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SUCROSE:SUCROSE FRUCTOSYLTRANSFERASE AND FRUCTAN:FRUCTAN FRUCTOSYLTRANSFERASE FROM *ALLIUM CEPA*

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Key Word Index—*Allium cepa*; Liliaceae; onion; fructan synthesis; fructosyltransferases.

Abstract—Sucrose:sucrose fructosyltransferase and fructan:fructan fructosyltransferase were isolated from the inner leaf bases of bulbing onion plants (*Allium cepa*) and separated by gel filtration on Bio-Gel P-150. Sucrose:sucrose fructosyltransferase produced only one trisaccharide, 1^F-fructosylsucrose, from sucrose. Fructan:fructan fructosyltransferase produced tetrasaccharide and higher polymers from trisaccharide. The trisaccharide found in the greatest concentration in onion, 6^G-fructosylsucrose, was produced from 1^F-fructosylsucrose by fructan:fructan fructosyltransferase and was not a product of sucrose:sucrose fructosyltransferase.

INTRODUCTION

Fructans are oligosaccharides and polysaccharides of fructose containing a single glucose moiety. Despite the widespread occurrence of fructans, they have been studied very little in comparison to starch, the other major non-structural carbohydrate polymer found in plants.

Synthesis of the β 2-1 linked fructan, inulin, by artichoke (*Helianthus tuberosus*) was attributed to inulosucrase (EC 2.4.1.9) by Dedonder [1]. Subsequently Edelman and Jefford [2] suggested that inulosucrase activity in artichoke was due to the activity of two separate enzymes, a sucrose:sucrose fructosyltransferase and a fructan:fructan fructosyltransferase. In this scheme, after sucrose synthesis, trisaccharide production can be considered to be the primary step in a series of transfer reactions resulting in fructan synthesis. Sucrose:sucrose fructosyltransferase catalyses this initial reaction by transferring a fructosyl group from a sucrose donor to a sucrose acceptor to produce trisaccharide (fructosylsucrose) and glucose. Fructan:fructan fructosyltransferase continues the polymerization with trisaccharide as the smallest possible donor.

Scott [3] isolated sucrose:sucrose fructosyltransferase activity from onions (*Allium cepa*) but was unable to completely separate this activity from fructan:fructan fructosyltransferase activity. This paper reports the isolation and separation of these activities from onion. Because of the presence of two trisaccharide isomers in onion [4], 1^F-fructosylsucrose (1-kestose) and 6^G-fructosylsucrose (neo-kestose), the product of the separated sucrose:sucrose fructosyltransferase was identified.

RESULTS

Purification of fructan-synthesizing enzymes

Attempts were made to isolate sucrose:sucrose fructosyltransferase and to free this enzyme from fructan:fructan fructosyltransferase activity. Sucrose:sucrose fructosyltransferase and fructan:fructan fructosyltransferase were not separated by $(NH_4)_2SO_4$ fractionation but invertase (EC 3.2.1.26) and fructan hydrolase activities (EC 3.2.1.7) were partially removed. Chromatography on Sephadex G-100 and DEAE-cellulose resulted in low yields of enzyme activity possibly because of enzyme binding to these carbohydrate columns. Fractions containing sucrose:sucrose fructosyltransferase activity eluted from Sephadex G-100 also contained fructan:fructan fructosyltransferase activity.

Sucrose:sucrose fructosyltransferase was most successfully purified by $(NH_4)_2SO_4$ fractionation followed by gel filtration on Bio-Gel P-150. The enzyme was separated from fructan:fructan fructosyltransferase on Bio-Gel P-150 (Fig. 1). The fraction containing most sucrose:sucrose fructosyltransferase activity was purified 40-fold (Table 1) and contained almost no fructan:fructan fructosyltransferase activity (Fig. 1). Fractions from the gel filtration column containing fructan-synthesizing enzymes did not contain invertase or fructan hydrolase activities.

Properties of sucrose:sucrose fructosyltransferase

Because of the important role of sucrose:sucrose fructosyltransferase in initiating fructan synthesis, some properties of the enzyme partially purified by $(NH_4)_2SO_4$ fractionation and Bio-Gel P-150

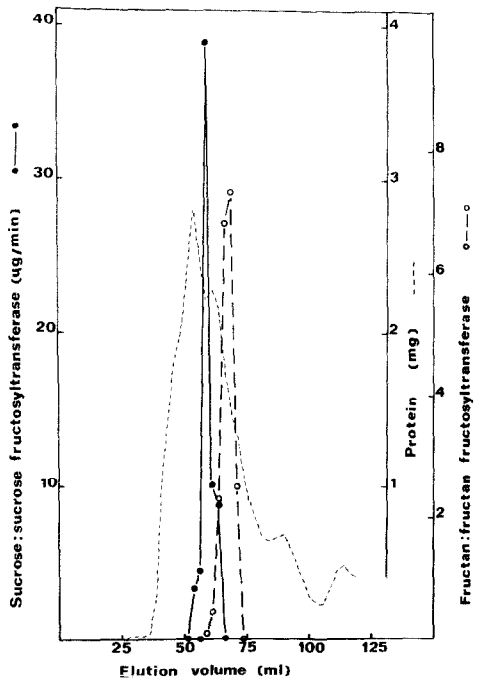

Fig. 1. Separation of sucrose:sucrose fructosyltransferase and fructan:fructan fructosyltransferase on a 2.6 × 30 cm column of Bio-Gel P-150. Fructan:fructan fructosyltransferase activity is the % of radioactivity in tetrasaccharide after 4 hr incubation with trisaccharide-[^{14}C].

chromatography were examined. The enzyme prepared in this way contained a small amount of fructan:fructan fructosyltransferase activity but no detectable hydrolytic activity. Maximum sucrose:sucrose fructosyltransferase activity was at pH 5.2 in 0.1 M phosphate–citrate buffer with less than 10% of this activity at pH 3 and 7. The influence of substrate concentration on enzyme activity was determined in 0.1 M phosphate–citrate buffer, pH 5.4. The apparent K_m for sucrose was 0.23 M. Gel filtration on a calibrated Sephadex G-100 column indicated a MW of 69 000.

Product of sucrose : sucrose fructosyltransferase

Onions contain two isomeric trisaccharides, 1^F-fructosylsucrose and 6^G-fructosylsucrose. In the cultivar Creamgold the 6^G isomer is present in the greatest concentration: one quantitative estimation gave a ratio of $6^G/1^F$ of 2.6 [4]. However the product of the purified sucrose:sucrose fructosyltransferase was 1^F-fructosylsucrose and not 6^G-fructosylsucrose (Table 2).

Properties of fructan : fructan fructosyltransferase

The purified fructan:fructan fructosyltransferase produced tetrasaccharide and sucrose from trisaccharide. After 4 hr incubation of the most active fractions eluted from Bio-Gel P-150 (Fig. 1) with trisaccharide-[^{14}C] as substrate, *ca* 7% of ratioactivity was found in tetrasaccharides. Smaller amounts of pentasaccharides and higher polymers were also formed. The presence of fructan:fructan fructosyltransferase in the sucrose:sucrose fructosyltransferase assays resulted in the conversion of some of the 1^F-fructosylsucrose produced by sucrose:sucrose fructosyltransferase to 6^G-fructosylsucrose (Table 2).

DISCUSSION

The proposal [2] that sucrose:sucrose fructosyltransferase and fructan:fructan fructosyltransferase are separate enzymes was largely based upon the reported separation of these two activities from artichoke on DEAE-cellulose [3]. Despite the initial report of inulosucrase by Dedonder [1], Edelman and Dickerson [5] had difficulty in demonstrating the synthesis of fructans from sucrose by enzyme preparations from artichoke. Fructan:fructan fructosyltransferase activity was always observed, leading to the suggestion [2] that the absence of sucrose:sucrose fructosyltransferase activity in the earlier studies was caused by the use of dormant tissues. We have separated sucrose:sucrose fructosyltransferase and fructan:fructan fructosyltransferase from onion by gel filtration on Bio-Gel P-150 minus 400 mesh (Fig. 1) sufficiently to establish that these two activities are due to separate enzymes.

The separation of these two activities from onion and the reported separation from artichoke [3] indicates that inulosucrase (EC 2.4.1.9) activity is probably the result of the activity of two separate enzymes in all species.

The product of the onion sucrose:sucrose fructosyltransferase is 1^F-fructosylsucrose (Table 2). The trisaccharide present in the greatest concentration in onion, 6^G-fructosylsucrose, was only formed when fructan:fructan fructosyltransferase activity was also present. Thus sucrose:sucrose fructosyltransferase from artichoke which contains only 1^F-fructosylsucrose [2] and the enzyme from onion both

Table 1. Purification of sucrose:sucrose fructosyltransferase

Step	Activity (μg/min)*	Protein (mg)	Sp. act. (μg/min/mg)	Yield (%)	Purification factor
Crude	160	373	0.429	100	—
50–80% $(NH_4)_2SO_4$ fraction	112	52.5	2.13	70.0	5.0
Bio-Gel P-150 total activity	65.7	12.0	5.48	41.1	12.8
Bio-Gel P-150 peak fraction	38.9	2.22	17.5	24.3	40.8

* μg of trisaccharide produced per min.

Table 2. Isomeric composition of trisaccharides produced from sucrose by onion enzymes

Enzyme preparation	dpm 1^F-fructosyl-sucrose	dpm 6^G-fructosyl-sucrose	% dpm in 1^F-fructosyl-sucrose
50–80% $(NH_4)_2SO_4$ fraction*	158 000	15 200	91.2
Sucrose : sucrose fructosyltransferase†	95 000	1 230‡	98.7
Sucrose : sucrose fructosyltransferase and fructan : fructan fructosyltransferase§	49 000	4 260	92.0

Enzymes were incubated for 2 hr with sucrose-[^{14}C] as substrate and the trisaccharide isomers produced were separated by TLC following removal of substrate by gel filtration.

* Contains both sucrose : sucrose fructosyltransferase and fructan : fructan fructosyltransferase.

† Peak sucrose : sucrose fructosyltransferase fraction from Bio-Gel P-150 column, 41-fold purified; contains very little fructan : fructan fructosyltransferase activity.

‡ Some may be residual sucrose with similar R_f to 6^G-fructosylsucrose.

§ Fraction between two enzyme peaks from Bio-Gel P-150 column.

produce the same trisaccharide. The initial product of bacterial levansucrase (EC 2.4.1.10) action on sucrose has also been reported to be 1^F-fructosylsucrose [6]. By analogy with inulosucrase, further studies of levansucrase may reveal the presence of two component activities. A sucrose : sucrose 1^F-fructosyltransferase may be common to both inulosucrase and levansucrase. Thus, the synthesis of β 2-1 linked fructans by inulosucrase and β 2-6 linked fructans by levansucrase may be due to the specificity of the fructan : fructan fructosyltransferase component.

The presence of 6^G-fructosylsucrose in onion and not in artichoke and the presence of higher MW fructans in artichoke may be attributed to differences between the substrate specificity of the fructan : fructan fructosyltransferases from onion and artichoke. The onion enzyme may transfer fructosyl groups from higher polymers to either the 1^F or the 6^G hydroxyl of sucrose to produce both trisaccharide isomers while the artichoke enzyme may transfer specifically to only the 1^F hydroxyl. Alternatively, fructan : fructan fructosyltransferase may contain separate component activities not yet detected.

Amylosucrase (EC 2.4.1.4) and dextransucrase (EC 2.4.1.5) synthesize α 1-4 linked and α 1-6 linked glucans from sucrose by glucosyltransfer. The separation of the fructosyltransfer enzymes acting on sucrose into two component activities suggests that the glucosyltransfer enzymes may be found to have component activities upon further investigation.

Edelman and Jefford [2] proposed that fructans are synthesized by fructosyl transfer reactions and that fructans, unlike many other carbohydrates, are not synthesized from nucleotide sugars. Others [7, 8] have isolated UDP-fructose from plants containing fructans as a possible intermediate in fructan synthesis. The amount of sucrose : sucrose fructosyltransferase and fructan : fructan fructosyltransferase activity isolated from onion plants, although low, (sucrose : sucrose fructosyltransferase 2–4 μg trisaccharide min/g fr. wt) was sufficient to account for the synthesis of all the fructans in the plant. Since all the low MW fructans of the onion [4] may be synthesized by these fructosyl transfer reactions, mechanisms of oligosaccharide synthesis involving nucleotide sugars such as UDP-fructose need not be invoked.

Fructans are not the only oligosaccharides and polysaccharides synthesized from sucrose without the participation of nucleotide sugars. Amylosucrase and dextransucrase produce glucans from sucrose by glucosyltransfer and galactans of the raffinose series are produced by galactosyltransfer to sucrose [9]. The unique properties of sucrose [10] such as its high free energy of hydrolysis (sucrose—29 kJ/mol, UDP-glucose—(32–33) kJ/mol [10]) may allow oligosaccharide synthesis with sucrose as glycosyl donor while other oligosaccharides and polysaccharides are formed by glycosyltransfer from nucleotide sugar donors.

EXPERIMENTAL

Source of enzyme. When the 5th leaf lamina had emerged, onion seedlings (*A. cepa* L. cv Creamgold) were exposed to long days (16 hr) to induce bulbing. Bulbing plants were used because juvenile plants contain very little fructan [11]. After 28 days under bulb-inducing conditions, the plants were harvested and immediately placed in a freezer at −18° before dissecting out the leaf bases at 4°. Inner leaf bases were used for purification because of the presence of higher sucrose : sucrose fructosyltransferase and lower fructan hydrolase activities than in outer leaf bases [12].

Enzyme extraction. All steps in enzyme purification were performed at 4°. The frozen inner leaf bases from 100–500 plants (300–900 g fr. wt) were homogenized in 0.5–1 l. 0.1 M Pi buffer, pH 7, containing 0.1 mM dithiothreitol and 50 mg/ml insoluble PVP. The homogenate was filtered through Miracloth and centrifuged at 10 000 g for 15 min. The protein in the supernatant was concd by the addition of $(NH_4)_2SO_4$ to 100% satn. The pellet was resuspended in *ca* 50 ml 1 mM Pi buffer, pH 7, and dialysed for 18 hr against 4 l.

of the same buffer. The dialysed 0–100% $(NH_4)_2SO_4$ prepn was subjected to $(NH_4)_2SO_4$ fractionation followed by dialysis as above.

Gel filtration chromatography. The 50–80% $(NH_4)_2SO_4$ fraction (3 ml) was loaded onto a 2.6 × 30 cm column of Sephadex G-100 or Bio-Gel P-150 (minus 400 mesh) which had been equilibrated with 5 mM Pi buffer, pH 7. The enzyme was eluted with the same buffer and 2.5 ml fractions were collected.

Substrate preparation. Labelled substrates were produced by feeding 37 M Bq of $^{14}CO_2$ to 12 bulbing onion plants in the light. Most of the ^{14}C was consumed by the plants in 100 min. After allowing a further 2 hr for ^{14}C to move into fructans in the leaf bases, the leaf bases were extracted with EtOH–H_2O (4:1) heated at 90°. The EtOH extract which contains all the fructans of onion [4] was concd by rotary evapn at 40°. Carbohydrates were separated be applying 0.5–1 ml (*ca* 50 mg) to a 1.6 × 210 cm column of Bio-Gel P2 (minus 400 mesh) and eluting with H_2O from a reservoir maintained at a constant pres. of 30 KPa [4]. Fractions (2.5 ml) were assayed for fructose using the resorcinol–HCl method [13]. Fractions containing trisaccharide were combined and concd by rotary evapn at 40°.

Sucrose : sucrose fructosyltransferase assay. The enzme was assayed using a method previously described [14] involving PhOH partition to stop the reaction and GLC to separate the substrate and products. The published assay procedure was slightly modified for sucrose concns above 0.1 M. Larger vols (0.5–1 ml) of reagent were required to dissolve all the sugars and form TMSi derivatives for GLC. The standard assay mixture contained 0.2 ml 0.1 M NaOAc buffer (pH 5.5), 0.2 ml 0.3 M sucrose and 0.2 ml of enzyme at 30°. Invertase activity was also determined by this assay [14].

Identification of product of sucrose : sucrose fructosyltransferase. Sucrose : sucrose fructosyltransferase was assayed using sucrose-[^{14}C] as substrate by adding 10 μl of EtOH–H_2O (9:1) containing 2.5 μg 74 k Bq/μg sucrose-[^{14}C] to the standard assay mixture. The reaction was stopped after 2 hr by PhOH partition and 0.5 ml of the aq. phase was applied to a 1.6 × 90 cm column of Bio-Gel P-2 (minus 400 mesh). Radioactivity was determined by counting a 0.2 ml aliquot of each 2.5 ml fraction. Fractions containing trisaccharide were combined and concd by rotary evapn at 40°. The composition of the trisaccharide fraction was determined by TLC.

Fructan : fructan fructosyltransferase assay. The assay mixture contained 0.2 ml 0.1 M NaOAc buffer (pH 5.5), 0.2 ml 0.15 M trisaccharide-[^{14}C] and 0.2 ml of enzyme prepn incubated at 30° for 1–4 hr. The reaction was stopped by the addition of 0.5 ml 90% w/v PhOH–H_2O. After centrifugation 0.5 ml of the upper aq. phase was chromatographed on a 1.6 × 90 cm column of Bio-Gel P2 as described above. Radioactivity in substrate and products was calculated following scintillation counting of each fraction. Fructan hydrolase activity was also determined by this assay.

TLC of trisaccharides. Trisaccharide isomers were separated by TLC on Si gel plates, previously equilibrated in 0.2 M NaH_2PO_4, with propan-2-ol–Me_2CO–H_2O (2:2:1) as solvent. Isomers were located using the urea–phosphoric acid reagent [15].

Protein determination. Protein was determined by an automated adaptation of the method of ref. [16].

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