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PURIFICATION AND CHARACTERISATION OF 6^G-FRUCTOSYLTRANSFERASE FROM THE ROOTS OF ASPARAGUS (*Asparagus officinalis* L.)

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ABSTRACT

A fructosyltransferase that catalyses the transfer of the terminal (2→1)-β-linked D-fructosyl group of fructo-oligosaccharides [1^F(1-β-D-fructofuranosyl)$_m$sucrose, $m > 0$] to HO-6 of the glucosyl group of similar saccharides [1^F(1-β-D-fructofuranosyl)$_n$sucrose, $n \geq 0$] has been purified (760-fold) from an extract of the roots of asparagus (*Asparagus officinalis* L.) by successive fractionation with ammonium sulfate, treatment with calcium phosphate gel, and then chromatography on octyl-Sepharose, DEAE-cellulose, Sephadex G-200, and raffinose-coupled Sepharose 6B. The enzyme, tentatively termed 6^G-fructosyltransferase, was homogeneous in disc electrophoresis, had a mol. wt. of ~69,000 and an optimum pH of ~5.5, was stable at pH 5.0–6.0 on heating for 20 mins at 45° and for 10 min at 20–37°, and was inhibited by Hg^{2+}, *p*-chloromercuribenzoate, and Ag^+.

INTRODUCTION

Nine oligosaccharides of the 1^F(1-β-D-fructofuranosyl)$_n$sucrose, 6^G(1-β-D-fructofuranosyl)$_n$sucrose, and 1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$-sucrose series have been found in the roots of asparagus[1,2] and can be synthesised *in vitro* by using an enzyme preparation obtained from asparagus roots[3]. Thus, three different types of fructosyltransferase appear to be present in asparagus roots. The isolation and characterisation of sucrose:sucrose 1-fructosyltransferase (SST) has been reported[4], and we now describe the purification and characterisation of a fructosyltransferase [6^G-fructosyltransferase (6^G-FT)].

MATERIALS AND METHODS

Materials. — Roots of asparagus (*Asparagus officinalis* L.) were collected from an experimental field near Sapporo in September, 1976, and stored in the frozen state. [U-^{14}C]Sucrose and [6,6′-^{3}H]sucrose were 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*], [U-^{14}C]neokestose [*O*-β-D-Fru*f*-(2→6)-*O*-α-D-Glc*p*-(1↔2)-

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β-D-Fruf], and [U-^{14}C]nystose [*O*-β-D-Fruf-(2→1)-*O*-β-D-Fruf-(2→1)-*O*-β-D-Fruf-(2↔1)-α-D-Glcp] were prepared from [U-^{14}C]sucrose using a preparation of asparagus fructosyltransferase[3].

1-Kestose, neokestose, nystose, 6^G(1-β-D-fructofuranosyl)$_n$sucrose (n = 2 and 3), and 1^F(1-β-D-fructofuranosyl)$_m$-6^G(1-β-D-fructofuranosyl)$_n$sucrose (m = 1, n = 1–3; m = 2, n = 1) were isolated from asparagus roots, and 1^F(1-β-D-fructofuranosyl)$_n$sucrose (n = 3–5) were obtained from Jerusalem artichoke tubers[1–4].

Determination of sugars and proteins. — Total hexose was determined by the anthrone method[5]. Proteins were determined by measuring absorbance at 280 nm referred to $E^{1\%}_{1\,cm}$ = 9.38 in aldolase[6].

P.c. and t.l.c. — Toyo No. 50 paper was used together with 1-propanol–ethyl acetate–water mixtures (*A*, 7:1:2: *B*, 6:1:3) and Kieselgel 60 (Merck) with 3–5 developments with *C* 1-butanol–2-propanol–water (10:5:4). Sugars were detected with anisidine phosphate[7].

Charcoal–Celite column chromatography. — A column (1.5 × 54 cm) of charcoal (Tokusei-shirasagi Bland, Takeda Chemical Industries Ltd.) and Celite 535 (Nakarai Chemical Industries Ltd.) (1:1) was washed with hydrochloric acid and then with water. Sugars were eluted by successive application of water, and 10, 13, and 20% ethanol[1,3].

Measurement of enzyme activity. — The activity of 6^G-fructosyltransferase (6^G-FT) was defined as the synthesis of 1^F,6^G-di-β-D-fructofuranosylsucrose{*O*-β-D-Fruf-(2→1)-*O*-[β-D-Fruf-(2→6)-*O*-α-D-Glcp-(1↔2)]-β-D-Fruf} from 1-kestose; 1 unit of activity refers to the amount of the enzyme which transfers 1 μmol of D-fructose during 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 5.5, 20 μl) was incubated at 30° for 1 h. The reaction was terminated by adding 0.1M mercuric chloride (10 μl), a carrier sugar [1^F,6^G-di-β-D-fructofuranosylsucrose] was added, and the mixture was subjected to charcoal–Celite chromatography and then p.c. The fructose transferred was calculated from the radioactivity in the 1^F,6^G-di-β-D-fructofuranosylsucrose isolated. Radioactivity measurements were performed with an Aloka Liquid Scintillation Spectrometer, Model LSC 900 or Model LSC 502[3].

Disc electrophoresis. — 7.5% Polyacrylamide gel (pH 8.0) was used[8,9] for 2 h at room temperature and 2 mA/tube. Protein bands were stained with Amido Black 10B.

Determination of molecular weight by gel filtration. — The ascending method was used with a column (2.64 × 98 cm) of Sephadex G-200 pre-equilibrated with 0.01M phosphate buffer containing 0.25M sodium chloride (pH 6.5) and elution with the same buffer at 6 ml/h. Chymotrypsinogen A (mol. wt., 25,000), hen-egg albumin (45,000), bovine serum albumin (67,000), and aldolase (147,000) were purchased from Boehringer Mannheim GmbH and employed as the reference proteins.

Preparation of raffinose-coupled Sepharose 6B. — Raffinose was coupled with epoxy-activated Sepharose 6B as previously described[4].

RESULTS

Purification of the enzyme. — (*a*) *Fractionation with ammonium sulfate.* Asparagus roots (200 g each; total 18 kg), previously washed with water and chopped, were homogenised in 0.05M phosphate buffer (pH 6.5, 1 litre). The homogenate was filtered through cheese cloth and centrifuged (10,000*g*, 15 min). 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 (5.7 litres) comprised fraction A.

The supernatant was treated with solid ammonium sulfate to 0.3–0.5 saturation. The precipitate was collected by centrifugation, and a solution in 0.01M phosphate buffer (pH 6.5) was dialysed for 4 days against the same buffer and then centrifuged. The supernatant (800 ml) comprised fraction B.

(*b*) *Treatment with calcium phosphate gel.* A suspension of calcium phosphate gel in fraction B (800 ml; gel 6.5 g/protein 1 g) was stored at 5° for 30 min and then centrifuged. The supernatant solution was concentrated by ultrafiltration (Amicon; Diaflo PM-10 filter) and dialysed overnight against 0.01M phosphate buffer (pH 6.5), to give fraction C (1.05 litre) which had little hydrolytic activity.

(*c*) *Octyl-Sepharose column chromatography.* Fraction C was concentrated to 500 ml by ultrafiltration, saturated to 35% with ammonium sulfate, and stored overnight in a cold chamber. The resulting precipitate was removed by centrifugation and the supernatant was loaded on to a column (5.5 × 30 cm) of octyl-Sepharose CL-4B previously equilibrated with 0.01M phosphate buffer (pH 6.5) saturated to 35% with ammonium sulfate. The column was eluted successively with 0.01M phosphate buffers (pH 6.5; 1.5 litres each) saturated with ammonium sulfate to 35, 30, 20, and 10%, respectively, and then with the buffer alone (2 litres). 6^G-Fructosyltransferase (6^G-FT) and 1^F-fructosyltransferase[3] (1^F-FT) activities were present in the buffer eluate saturated to 20% with ammonium sulfate and in the 0.01M phosphate buffer, respectively. The former eluate was concentrated by ultrafiltration to ~500 ml, 0.01M phosphate buffer (~500 ml) was added, and the solution was concentrated. The process was repeated and the final solution (~500 ml), which was almost free from ammonium sulfate, was dialysed against 0.01M phosphate buffer (pH 7.0) for 2 days to give fraction D (1 litre).

(*d*) *DEAE-cellulose chromatography.* Fraction D was chromatographed on a column (5 × 12 cm) of DEAE-cellulose equilibrated with 0.01M phosphate buffer (pH 7.0) by successive elution with 0.01, 0.02, and 0.07M phosphate buffers (pH 7.0, 1 litre each). The 0.07M buffer eluate showed 6^G-FT activity; it was concentrated and dialysed overnight against 0.01M phosphate buffer to give fraction E (65 ml) which was further chromatographed on a column (2.8 × 10 cm) of DEAE-cellulose by successive elution with 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, and 0.07M phosphate buffers (pH 7.0, 300 ml each). The 0.05 and 0.06M phosphate buffer eluates showed 6^G-FT activity. These eluates were combined, concentrated by ultrafiltration, and dialysed

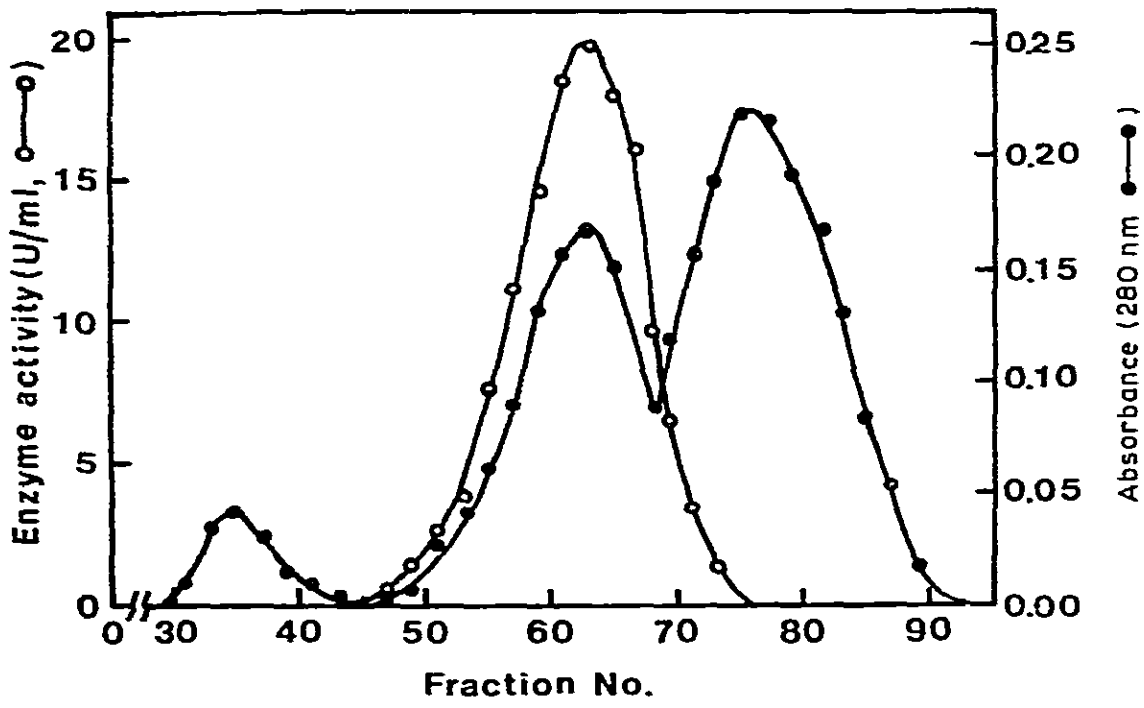


Fig. 1. Chromatography of asparagus 6^G-fructosyltransferase on Sephadex G-200; elution with 0.01M phosphate buffer containing 0.25M NaCl (5-ml fractions).

overnight against 0.25M sodium chloride in 0.01M phosphate buffer (pH 6.5) to give fraction F (50 ml).

(*e*) *Sephadex G-200 chromatography*. Fraction F was concentrated to 3 ml by ultrafiltration and chromatographed on a column (2.64 × 98 cm) of Sephadex G-200 equilibrated with 0.25M sodium chloride in 0.01M phosphate buffer (pH 6.5) by elution with the same buffer (Fig. 1). Thus, active fractions 55–67 were combined and concentrated to give fraction G (10 ml).

(*f*) *Raffinose-coupled Sepharose 6B chromatography*. Fraction G was dialysed overnight against 0.01M acetate buffer (pH 5.6) and then subjected to chromatography on a column (1 × 8 cm) of raffinose-coupled Sepharose 6B. Elution with

TABLE I

PURIFICATION OF ASPARAGUS 6^G-FT

Fraction[a]	*Step*	*Total activity (U)*	*Total protein (mg)*	*Volume (ml)*	*Specific activity (U/mg of protein)*
A	$(NH_4)_2SO_4$, 0–1 saturation	12600	70380	5700	0.18
B	$(NH_4)_2SO_4$, 0.3–0.5 saturation	9580	24520	800	0.39
C	Calcium phosphate gel	7172	6410	1050	1.12
D	Octyl-Sepharose	5710	715	1000	7.99
E	DEAE-cellulose, 1st	3098	104	65	29.8
F	DEAE-cellulose, 2nd	1785	42.8	50	41.7
G	Sephadex G-200	931	8.01	10	116.2
H	Sepharose 6B	684	5.00	10	136.8

[a]See Results.

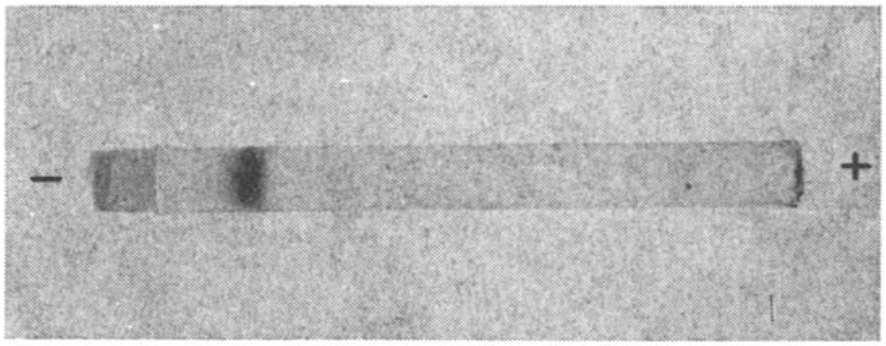


Fig. 2. Disc electrophoresis of asparagus 6^G-fructosyltransferase (60 μg as protein) on polyacrylamide gel (see Results).

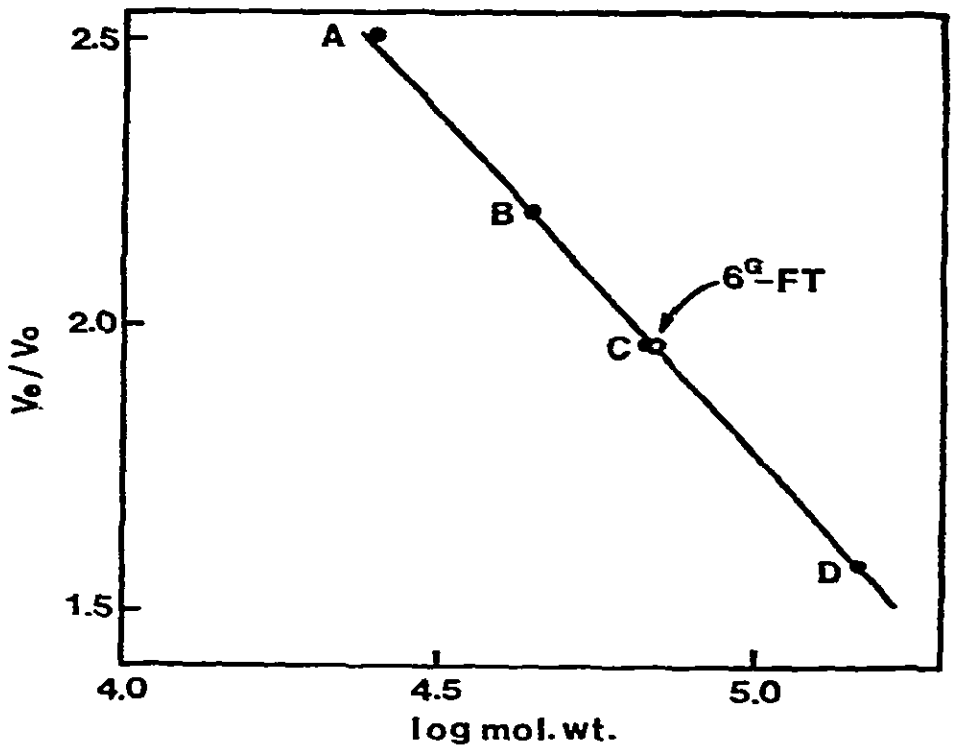
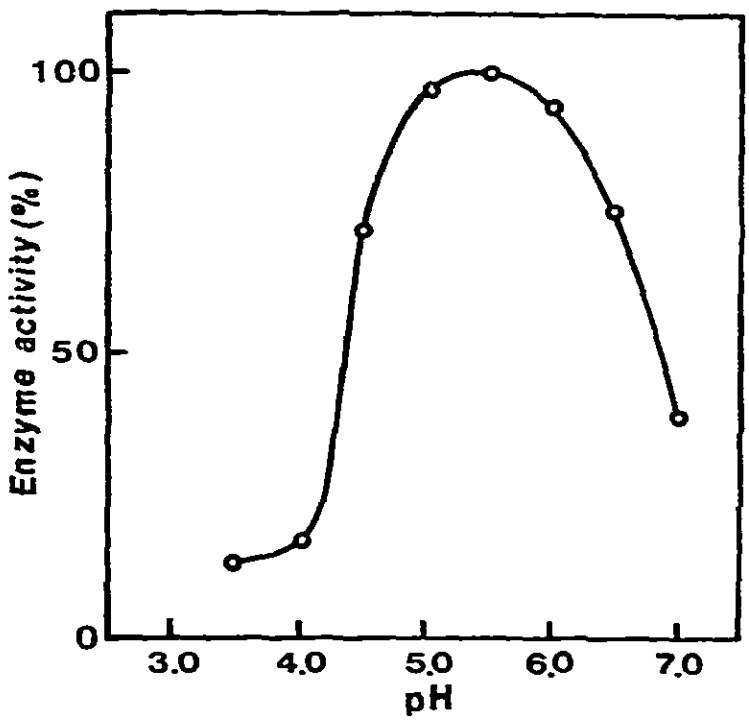


Fig. 3. Estimation of molecular weight of asparagus 6^G-fructosyltransferase by gel filtration: Ve, elution volume; Vo, void volume. Standard proteins: A, chymotrypsinogen A (mol. wt. 25,000); B, hen-egg albumin (45,000); C, bovine serum albumin (67,000); D, aldolase (147,000).



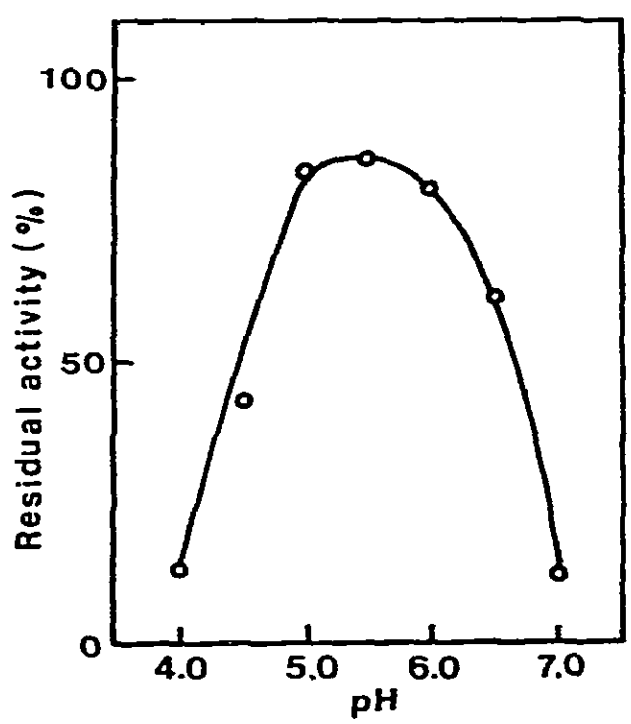


Fig. 4. Effect of pH on the activity of asparagus 6^G-fructosyltransferase (13.9 U/ml).

Fig. 5. Effect of pH on the stability of asparagus 6^G-fructosyltransferase (24 U/ml).

0.01M acetate buffer (pH 5.6, 100 ml) removed a trace of sucrose:sucrose 1-fructosyltransferase (SST). Elution with 0.01M phosphate buffer (pH 6.0, 100 ml) then gave the 6^G-FT. The eluate was concentrated, and dialysed against 5mM phosphate buffer (pH 6.5), to give a finally purified enzyme preparation fraction H (10 ml).

Thus, as shown in Table I, this enzyme could be purified 760-fold from fraction A, and its specific activity was 136.8 U/mg of protein. The enzyme preparation was free from β-D-fructofuranosidase, SST, and 1^F-FT, because it failed to hydrolyse sucrose, 1-kestose, neokestose, or 1^F(1-β-D-fructofuranosyl)$_n$sucrose (n = 2–5), to transfer a fructosyl group of sucrose to sucrose, and to synthesise nystose from 1-kestose.

General properties of the enzyme. — (*a*) *Disc electrophoresis.* Fraction H from (*f*) above was subjected to electrophoresis on polyacrylamide gel (Fig. 2). One gel was stained with Amido Black 10B, and a second gel was cut into discs (2 mm width) each of which was homogenised with McIlvaine buffer (pH 5.5, 0.2 ml). 0.2M 1-Kestose (0.2 ml) was added and each mixture was incubated at 30° for 15 h. The 6^G-FT activity was assayed by t.l.c. of the reaction products. The 6^G-FT activity resided in a single disc which showed a protein band.

(*b*) *Molecular weight.* Molecular weight was estimated to be ~69,000, in comparison with those of the reference proteins, by Sephadex G-200 gel-filtration (Fig. 3).

(*c*) *Optimum pH.* A pH-dependence curve of the reaction between the enzyme and 1-kestose is given in Fig. 4; the optimum pH was ~5.5.

(*d*) *pH Dependence of stability.* Enzyme solutions variously at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 were pre-incubated at 45° for 20 min, cooled to 0°, and adjusted to pH 5.5, and the residual enzyme activity was measured (Fig. 5). At pH 5–6, 80% or more of the initial activity was detected; but at pH 4 and 7, ~87% of the activity was lost.

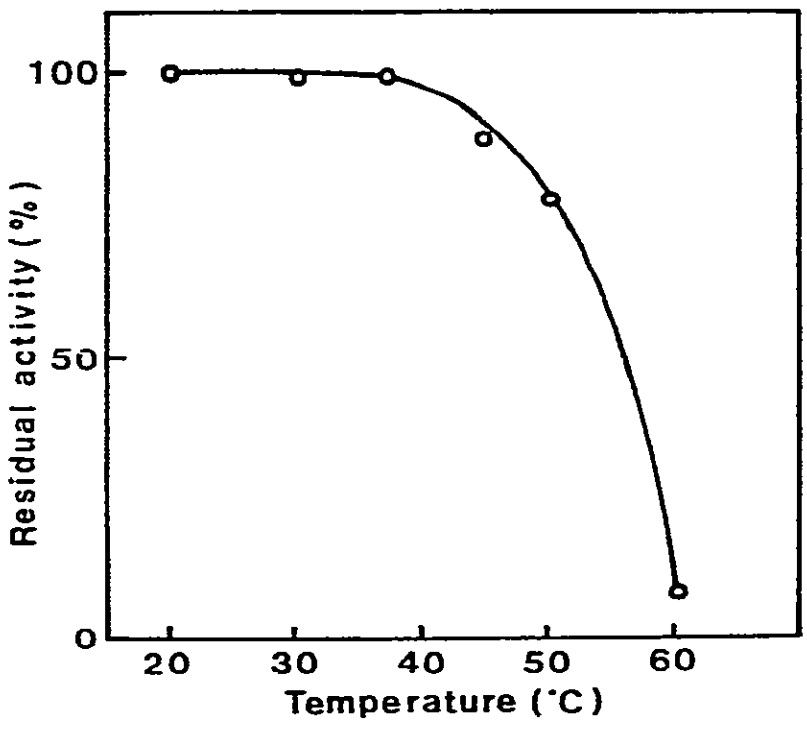


Fig. 6. Effect of temperature on asparagus 6^G-fructosyltransferase (18 U/ml).

TABLE II

EFFECTS[a] OF VARIOUS COMPOUNDS ON ASPARAGUS 6^G-FT

Compound	*Concentration (mM)*	*Relative activity*
Control	—	100.0
$HgCl_2$	0.1	8.4
*p*CMB	0.12	17.5
$AgNO_3$	1.0	63.8
$CuSO_4$	1.0	96.0
$BaCl_2$	1.0	98.3
$CaCl_2$	1.0	101.4
$ZnSO_4$	1.0	102.6
$MgCl_2$	1.0	105.8
$AlCl_3$	1.0	108.3

[a]See Results.

(*e*) *Thermal stability.* Enzyme solutions were heated for 10 min at 30, 37, 45, 50, or 60° (20° was taken as the control), and the residual activity was measured (Fig. 6); the enzyme was stable at 20–37°, but inactivated at 60°.

(*f*) *Effects of inhibitors.* A mixture of enzyme (9.5 U/ml, 20 μl) and 0.4M [U-^{14}C]-1-kestose in McIlvaine buffer (pH 5.5, 10 μl) was incubated at 30° for 1 h in the presence of water or one of the inhibitors (10 μl). After the reaction had been stopped by the addition of 0.1M mercuric chloride (10 μl), the mixture was subjected to p.c. to separate the mono-, di-, and higher saccharides. Each fraction was assayed for radioactivity. Residual activity of the enzyme was calculated from the radioactivity incorporated into the tetrasaccharide fraction, and expressed in terms of relative activity.

As shown in Table II, mercuric chloride (0.1mM), *p*-chloromercuribenzoate

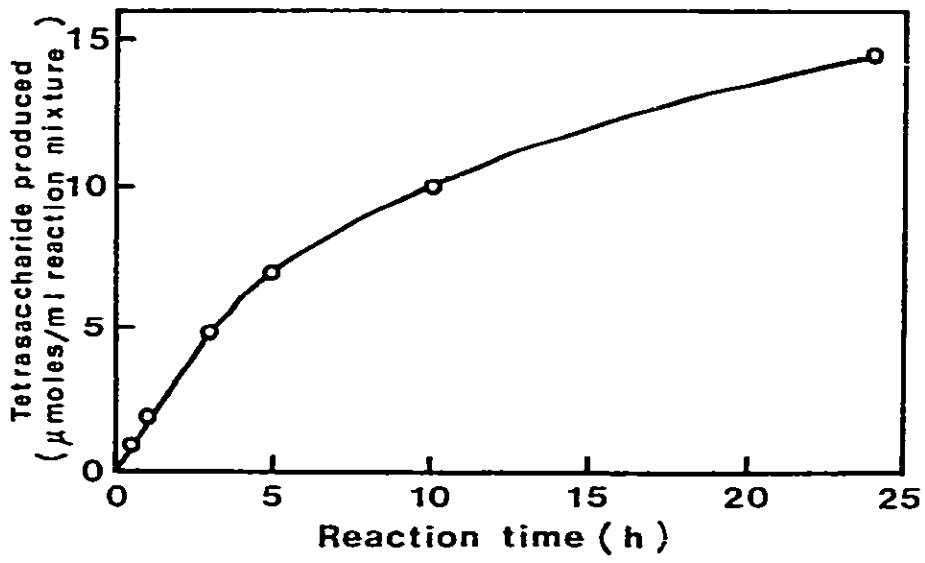


Fig. 7. Synthesis of tetrasaccharide from 1-kestose by asparagus 6^G-fructosyltransferase. A mixture of 0.5M [U-^{14}C]-1-kestose (0.045 μCi, 10 μl), McIlvaine buffer (pH 5.5, 10 μl), and enzyme (4.7 U/ml, 20 μl) was incubated in the presence of a small amount of toluene at 30°. The reaction was terminated with 0.1M mercuric chloride (10 μl), and the mixture, together with carrier sugars [D-fructose, D-glucose, sucrose, and 1^F(1-β-D-fructofuranosyl)$_n$sucrose (n = 2–5), 100 μg of each], was subjected to p.c. (5 developments with solvent *A*). The eight fractions thus obtained were concentrated *in vacuo* and solutions of the residues in water (2 ml) were assayed for radioactivity.

(0.12mM), and silver nitrate (mM) inhibited the enzyme by ~92, ~83, and ~36%, respectively. The enzyme activity was accelerated by ~6 and ~8% with magnesium chloride and aluminum chloride, respectively. Calcium sulfate, barium chloride, calcium chloride, and zinc sulfate were ineffective.

Substrate specificity of 6^G*-FT.* — (*a*) *1-Kestose.* The formation of tetrasaccharides was monitored when radioactive 1-kestose was incubated with the 6^G-FT preparation. The formation of tetrasaccharides was accompanied by liberation of sucrose, which increased during ~3 h and then decreased (Fig. 7).

A portion (1 ml) of the tetrasaccharide fraction (2 ml) in Fig. 7 was subjected to chromatography, together with carrier sugars {nystose, 1^F,6^G-di-β-D-fructofuranosylsucrose, and 6^G(1-β-D-fructofuranosyl)$_2$sucrose [*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*]; 1 mg each}, on charcoal–Celite. The isomers isolated were assayed for their radioactivities. The results indicated that only 1^F,6^G-di-β-D-fructofuranosylsucrose was produced during ~10 h, and traces of 6^G(1-β-D-fructofuranosyl)$_2$sucrose and nystose appeared after prolonged reaction.

(*b*) *Nystose.* An experiment similar to that described in (*a*) was performed with [U-^{14}C]nystose, and the radioactivity incorporated into the pentasaccharides was measured (Fig. 8). The pentasaccharides were isolated by charcoal–Celite chromatography, 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.

The synthesis of pentasaccharides with liberation of 1-kestose was proportional to the reaction time up to ~10 h. The pentasaccharide synthesised was 1^F(1-β-D-fructofuranosyl)$_2$-6^G-β-D-fructofuranosylsucrose{*O*-β-D-Fru*f*-(2→1)-*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*} by charcoal–Celite chromatography.

(*c*) *1*^{F}(1-β-D-*Fructofuranosyl*)$_n$*sucrose* (n = *3 and 4*). Unlabelled saccharides were incubated with 6^G-FT for 24 h as described above, and the products were analysed by t.l.c.

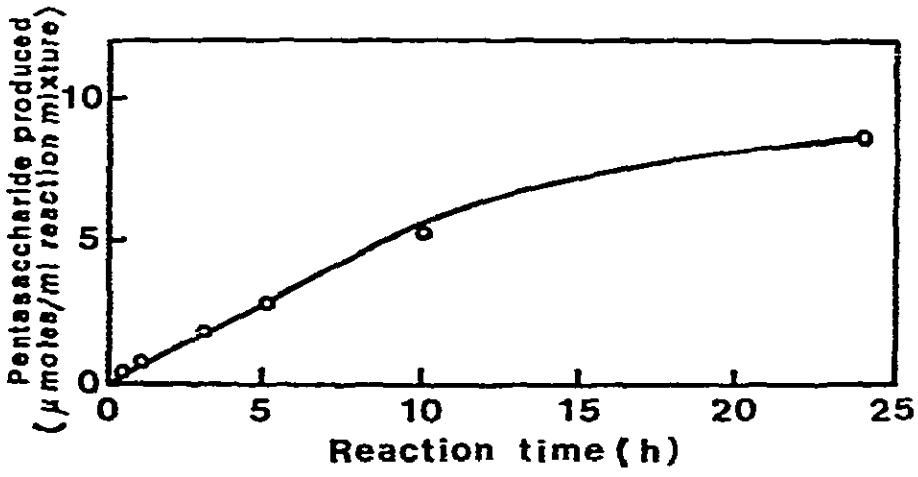


Fig. 8. Synthesis of pentasaccharide from nystose by asparagus 6^G-fructosyltransferase. The experiment was conducted in a manner similar to that described for Fig. 7, and the formation of pentasaccharide was monitored on the basis of radioactivity incorporated from 0.4M [U-^{14}C]nystose (0.03 μCi, 10 μl).

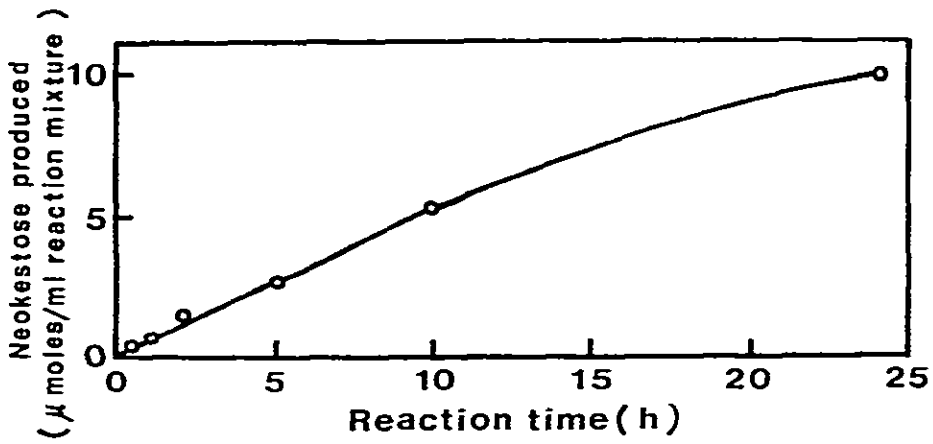


Fig. 9. Formation of neokestose by asparagus 6^G-FT-catalysed fructosyl transfer from 1-kestose to sucrose. A mixture of enzyme (4.7 U/ml, 20 μl), 0.2M 1-kestose, and 0.2M [U-^{14}C]sucrose (0.1 μCi) in McIlvaine buffer (pH 5.5, 20 μl) was incubated at 30°. After addition of 0.1M mercuric chloride (10 μl) to terminate the reaction, the mixture was subjected to p.c. (solvent *A*) to isolate the trisaccharide fractions; the radioactivity of each fraction was assayed.

Small amounts of 1^F(1-β-D-fructofuranosyl)$_3$-6^G-β-D-fructofuranosylsucrose and 1^F(1-β-D-fructofuranosyl)$_4$-6^G-β-D-fructofuranosylsucrose were produced from 1^F(1-β-D-fructofuranosyl)$_3$sucrose and 1^F(1-β-D-fructofuranosyl)$_4$sucrose, respectively.

The above findings indicate that the asparagus 6^G-FT is highly specific for the transfer of a single, terminal (2→1)-linked fructofuranosyl group to HO-6 of the glucose residue in another saccharide molecule.

Fructosyl transfer reactions from 1-kestose. — (*a*) *To sucrose.* The results are shown in Fig. 9. Chromatography of the trisaccharide fraction on charcoal–Celite

TABLE III

EFFECT OF SUCROSE CONCENTRATION ON THE FORMATION OF NEOKESTOSE AND 1^F,6^G-DI-β-D-FRUCTOFURANOSYLSUCROSE FROM 1-KESTOSE BY ASPARAGUS 6^G-FT[a]

Reaction mixture		*Saccharide produced (μmol/reaction mixture)*	
[U-^{14}C]-1-Kestose (M)[b]	*[^{3}H]Sucrose* (M)[b]	*Neokestose*[c]	*1^F,6^G-Di-β-D-fructofuranosylsucrose*[d]
0.1	0	—	10.00
0.1	0.010	1.24	9.05
0.1	0.025	2.50	8.41
0.1	0.050	3.89	8.27
0.1	0.1	3.90	5.21
0.1	0.2	4.01	2.25
0.1	0.4	5.45	1.88
0.1	0.5	5.53	1.97

[a]A mixture of enzyme (20 μl), 0.2M [U-^{14}C]-1-kestose (0.045 μCi), and 0.02–1M [^{3}H]sucrose (0.1 μCi) in McIlvaine buffer (pH 5.5, 20 μl) was incubated at 30° for 2 h. After addition of 0.1M mercuric chloride (10 μl) to stop the reaction, the mixture was subjected to p.c. (solvent *A*) to isolate the tri- and tetra-saccharide fractions. Neokestose and 1^F,6^G-di-β-D-fructofuranosylsucrose were isolated from the tri- and tetra-saccharide fractions by chromatography on charcoal–Celite and assayed for radioactivity. [b]Concentration in reaction mixture. [c]Calculated from [^{3}H]-radioactivity incorporated. [d]Calculated from [^{14}C]-radioactivity incorporated.

TABLE IV

FRUCTOSYL TRANSFER FROM 1-KESTOSE TO $1^F(1\text{-}\beta\text{-D-FRUCTOFURANOSYL})_n$SUCROSE BY ASPARAGUS 6^G-FT[a]

Donor	*Acceptor (unlabelled)*	*Fructose transferred (μmol/ml of reaction mixture)*
[U-^{14}C]-1-Kestose	None (Control)	5.00
[U-^{14}C]-1-Kestose	Nystose	1.42
[U-^{14}C]-1-Kestose	$1^F(1\text{-}\beta\text{-D-Fructofuranosyl})_3$sucrose	0.87
[U-^{14}C]-1-Kestose	$1^F(1\text{-}\beta\text{-D-Fructofuranosyl})_4$sucrose	0.69
[U-^{14}C]-1-Kestose	$1^F(1\text{-}\beta\text{-D-Fructofuranosyl})_5$sucrose	0.48
[U-^{14}C]-1-Kestose	Inulin	Nil

[a]A mixture of 0.2M [U-^{14}C]-1-kestose (0.1 μCi) in McIlvaine buffer (pH 5.5, 10 μl), an acceptor [0.4M $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_n$sucrose ($n$ = 2–5) or 5% inulin] in the same buffer (10 μl), and enzyme (20 μl) was incubated at 30° for 3 h. 0.1M Mercuric chloride (10 μl) was added to terminate the reaction, and the mixture was subjected to p.c. (5 developments) together with carrier sugars. Zones corresponding to mono- to octa-saccharides were extracted with water, and the extracts were concentrated *in vacuo* to dryness. Each residue was dissolved in water (2 ml) and assayed for radioactivity. The fructose transferred was calculated from the radioactivity of the synthesised saccharide having a d.p. higher by one fructose residue than that of the acceptor.

gave only neokestose, which was produced proportionally to the reaction time during 10 h. Traces of another trisaccharide had appeared after 24 h. Thus, the 6^G-FT preparation catalyses fructosyl transfer from 1-kestose to HO-6 of the glucose residue of sucrose.

A small amount of $1^F,6^G$-di-β-D-fructofuranosylsucrose was present in the tetrasaccharide fraction; hence, the effect of sucrose on the formation of neokestose and $1^F,6^G$-di-β-D-fructofuranosylsucrose from 1-kestose was investigated. As shown in Table III, the formation of neokestose increased with increase in the concentration of sucrose, whereas that of $1^F,6^G$-di-β-D-fructofuranosylsucrose decreased. The decrease was ~50% with 0.1M sucrose and 80% at 0.5M; the ratios of neokestose to the tetrasaccharide were ~0.5 at 0.05M, 0.7 at 0.1M, 1.8 at 0.2M, and 2.8 at 0.5M sucrose.

(*b*) *To* $1^F(1\text{-}\beta\text{-D-}fructofuranosyl)_n$*sucrose* (n = *3–5*) *and inulin.* Table IV shows that the fructosyl transfer from 1-kestose to these oligosaccharides was highly catalysed for the saccharides, but that inulin was not an acceptor.

The pentasaccharides produced from the reaction between [U-^{14}C]-1-kestose and nystose were isolated by p.c. (Table IV), and, together with the carrier sugars $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_3$sucrose, $6^G(1\text{-}\beta\text{-D-fructofuranosyl})_3$sucrose, $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_2\text{-}6^G\text{-}\beta\text{-D-fructofuranosylsucrose}$, and $1^F\text{-}\beta\text{-D-fructofuranosyl-}6^G(1\text{-}\beta\text{-D-fructofuranosyl})_2$sucrose (1 mg each), were subjected to re-chromatography on charcoal–Celite. Two radioactive pentasaccharides were isolated and identified as $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_3$sucrose and $1^F(1\text{-}\beta\text{-D-fructofuranosyl})_2\text{-}6^G\text{-}\beta\text{-D-fructofuranosylsucrose}$. The incorporation of radioactivity was 42 c.p.m. into the former and 804 c.p.m. into the latter.

Thus, fructosyl transfer from 1-kestose to nystose occurs overwhelmingly at HO-6 of the glucosyl group of nystose and negligibly at the terminal fructosyl group. Fructosyl transfer from 1-kestose to 1^F(1-β-D-fructofuranosyl)$_{3-5}$sucrose probably occurs in the same fashion.

DISCUSSION

Three types of enzyme which catalyse fructosyl transfer have been reported in higher plants, namely, sucrose:sucrose 1-fructosyltransferase (SST) in Jerusalem artichoke[10,11], *Polianthes tuberosa*[12], onion[10,11], chicory[13,14], lettuce[15], and agave[16], (2→1)-β-D-fructan:(2→1)-β-D-fructan 1-fructosyltransferase (FFT) in Jerusalem artichoke[11], onion[11], and agave[17], and invertase in sugar beet[18] and banana[19].

These fructosyltransferases catalyse fructosyl transfer from (2→1)-β-linked fructosaccharides to HO-1 of the fructosyl group of another molecule, although invertase of sugar beet[18] and banana[19] and the "transfructosylase" of agave plants[17] show wide substrate specificity.

Hitherto, no enzyme which catalyses fructosyl transfer from a (2→1)-β-linked fructosaccharide to HO-6 of the glucosyl group of another saccharide molecule has been purified highly and characterised. Scott[10] and Edelman and Jefford[11] assumed that an onion enzyme fraction containing SST and FFT catalysed fructosyl transfer from 1-kestose to the glucosyl (HO-6) group of sucrose, producing neokestose. Recently, Satyanarayana[17] reported that the "transfructosylase" fraction, with SST and other fructosyltransferase activities, from agave plants catalysed the formation of pentasaccharides composed of 1-kestose and neokestose from nystose.

We have shown[3] that 6^G-fructosyltransferase (6^G-FT) is present in asparagus roots and now report on the purification and properties of the enzyme.

The enzyme was free from hydrolytic activity and was purified 760-fold from asparagus roots by eight steps (see Table I) to give a single protein band on polyacrylamide gel (pH 8.0). The use of octyl-Sepharose and raffinose-coupled Sepharose 6B allowed 1^F-fructosyltransferase[3] (1^F-FT) and SST, respectively, to be removed.

The general properties of the enzyme (mol. wt., ~69,000; optimum pH 5.5; stable at pH 5.0–6.0 on heating at 45°; stable at 20–37°; inhibited by Hg^{2+}, *p*CMB, and Ag^+) were similar to those[4] of asparagus SST (mol. wt., ~65,000; optimum pH 5.0; stable at pH 5.0–6.5 on heating at 45°; stable at 15–45°; inhibited by Hg^{2+}, *p*CMB, Mn^{2+}, and Ag^+), but the substrate specificity and the chromatographic behaviour of the enzyme were different.

The enzyme catalysed reaction *1* (self-transfer); no 1^F(1-β-D-fructofuranosyl)$_{n+1}$-sucrose was formed. The reaction was more rapid with the lower members of the series, but sucrose was not a substrate.

$$2\ 1^F\text{(1-}\beta\text{-D-Fructofuranosyl)}_n\text{sucrose} \rightleftharpoons 1^F\text{(1-}\beta\text{-D-fructofuranosyl)}_n\text{-}6^G\text{-}\beta\text{-D-fructofuranosylsucrose} + 1^F\text{(1-}\beta\text{-D-fructofuranosyl)}_{n-1}\text{sucrose}\ (n > 0) \qquad (1)$$

The enzyme catalysed reaction *2*, *i.e.*, the transfer of the terminal fructosyl group of 1-kestose to HO-6 of the glucosyl group of 1^F(1-β-D-fructofuranosyl)$_n$sucrose. Inulin was not an acceptor, and the reaction proceeded most rapidly when $n = 1$.

1-Kestose + 1^F(1-β-D-fructofuranosyl)$_n$sucrose ⇌
1^F(1-β-D-fructofuranosyl)$_n$-6^G-β-D-fructofuranosylsucrose + sucrose ($n \geqslant 0$) (*2*)

Reactions *1* and *2* can be combined to give reaction *3*.
1^F(1-β-D-Fructofuranosyl)$_m$sucrose + 1^F(1-β-D-fructofuranosyl)$_n$sucrose
⇌1^F(1-β-D-fructofuranosyl)$_n$-6^G-β-D-fructofuranosylsucrose +
1^F(1-β-D-fructofuranosyl)$_{m-1}$sucrose ($m > 0$, $n \geqslant 0$) (*3*)

Thus, the enzyme isolated from asparagus roots may be a new type of fructosyltransferase, and the name 6^G-fructosyltransferase [1^F(1-β-D-fructofuranosyl)$_m$sucrose:1^F(1-β-D-fructofuranosyl)$_n$sucrose 6^G-fructosyltransferase] is proposed. Studies of the reverse reactions will be reported elsewhere.

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