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PURIFICATION AND CHARACTERISATION OF 1^F-FRUCTOSYLTRANSFERASE FROM THE ROOTS OF ASPARAGUS (*Asparagus officinalis* L.)

NORIO SHIOMI
Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060 (Japan)

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ABSTRACT

A fructosyltransferase that transfers a terminal D-fructosyl group from a (2→1)-β-linked fructosaccharide to HO-1 of another D-fructosyl group has been purified from an extract of asparagus roots by successive chromatography with DEAE-cellulose, octyl-Sepharose, Sephadex G-200, and raffinose-coupled Sepharose 6B. The disc-electrophoretically homogeneous enzyme was free from β-D-fructofuranosidase, sucrose:sucrose 1-fructosyltransferase, and 6^G-fructosyltransferase activity, and catalysed the D-fructosyl transfer from 1-kestose more rapidly to saccharides of the neokestose series [1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucrose] than to those of the 1-kestose series [1^F(1-β-D-fructofuranosyl)$_n$sucrose]. The enzyme was tentatively termed 1^F-fructosyltransferase. The general properties of the enzyme were as follows: mol. wt., ~64,000; optimum pH, ~5.0; stable at pH 5.0–5.5 at 45° for 20 min; stable at 30–45° for 10 min; inhibited by Hg^{2+}, *p*-chloromercuribenzoate, and Ag^+.

INTRODUCTION

Eleven fructo-oligosaccharides of the 1-kestose and neokestose series have been reported to be present in asparagus roots[1–3], and these saccharides could be synthesised *in vitro* with an enzyme preparation from the same source[4]. As this enzyme preparation was presumed to contain three types of fructosyltransferase[4], their purification and characterisation were attempted. The properties of sucrose:sucrose 1-fructosyltransferase[5] (SST) and 6^G-fructosyltransferase[6] (6^G-FT) have been described, and we now report on 1^F-fructosyltransferase (1^F-FT).

MATERIALS AND METHODS

Materials. — Roots of asparagus (*Asparagus officinalis* L.) were harvested at the Experimental Farm, Faculty of Agriculture, Hokkaido University, in September, 1977. They were stored in the frozen state.

[U-^{14}C]Sucrose was purchased from The Radiochemical Centre (Amersham,

Bucks, U.K.). [U-^{14}C]-1-Kestose [*O*-β-D-Fru*f*-(2→1)-*O*-β-D-Fru*f*-(2↔1)-α-D-Glc*p*] and [U-^{14}C]nystose [*O*-β-D-Fru*f*-(2→1)-*O*-β-D-Fru*f*-(2→1)-*O*-β-D-Fru*f*-(2↔1)-α-D-Glc*p*] were prepared from [U-^{14}C]sucrose by using a preparation of asparagus fructosyltransferase[4].

1-Kestose, nystose, neokestose [*O*-β-D-Fru*f*-(2→6)-*O*-α-D-Glc*p*-(1↔2)-β-D-Fru*f*], 6^G(1-β-D-fructofuranosyl)$_n$sucrose {*n* = 2 [*O*-β-D-Fru*f*-(2→1)-*O*-β-D-Fru*f*-(2→6)-*O*-α-D-Glc*p*-(1↔2)-β-D-Fru*f*] and *n* = 3}, and 1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucrose {*m* = 1, *n* = 1, *O*-β-D-Fru*f*-(2→1)-*O*-[β-D-Fru*f*-(2→6)-*O*-α-D-Glc*p*]-β-D-Fru*f*; *m* = 1, *n* = 2; and *m* = 2, *n* = 1} were obtained from asparagus roots. 1^F(1-β-D-Fructofuranosyl)$_n$sucrose {*n* = 3 [*O*-β-D-Fru*f*-(2→1)-*O*-β-D-Fru*f*-(2→1)-*O*-β-D-Fru*f*-(2→1)-*O*-β-D-Fru*f*-(2↔1)-α-D-Glc*p*], *n* = 4 and 5} were prepared from Jerusalem artichoke tubers[1,2,4,5].

Methods. — Sugars were detected and determined by the anthrone method[7]. Proteins were determined by measuring the absorbance at 280 nm referred to $E^{1\%}_{1\,cm}$ = 9.38 in aldolase[8].

Chromatography. — P.c. was performed on Toyo No. 50 filter paper with 1-propanol–ethyl acetate–water mixtures (*A*, 7:1:2; *B*, 6:1:3). T.l.c. (3–5 developments with *C* 1-butanol–2-propanol–water, 10:5:4) was performed on Kieselgel 60 (Merck) with detection by anisidine phosphate[9]. Charcoal–Celite chromatography involved a column (1.5 × 54 cm) packed with charcoal (Tokusei-shirasagi Brand, Takeda Chemical Industries, Ltd.) and Celite 535 (Nakarai Chemical Industries, Ltd.) (1:1) that was washed with hydrochloric acid and then water. Sugar solutions were placed on the column and eluted successively with water, and 10, 13, and 20% ethanol.

Measurement of enzyme activity. — One unit of 1^F-fructosyltransferase (1^F-FT) is defined as the amount of enzyme transferring a D-fructosyl group from 1-kestose to 1-kestose that produces 1 μmol of nystose in 1 h under the conditions described below.

A mixture of enzyme (20 μL) and 0.4M [U-^{14}C]-1-kestose (0.045 μCi) in McIlvaine buffer (pH 5.0, 20 μL) was incubated for 1 h at 30° and then treated with 0.1M mercuric chloride (10 μL). Carrier sugars (D-fructose, D-glucose, sucrose, and nystose, 100 μg of each) were added and subjected to p.c. (solvent *A*). Each saccharide was extracted with water, and the extract was concentrated *in vacuo* to dryness. The residue was dissolved in water (1 mL) and the radioactivity of a portion (0.5 mL) was measured[4] by using an Aloka Liquid Scintillation Spectrometer, Model LSC 502 and LSC 651. The activity of 1^F-FT was calculated from the radioactivity in nystose and the total radioactivity recovered.

Disc electrophoresis. — 7.5% Polyacrylamide gel (pH 8.0) was used[10,11] for 2 h at room temperature and 2 mA/tube. Protein bands on the gel were stained with Amido Black 10B.

Determination of molecular weight. — The method involved gel filtration by the ascending method with a column (2.64 × 98 cm) of Sephadex G-200 pre-equilibrated with 0.01M phosphate buffer (pH 6.5) containing 0.25M sodium chloride.

Elution was effected with the same buffer at 6 ml/h. Cytochrome c (mol. wt., 12,400), chymotrypsinogen A (25,000), hen-egg albumin (45,000), bovine serum albumin (67,000), and aldolase (147,000) were used as marker proteins.

Raffinose-coupled Sepharose 6B. — This material was prepared by coupling[5] raffinose with epoxy-activated Sepharose 6B.

RESULTS

Purification of enzyme. — (*a*) *Fractionation with ammonium sulfate.* Asparagus roots (200 g of each; total, 25 kg), previously washed with water and chopped, were homogenised in cold 0.05M phosphate buffer (pH 6.5, 1 litre). After filtering through cheese cloth and centrifuging (standard conditions of 10,000*g* for 15 min were used throughout), the supernatant solution was saturated with solid ammonium sulfate and stored overnight in a cold chamber. The resulting precipitate was collected by centrifugation, and a solution in 0.05M phosphate buffer (pH 6.5) was dialysed for 5 days against the same buffer and then centrifuged. The supernatant solution (6.42 litres) was the "$(NH_4)_2SO_4$, 0–1 saturation" fraction.

This fraction was treated with solid ammonium sulfate, to give 0.3 saturation, and stored overnight in a cold chamber. After centrifuging, ammonium sulfate was added to bring the supernatant solution to 0.8 saturation. A solution of the precipitate in 0.01M phosphate buffer (pH 6.5) was dialysed for 4 days against the same buffer, to give the "$(NH_4)_2SO_4$, 0.3–0.8 saturation" fraction (2.77 litres).

(*b*) *Treatment with calcium phosphate gel.* A suspension of calcium phosphate gel in the "$(NH_4)_2SO_4$, 0.3–0.8 saturation" fraction (gel, 7 g/g of protein) was stored for 30 min in a cold chamber and then centrifuged. The supernatant solution (3.09 litres) was the "calcium phosphate gel" fraction. Almost all of the hydrolytic activity was removed by this gel treatment.

(*c*) *First chromatography on DEAE-cellulose.* The "calcium phosphate gel" fraction was concentrated to 500 ml by ultrafiltration (Amicon; Diaflo PM-30 filter), dialysed overnight against 0.01M phosphate buffer (pH 6.5), and added to a column (4.8 × 23 cm) of DEAE-cellulose previously equilibrated with the same buffer. Elution was effected with 0.01M (3 litres), 0.02M (1.5 litres), and 0.07M phosphate buffers (3 litres) in sequence. The eluate emerging with the 0.07M buffer was concentrated and then dialysed overnight against 0.01M phosphate buffer, to give the "DEAE-cellulose, 1st" fraction (350 ml).

(*d*) *Octyl-Sepharose chromatography.* The "DEAE-cellulose, 1st" fraction was saturated to 35% with solid ammonium sulfate and stored overnight, and then centrifuged to remove the precipitate, placed on a column (2.6 × 20 cm) of octyl Sepharose CL-4B, and eluted successively with 0.01M phosphate buffer (pH 6.5) saturated with ammonium sulfate by 35, 30, 20, 10, and 0% (500 ml of each). The 1^F-fructosyltransferase (1^F-FT) activity was found only in the final eluate, and was separated from sucrose:sucrose 1-fructosyltransferase (SST) and 6^G-fructosyltransferase (6^G-FT) activities.

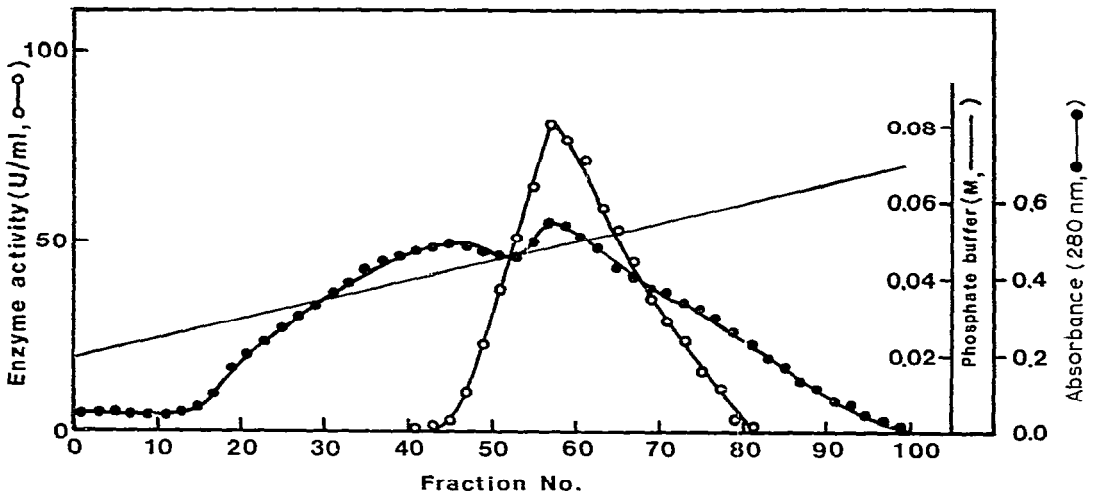


Fig. 1. Second chromatography on DEAE-cellulose of asparagus 1^F-fructosyltransferase; elution with a linear gradient 0.02→0.07M phosphate buffer (5-ml fractions).

The 1^F-FT-active fraction was concentrated (Amicon, PM-30 filter) and dialysed for 2 days against 0.01M phosphate buffer (pH 6.5), to give the "octyl-Sepharose" fraction (57 ml).

(*e*) *Second chromatography on DEAE-cellulose.* The "octyl-Sepharose" fraction was concentrated to 20 ml and eluted from a column (2 × 19 cm) of DEAE-cellulose with phosphate buffers (pH 6.5, 500 mL of each) of 0.01M, 0.02M, and then with a linear gradient from 0.02→0.07M (Fig. 1). Fractions 47–73 were combined, concentrated (Amicon, PM-10 filter), and dialysed overnight against 0.01M acetate buffer (pH 5.6), to give the "DEAE-cellulose, 2nd" fraction (30 ml).

(*f*) *Raffinose-coupled Sepharose 6B chromatography.* The "DEAE-cellulose, 2nd" fraction was further concentrated (Amicon, PM-10 filter) to 10 mL, and subjected to chromatography on a column (1 × 10 cm) of raffinose-coupled Sepharose 6B equilibrated with 0.01M acetate buffer (pH 5.6). The column was washed with the same buffer (100 mL), and the 1^F-FT was eluted with 0.01M phosphate buffer (pH 6.0, 100 mL) ("Sepharose 6B" fraction; 100 mL).

(*g*) *Sephadex G-200 chromatography.* The "Sepharose 6B" fraction (100 mL) was concentrated to 3 mL (Amicon, PM-10 filter), dialysed overnight against 0.01M phosphate buffer (pH 6.5) containing 0.25M sodium chloride, and then eluted from a column (2.64 × 98 cm) of Sephadex G-200 with the same buffer. Fractions 41–51 (92 ml) were the "Sephadex G-200, 1st" fraction (Fig. 2).

This 1^F-FT-active fraction (92 mL) was concentrated to 3 mL (Amicon, PM-10 filter) and re-chromatographed (Fig. 3). Fractions 43–59 were combined, and dialysed against 5mM phosphate buffer (pH 6.5), to give the "Sephadex G-200, 2nd" fraction (12 mL). This highly purified enzyme is tentatively termed 1^F-fructosyltransferase (1^F-FT).

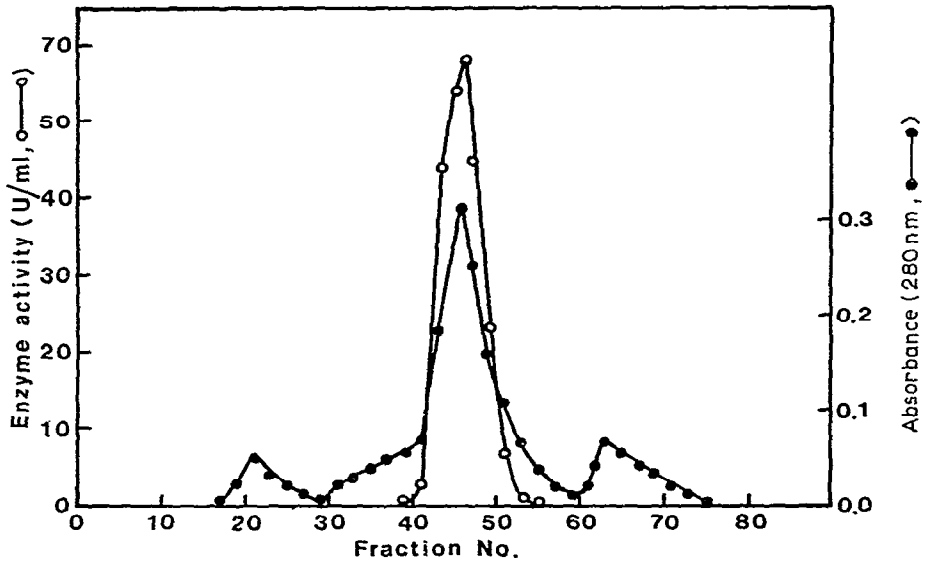


Fig. 2. Chromatography on Sephadex G-200 of asparagus 1^F-fructosyltransferase; elution with 0.01M phosphate buffer containing 0.25M NaCl (7.5-ml fractions).

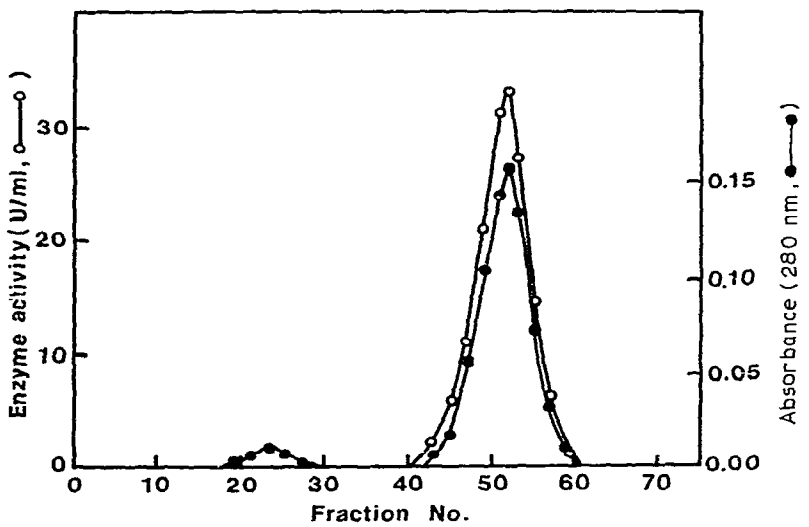


Fig. 3. Second chromatography on Sephadex G-200 of asparagus 1^F-fructosyltransferase; elution with 0.01M phosphate buffer containing 0.25M NaCl (6.6-ml fractions).

Table I shows that the enzyme was purified 298-fold from the original precipitate in (*a*) in a yield of 1.7% and with a specific activity of ~185 U/mg of protein. The preparation was free from β-D-fructofuranosidase, SST, and 6^G-FT, because it could not hydrolyse sucrose, 1-kestose, neokestose, and $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_n$sucrose ($n$ = 2–5), catalyse self-transfer of a D-fructosyl group between sucrose mole-

TABLE I

PURIFICATION PROCESS OF ASPARAGUS 1^F-FRUCTOSYLTRANSFERASE

Step	*Activity (U)*	*Protein (mg)*	*Volume (ml)*	*Specific activity (U/mg of protein)*
$(NH_4)_2SO_4$, 0–1 saturation	93188	149226	6420	0.62
$(NH_4)_2SO_4$, 0.3–0.8 saturation	54760	60940	2770	0.90
Calcium phosphate gel	38970	17216	3090	2.26
DEAE-cellulose 1st	20507	2499	350	8.21
Octyl-Sepharose	12998	182.40	57	71.3
DEAE-cellulose, 2nd	6735	68.19	30	98.8
Sepharose 6B	4145	28.03	100	147.9
Sephadex G-200, 1st	2789	17.09	92	163.2
Sephadex G-200, 2nd	1590	8.60	12	184.9

cules, or synthesise neokestose and $1^F,6^G$-di-β-D-fructofuranosylsucrose from 1-kestose.

General properties of the enzyme. — (*a*) *Disc electrophoresis.* The purified 1^F-FT preparation (~80 μg as protein) was subjected to electrophoresis on polyacrylamide gel. One gel was stained with Amido Black 10B. Another gel was cut into discs (2-mm width), and each was homogenised with McIlvaine buffer (pH 5.0, 0.2 ml). Each homogenate was incubated with 0.2M 1-kestose (0.2 ml) for 15 h at 30°, and then subjected to t.l.c. to examine the formation of nystose. The enzyme preparation showed a single protein band in the gel, and 1^F-FT activity resided only in this band.

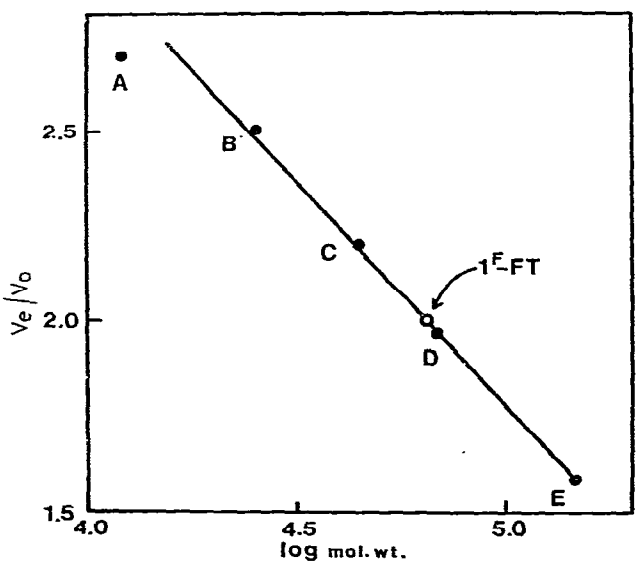
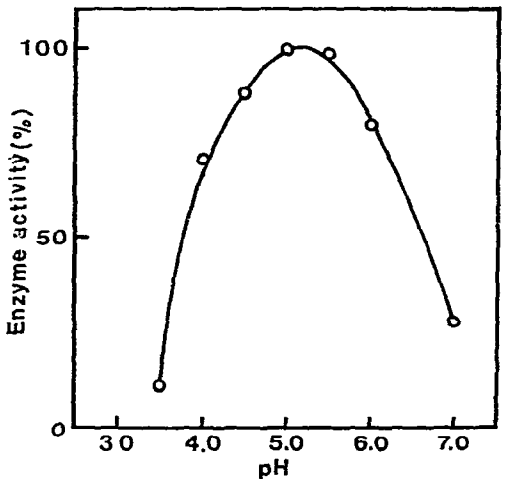


Fig. 4. Molecular weight of asparagus 1^F-fructosyltransferase; V_e, elution volume; V_o, void volume; A, chymotrypsinogen A (mol. wt. 25,000); B, hen-egg albumin (45,000); C, bovine serum albumin (67,000); D, aldolase (147,000) (see text for details).



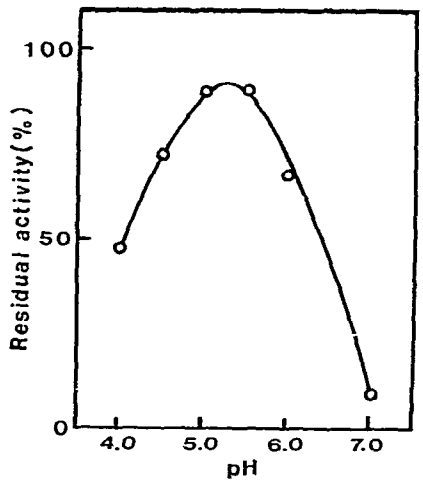


Fig. 5. Effect of pH on the activity of asparagus 1^F-fructosyltransferase (12 U/ml).

Fig. 6. Effect of pH on the heat stability of asparagus 1^F-fructosyltransferase (15.8 U/ml) (see text for details).

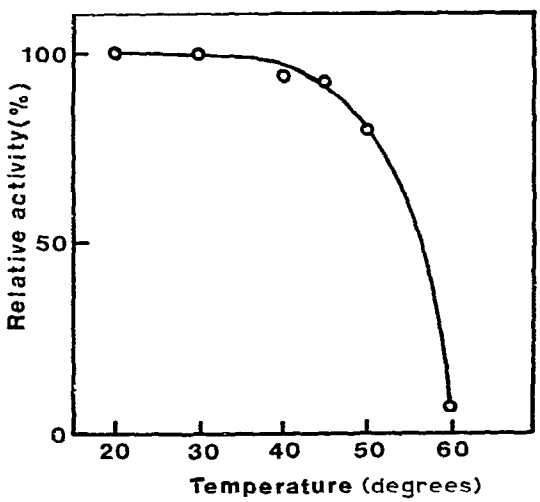


Fig. 7. Effect of temperature on asparagus 1^F-fructosyltransferase (17.8 U/ml) (see text for details).

(*b*) *Molecular weight.* The molecular weight of the enzyme was estimated to be ~64,000 by comparison with those of the marker proteins by Sephadex G-200 gel-filtration (Fig. 4).

(*c*) *Optimum pH.* The optimum pH of the enzyme was ~5 in the reaction with 1-kestose (Fig. 5).

(*d*) *pH-Stability.* Solutions of enzyme at pH 4.0, 4.5, 5.0, 5.5, 6.0 or 7.0 were incubated for 20 min at 45°, cooled to 0°, adjusted to pH 5.0, and then assayed for residual enzyme activity. As shown in Fig. 6, the residual activities were ~90%

at pH 5.0 and 5.5, ~70% at pH 4.5 and 6.0, ~50% at pH 4.0, and ~10% at pH 7.0.

(*e*) *Thermal stability.* After the enzyme solution in McIlvaine buffer (pH 5.0) had been heated for 10 min at 20, 30, 40, 45, 50, or 60°, the residual 1^F-FT activity was measured (Fig. 7). The enzyme was stable at 30–45°, but inactivated at 60°.

(*f*) *Effects of inhibitors.* A mixture of enzyme (20 μl) and 0.4M [U-^{14}C]-1-kestose in McIlvaine buffer (pH 5.0, 10 μl) was incubated at 30° for 1 h in the presence of water or the reagent solution (10 μl), and then treated with 0.1M mercuric chloride (10 μl) and subjected to p.c. Each oligosaccharide was assayed for radioactivity. The residual activity of the enzyme was calculated from the radioactivity in the tetrasaccharide fraction.

Mercuric chloride (10^{-4}M), *p*-chloromercuribenzoate (1.2×10^{-4}M), silver nitrate (10^{-3}M), and copper sulfate (10^{-3}M) inhibited by ~95, 88, 24, and 13%, respectively. At 10^{-3}M, lithium sulfate and the chlorides of magnesium, calcium, and barium had no effect, and activity was stimulated by ~19 and 26% with zinc sulfate and manganese sulfate, respectively.

Substrate specificity. — (*a*) "*Self-transfer*" *of* D-*fructosyl residue.* Transfer of a D-fructosyl residue between identical saccharide molecules, *e.g.*, sucrose, 1-kestose, nystose, 6^G(1-β-D-fructofuranosyl)$_2$sucrose, 1^F,6^G-di-β-D-fructofuranosylsucrose, or 1^F(1-β-D-fructofuranosyl)$_3$sucrose, was investigated by t.l.c. using purified asparagus 1^F-FT.

A mixture of enzyme (10 U/ml, 20 μl) and substrate in McIlvaine buffer (pH 5.0; 0.1M, 20 μl) was incubated for 5 h at 30°, and then treated with 0.1M mercuric chloride (10 μl) and subjected to t.l.c.

The enzyme catalysed D-fructosyl transfer to produce tetrasaccharide and sucrose from 1-kestose; pentasaccharide and 1-kestose from nystose; pentasaccharide(s) and neokestose from 6^G(1-β-D-fructofuranosyl)$_2$sucrose or 1^F,6^G-di-β-D-fructofuranosylsucrose; and hexasaccharide and tetrasaccharide (nystose) from 1^F(1-β-D-fructofuranosyl)$_3$sucrose; but no sugar from sucrose or neokestose.

A mixture of enzyme (20 U/ml, 20 μl) and 0.2M [U-^{14}C]-1-kestose (0.045 μCi) in McIlvaine buffer (pH 5.0, 20 μl) was incubated for 0.25, 0.5, 1, 2, 5, and 10 h, at 30° in the presence of a minimum amount of toluene. The reaction was stopped by

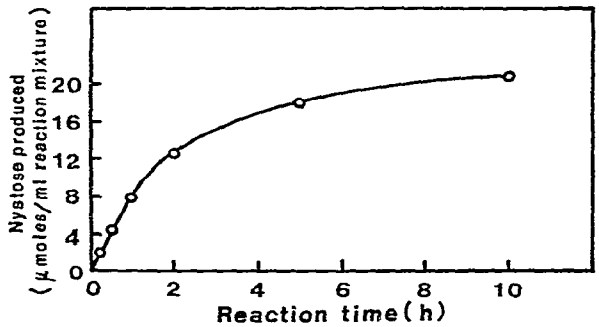


Fig. 8. Synthesis of nystose from 1-kestose by 1^F-fructosyltransferase (see text for details).

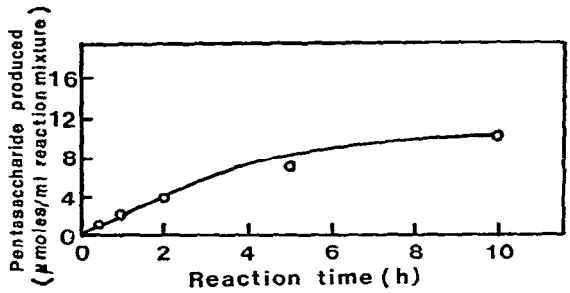


Fig. 9. Synthesis of pentasaccharide from nystose by asparagus 1^F-fructosyltransferase (see text for details).

the addition of 0.1M mercuric chloride (10 μl). Each mixture, together with carrier sugars [sucrose, 1-kestose, nystose, and 1^F(1-β-D-fructofuranosyl)$_3$sucrose, 100 μg of each], was subjected to p.c. (5 developments with solvent *A*). The resulting five fractions were concentrated *in vacuo* and a solution of each residue in water (1 ml) was assayed for radioactivity. The amount of tetrasaccharide was calculated from the radioactivity in tetrasaccharide fraction. Tetrasaccharide formation is shown in Fig. 8.

A portion (0.5 ml) of the tetrasaccharide fraction (1 ml) isolated after incubation for 5 or 10 h was chromatographed together with carrier sugars [nystose, 1^F,6^G-di-β-D-fructofuranosylsucrose and 6^G(1-β-D-fructofuranosyl)$_2$sucrose, 1 mg of each] on a column of charcoal–Celite. The isomers isolated were assayed for their radioactivities. The results showed that only nystose was produced in the incubation.

In a parallel experiment with [U-^{14}C]nystose (0.03 μCi, 10 μl), the radioactivity incorporated into pentasaccharide was measured (Fig. 9).

Chromatography on charcoal–Celite of the pentasaccharide fraction, using 1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucrose [$m = 0$, $n = 3$; $m = 1$, $n = 2$; $m = 2$, $n = 1$; and $m = 3$, $n = 0$] as carrier sugars, indicated the pentasaccharide to be 1^F(1-β-D-fructofuranosyl)$_3$sucrose.

Thus, the enzyme catalysed "self-transfer" between two similar saccharides of the 1-kestose series [1^F(1-β-D-fructofuranosyl)$_n$sucrose], to form the oligosaccharide having a d.p. higher by one D-fructose residue, and the D-fructosyl transfer involved HO-1 of a terminal D-fructosyl residue.

With the neokestose-series saccharide [1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucrose] as substrate, the same results as those described above were obtained. However, the enzyme did not transfer a D-fructosyl residue linked to HO-6 of the D-glucosyl group in 1^F(1-β-D-fructofuranosyl)$_n$-6^G-β-D-fructofuranosylsucrose, since neokestose did not serve as substrate for "self-transfer".

(*b*) D-*Fructosyl transfer from 1-kestose to sucrose.* A mixture of 0.4M [U-^{14}C]sucrose (0.1 μCi) in McIlvaine buffer (pH 5.0, 10 μl), 0.4M 1-kestose in the same buffer (10 μl), and enzyme (20 U/ml, 20 μl) was incubated for 0.5, 1, 2, 3, 5, and 10 h, at 30° in the presence of the minimum amount of toluene. The reaction was terminated by the addition of 0.1M mercuric chloride (10 μl). The amount of 1-kestose formed was calculated from the radioactivity incorporated into the trisaccharide fraction.

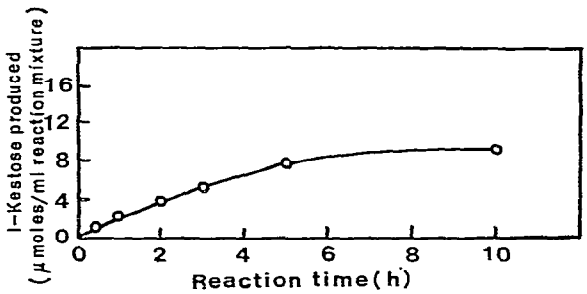


Fig. 10. D-Fructosyl transfer from 1-kestose to sucrose by asparagus 1^F-fructosyltransferase (see text for details).

The trisaccharide synthesised was proved to be 1-kestose only by t.l.c. As shown in Fig. 10, 1-kestose was produced proportionally to the reaction time until ~5 h.

The results indicate that the enzyme catalyses D-fructosyl transfer from 1-kestose to HO-1 of the D-fructosyl group of sucrose, to form another 1-kestose molecule.

(*c*) D-*Fructosyl transfer from 1-kestose to* 1^F(*1-β-*D-*fructofuranosyl*)$_n$*sucrose and* 1^F(*1-β-*D-*fructofuranosyl*)$_m$-6^G(*1-β-*D-*fructofuranosyl*)$_n$*sucrose*. A mixture of 0.4M [U-^{14}C]-1-kestose (0.045 μCi) in McIlvaine buffer (pH 5.0, 10 μl), one of the acceptors [0.4M 1^F(1-β-D-fructofuranosyl)$_n$sucrose (n = 2 or 3), 0.4M 1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucrose (m = 0, n = 1; m = 0, n = 2; m = 0, n = 3; m = 1, n = 1; m = 1, n = 2; or m = 2, n = 1) or 5% inulin] in the same buffer (10 μl), and enzyme (5.0 U/ml, 20 μl) was incubated for 3 h at 30°. After 0.1M mercuric chloride (10 μl) had been added, the mixture was subjected to p.c. together

TABLE II

FRUCTOSYLTRANSFER FROM 1-KESTOSE TO SEVERAL FRUCTO-OLIGOSACCHARIDES BY ASPARAGUS 1^F-FRUCTOSYLTRANSFERASE[a]

Acceptor	*Fructose transferred*	
	(mol/ml of reaction mixture)	*(ratio)*
1^F-β-D-Fructofuranosylsucrose (1-kestose) (control)	7.50	1.00
1^F (1-β-D-Fructofuranosyl)$_2$sucrose (nystose)	1.05	0.14
1^F (1-β-D-Fructofuranosyl)$_3$sucrose	0.73	0.10
6^G-β-D-Fructofuranosylsucrose (neokestose)	8.00	1.07
6^G (1-β-D-Fructofuranosyl)$_2$sucrose	4.76	0.63
6^G (1-β-D-Fructofuranosyl)$_3$sucrose	3.49	0.47
1^F,6^G-Di-β-D-fructofuranosylsucrose	5.26	0.70
1^F (1-β-D-Fructofuranosyl)$_2$-6^G-β-D-fructofuranosylsucrose	3.78	0.50
1^F-β-D-Fructofuranosyl-6^G (1-β-D-fructofuranosyl)$_2$sucrose	2.85	0.38
Inulin	Nil	—

[a]Details of the experiments are described in the text.

with carrier sugars (5 development with solvent *B*). Zones corresponding to mono- to hexa- and higher-saccharides were extracted with water, and each extract was concentrated *in vacuo* to dryness. Each residue was dissolved in water (1 ml) and assayed for radioactivity. The amount of D-fructose transferred was calculated from the radioactivity of each oligosaccharide.

As shown in Table II, the enzyme catalysed D-fructosyl transfer most rapidly from 1-kestose to neokestose, and quite rapidly to $1^F,6^G$-di-β-D-fructofuranosylsucrose, $6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose, $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_2\text{-}6^G\text{-}\beta\text{-D-fructofuranosylsucrose}$, $6^G(1\text{-}\beta\text{-D-fructofuranosyl})_3$sucrose, and 1^F-β-D-fructofuranosyl-$6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose. The rate of D-fructosyl transfer from 1-kestose to nystose and $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_3$sucrose was slow, and to inulin was zero.

These findings indicate that neokestose and related saccharides [$1^F(1\text{-}\beta\text{-D-fructofuranosyl})_m\text{-}6^G(1\text{-}\beta\text{-D-fructofuranosyl})_n$sucrose] are good acceptors, but the 1-kestose series saccharides [$1^F(1\text{-}\beta\text{-D-fructofuranosyl})_n$sucrose] (with the exception of 1-kestose) and inulin are not.

A portion (0.5 ml) of the tetrasaccharide fraction (1 ml) formed from [U-^{14}C]-1-kestose and neokestose [asparagus 1^F-FT (6.8 U/ml)], and isolated by p.c. as described above, was chromatographed together with carrier sugars [nystose, $6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose and $1^F,6^G$-di-β-D-fructofuranosylsucrose, 1 mg of each] on a charcoal–Celite column.

Two radioactive tetrasaccharides were isolated and identified as $6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose (4.78 μmol/ml of reaction mixture) and $1^F,6^G$-di-β-D-fructofuranosylsucrose (5.08 μmol).

Likewise, the pentasaccharide fractions from the reaction mixtures of [U-^{14}C]-1-kestose and $6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose or $1^F,6^G$-di-β-D-fructofuranosylsucrose were found to be 1^F-β-D-fructofuranosyl-$6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose (4.84 μmol) and $6^G(1\text{-}\beta\text{-D-fructofuranosyl})_3$sucrose (1.87 μmol) from the former, and 1^F-β-D-fructofuranosyl-$6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose (4.67 μmol) and $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_2\text{-}6^G\text{-}\beta\text{-D-fructofuranosylsucrose}$ (2.35 μmol) from the latter.

These results indicate that D-fructosyl transfer from 1-kestose occurs preferentially to HO-1 of the terminal D-fructosyl residue of the shorter chain in neokestose-series saccharides.

DISCUSSION

A fructosyltransferase catalysing the transfer of a terminal D-fructosyl group from fructosaccharides [$1^F(1\text{-}\beta\text{-D-fructofuranosyl})_n$sucrose] to HO-1 of the D-fructosyl group of similar saccharides [$1^F(1\text{-}\beta\text{-D-fructofuranosyl})_m$sucrose] was reported[12,13] in studies on the biosynthesis of fructosaccharides in tubers of the Jerusalem artichoke (*Helianthus tuberosus* L.), and named β-(2→1′)-fructan:β-(2→1′)-fructan 1-fructosyltransferase (FFT).

The FFT catalyses the fructosyl transfer reaction described below, to produce a series of oligofructosides and polyfructans. The enzyme also catalyses "self-

transfer" between two identical saccharide molecules ($n = m = 1$–7), to form a series of polymers with d.p. larger and smaller than that of the original substrate:

$$\text{Glc–Fru–(Fru)}_n + \text{Glc–Fru–(Fru)}_m \rightleftharpoons \text{Glc–Fru–(Fru)}_{n-1} + \text{Glc–Fru–(Fru)}_{m+1},$$

where $n = 1$–$\sim$35, $m = 0$–$\sim$35.

A fructosyltransferase of this type (FFT) was found in onion[13] and agave[14] plants. A "sucrose:sucrose fructosyltransferase" and "fructan:fructan fructosyltransferase" have been isolated[15] from the inner leaf bases of onion plants: the latter preparation was also able to produce neokestose from 1-kestose. Hitherto, the FFT of higher plants has not been purified highly and the specificity of its fructosyl transfer is obscure.

A fructosyltransferase from asparagus roots has now been purified and its general properties and substrate specificity have been established. The asparagus enzyme was purified 298-fold by nine successive purification steps and then showed a single protein band on polyacrylamide gel electrophoresis. The enzyme was freed from β-D-fructofuranosidase activity by calcium phosphate-gel treatment, and SST and 6^G-fructosyltransferase (6^G-FT) activities by column chromatography on octyl-Sepharose.

The general properties of the enzyme resembled those of SST and 6^G-FT from asparagus roots, but its substrate specificity and chromatographic behaviour differed. The enzyme catalysed "self-transfer" between identical oligosaccharides of the 1-kestose series [1^F(1-β-D-fructofuranosyl)$_n$sucrose]. The terminal D-fructosyl group is transferred to HO-1 of another saccharide molecule. Sucrose does not serve as a substrate for "self-transfer". The enzyme also catalyses similar "self-transfer" in the neokestose series [1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucrose]. Since neokestose cannot serve as substrate of "self-transfer", the enzyme appears not to be able to catalyse the transfer of a D-fructosyl residue linked to HO-6 of the D-glucosyl group in the neokestose-series.

The enzyme also catalyses D-fructosyl transfer from 1-kestose to HO-1 of a terminal D-fructosyl group of 1^F(1-β-D-fructofuranosyl)$_n$sucroses and 1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucroses (m and $n \geqslant 0$). The latter saccharides are much the better acceptors. The reaction is favoured by a low number of residues in the acceptor saccharide. For neokestose-series saccharides, D-fructosyl transfer tends to occur preferentially at the D-fructosyl group of the shorter chain in the acceptor molecule. Inulin does not accept D-fructosyl residues from 1-kestose.

The asparagus fructosyltransferase differs distinctly from the β-(2→1′)-fructan:β-(2→1′)-fructan 1-fructosyltransferase of Jerusalem artichoke tubers in the specificity towards neokestose-series saccharides, inulin, and its related polymer saccharides, the neokestose series being effective, but inulin and its related polymers ineffective, as substrates for the asparagus enzyme. For these reasons and by analogy with the asparagus 6^G-fructosyltransferase[6], the fructosyltransferase purified from asparagus roots is tentatively termed 1^F-fructosyltransferase.

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