM 2-mercaptoethanol and used as the enzyme source for all studies on the properties of the transferase.

Enzyme assays. All reactions for the galactosyltransferase were carried out for 30 min at pH 6.9 and at 30° (unless otherwise stated). The crude extracts, $(NH_4)_2SO_4$, G-200 and DEAE 50 fractions were assayed by incubating 40 μl of enzyme soln with 10 μl of a standard assay soln containing 20 mM raffinose and 4.3 mM [^{14}C]galactinol, sp. act. 0.26 μCi/μmol in H_2O. The reaction was terminated by the addition of 200 μl anhydrous EtOH, the soln evapd to dryness in an air stream, redissolved in 50 μl 50% EtOH and the reaction products sepd by descending PC using Whatman No. 4, single development for 24 hr with PrOH–EtOAc–H_2O (7:1:2). Individual assay strips were scanned with a radiochromatogram scanner, the radioactive peaks cut out, placed in vials containing 15 ml toluene and 0.5% PPO, and counted in a scintillation spectrometer. Identification of the radioactive peaks was done by co-chromatography with strips of a standard sugar soln visualized with $AgNO_3$–NaOH [22]. The unit of enzyme activity was defined as the quantity that synthesized 1 μmol of stachyose/hr under the above conditions. Sp. acts. are expressed in mU/mg protein. Protein determinations were made using the method of [23] using crystalline BSA as the standard. For rapid assay of fractions emerging from the G-200 and DEAE 50 columns 40 μl were incubated with 10 μl of a soln containing 5 mM raffinose and 1.5 mM galactinol in H_2O. The reaction was terminated by the addition of 200 μl dry EtOH, the soln evapd to dryness in an air stream, redissolved in 50 μl 50% EtOH and 35 μl spotted on 'Baker TLC' plates coated with Si Gel 60-F. The plates were developed for 10 cm twice in

EtOAc–HOAc–MeOH–H_2O (13:3:3:2) with a 5-min drying period in between runs. The plates were then sprayed with a soln containing 3 g PHOH and 5 ml conc H_2SO_4/100 ml EtOH and heated at 110° for 10–15 min. The pH–activity curve was determined by incubating 20 μl of enzyme soln with 20 μl of 100 mM McIlvaine buffer for range pH 3.5–6, 0.1 M Na–Pi buffer for range pH 6.5–7, 0.1 M glycine–NaOH buffer at pH 7.5 and 0.1 M 2-amino-2-methyl-1:3-propanediol buffer for range pH 8–8.5, and adding 10 μl standard assay soln. The reaction products of these assays and for all those described below were sepd by descending PC using Whatman No. 1, 3 × 24 hr in PrOH–EtOAc–H_2O (7:1:2). K_m values for raffinose and melibiose were determined by incubating 20 μl enzyme soln with 20 μl 2.5 mM or 12.5 mM [^{14}C]galactinol (sp. act. 0.12 μCi/μmol) in Na–Pi buffer and 10 μl of 5–40 mM raffinose or melibiose in H_2O. K_m values for galactinol were determined by incubating 20 μl enzyme soln with 5 μl 70 mM raffinose in H_2O and 10 μl of 0.3–3 mM [^{14}C]galactinol (sp. act. 0.12 μCi/μmol) in H_2O. The K_m value for the hydrolysis of galactinol was determined by incubating 20 μl enzyme soln with 20 μl Na–Pi buffer and 10 μl of 0.3–2.5 mM [^{14}C]galactinol in H_2O. The K_i value for myo-inositol was determined by preincubating 20 μl of enzyme soln with 20 μl of myo-inositol at 10–40 mM in Na–Pi buffer for 10 min and starting the reaction by adding 10 μl of a soln containing 50 mM raffinose and 5 mM [^{14}C]galactinol (sp. act. 0.12 μCi/μmol) in H_2O. Inhibitory studies were determined by incubating 20 μl enzyme soln and 20 μl of inhibitor solns in Na–Pi buffer with 10 μl standard assay soln. To study the effect of metal ions, 4 ml of the enzyme soln was dialysed against 1 l. 50 mM MOPS buffer pH 6.9 containing 20 mM 2-mercaptoethanol for 24 hr. 20 μl of the dialysed enzyme soln were preincubated for 10 min at 30° with 20 μl of 7.5 mM solns of the metal ions in 50 mM MOPS buffer pH 6.9 containing 20 mM 2-mercaptoethanol and the reaction started by adding 10 μl of the standard assay soln. Acceptor specificity was determined by incubating 20 μl enzyme soln with 20 μl 2.5 mM [^{14}C]galactinol (sp. act. 0.12 μCi/μmol) in Na–Pi buffer and 10 μl 20 mM solns of various sugar substrates in H_2O. Donor specificity was determined by incubating 20 μl enzyme soln, 10 μl 15.5 mM [^{14}C]raffinose (sp. act. 0.70 μCi/μmol) in H_2O, and 20 μl Na–Pi buffer containing one of the following, 25 mM UDPGal, 10 mM *p*-nitrophenyl-α-D-galactopyranoside, 40 mM melibiose or no addition. Other reactions catalysed by the galactosyltransferase prepn were studied by incubating 20 μl of enzyme soln with (a) 20 μl 20 mM stachyose in Na–Pi buffer and 10 μl 20 mM [^{14}C]myo-inositol (sp. act. 0.12 μCi/μmol) in H_2O, (b) 10 μl 20 mM [^{14}C]myo-inositol (sp. act. 0.12 μCi/μmol) in H_2O and 5 μl 15 mM galactinol in H_2O, (c) 10 μl 20 mM stachyose in Na–Pi buffer and 20 μl 15.5 mM [^{14}C]raffinose (sp. act. 0.70 μCi/μmol) in H_2O.

Assays of α-galactosidase activity were done by incubating 0.1 ml suitably diluted enzyme soln with 0.2 ml 0.3% *p*-nitrophenyl-α-D-galactopyranoside in 0.2 M NaOAc buffer pH 5 for 20 min at 30°. The reaction was stopped with 3 ml 5% Na_2CO_3 and the *p*-NO_2 PhOH released determined at 400 nm. The unit of enzyme activity was defined as the quantity that hydrolyses 1 μmol substrate/min under the above conditions.

PAGE was performed using 7.5% medium pore gels pH 8.9 at 4 mA/gel as described in ref. [24].

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