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Note

Reverse reactions of fructosyl transfer catalysed by asparagus 6^G-fructosyltransferase

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Three fructosyltransferases {a sucrose:sucrose 1-fructosyltransferase, a tentatively termed 6^G-fructosyltransferase [1^F(1-β-D-fructofuranosyl)$_m$sucrose:1^F(1-β-D-fructofuranosyl)$_n$ sucrose 6^G-fructosyltransferase], and a 1^F-fructosyltransferase} have been found in the roots of asparagus (*Asparagus officinalis* L.), and are involved in the synthesis[1] of fructo-oligosaccharides. Their general properties and substrate specificities have been investigated[2–4]. 6^G-Fructosyltransferase catalyses the transfer of the terminal (2→1)-linked β-D-fructosyl group of fructo-oligosaccharides to HO-6 of the D-glucosyl group of similar saccharides[3], and plays a significant role in the synthesis of neokestose and related saccharides [1^F(1-β-D-fructofuranosyl)$_n$-6^G-β-D-fructofuranosylsucrose][5]. We now report on several reverse reactions catalysed by the enzyme.

The purification of the enzyme to homogeneity in disc electrophoresis and determination of enzyme activity have been reported[3].

The first reverse reaction studied involved fructosyl transfer from 1^F,6^G-di-β-D-fructofuranosylsucrose to sucrose, which is the reverse of fructosyl transfer between 1-kestose molecules.

A mixture (*A*) of 0.2M [U-^{14}C]sucrose (0.1 μCi) and 0.2M 1^F,6^G-di-β-D-fructofuranosylsucrose in McIlvaine buffer (pH 5.5, 20 μL) and enzyme (20 μL) was incubated at 30° for 3, 5, and 10 h. Another mixture (*B*) of 0.2M [U-^{14}C]-1-kestose (0.045 μCi) in the same buffer (20 μL) and enzyme (20 μL) was incubated under the same conditions. After the reaction had been stopped by the addition of 0.1M mercuric chloride (10 μL), each mixture was subjected to p.c. [5 developments with 1-propanol–ethyl acetate–water (7:1:2), detection with anisidine phosphate[6]]. Each of the eight fractions thus obtained was assayed for radioactivity. A portion of the trisaccharide fraction from *A* or the tetrasaccharide fraction from *B* was subjected to chromatography, together with carrier sugars [1 mg of each; 1-kestose and neokestose for *A*; and nystose, 1^F,6^G-di-β-D-fructofuranosylsucrose, and 6^G(1-β-D-fructofuranosyl)$_2$sucrose for *B*] on a column of charcoal–Celite[1]. The isolated

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TABLE I

FORMATION OF 1-KESTOSE BY ASPARAGUS 6^G-FRUCTOSYLTRANSFERASE-CATALYSED FRUCTOSYL TRANSFER FROM $1^F,6^G$-DI-β-D-FRUCTOFURANOSYLSUCROSE TO SUCROSE

Reaction mixture	*Fructose transferred (μmol/mL of reaction mixture)*		
	Incubation time (h)		
	3	*5*	*10*
A $1^F,6^G$-Di-β-D-fructofuranosylsucrose + [U-^{14}C]sucrose	0.87	1.54	3.27
B [U-^{14}C]-1-Kestose	6.26	9.45	16.88

TABLE II

FORMATION OF 1-KESTOSE BY ASPARAGUS 6^G-FRUCTOSYLTRANSFERASE-CATALYSED FRUCTOSYL TRANSFER FROM NEOKESTOSE TO SUCROSE

Reaction mixture	*Fructose transferred (μmol/mL of reaction mixture)*		
	Incubation time (h)		
	3	*5*	*10*
C Neokestose + [U-^{14}C]sucrose	0.61	1.26	2.47
D 1-Kestose + [U-^{14}C]sucrose	1.15	2.13	4.10

saccharides (1-kestose from *A*, and $1^F,6^G$-di-β-D-fructofuranosylsucrose from *B*) were assayed for radioactivity, from which the amounts of fructose transferred were calculated.

As shown in Table I, radioactive 1-kestose was formed from $1^F,6^G$-di-β-D-fructofuranosylsucrose and sucrose. The ratios of the rates of the reverse (*A*) and normal reactions (*B*) were 0.14, 0.16, and 0.19 for the 3-, 5-, and 10-h reactions, respectively. Thus, the reverse reaction proceeds slowly.

The second reverse reaction studied involved fructosyl transfer from neokestose to sucrose, which is the reverse of the transfer from 1-kestose to sucrose.

A mixture (*C*) of 0.2M [U-^{14}C]sucrose (0.1 μCi) and 0.2M neokestose in McIlvaine buffer (pH 5.5, 20 μL) and enzyme (3.7 U/mL, 20 μL) was incubated at 30° for 3, 5, and 10 h. Another mixture (*D*) of 0.2M [U-^{14}C]sucrose (0.1 μCi) and 0.2M 1-kestose in the same buffer and enzyme (20 μL) was also incubated under the same conditions. After the reaction had been terminated by the addition of 0.1M mercuric chloride (10 μL), each mixture was subjected to p.c., together with carrier sugars (1-kestose for *C*, and neokestose for *D*; 1 mg of each), to isolate the trisaccharide fraction. Each trisaccharide fraction was chromatographed on a column of charcoal–Celite, to give 1-kestose from *C* and neokestose from *D*. Each saccharide was assayed for radioactivity, from which the amount of fructose transferred was calculated.

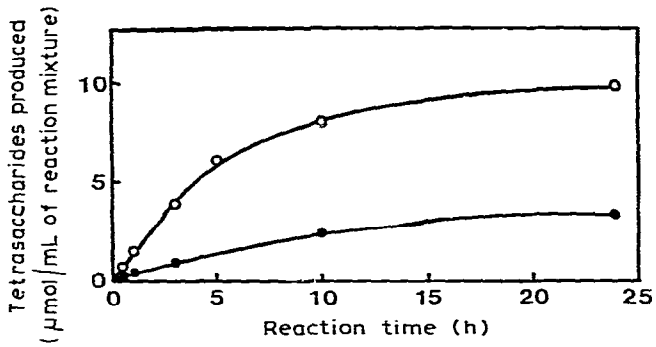

Fig. 1. Synthesis of tetrasaccharides from neokestose by asparagus 6^G-fructosyltransferase: —○—, $1^F,6^G$-di-β-D-fructofuranosylsucrose; —●—, 6^G(1-β-D-fructofuranosyl)$_2$sucrose.

The formation of radioactive 1-kestose from neokestose and [U-^{14}C]sucrose was confirmed (Table II), indicating that the reverse reaction is possible. The ratios of the rates for the reactions *C* and *D* were 0.53, 0.59, and 0.60 for the 3-, 5-, and 10-h reactions, respectively, indicating that the reverse reaction proceeds slowly.

The results show that the enzyme catalyses the transfer of a single, terminal, D-fructosyl group linked to HO-6 of the D-glucosyl residue of neokestose or $1^F,6^G$-di-β-D-fructofuranosylsucrose to HO-1 of the fructosyl group of sucrose. Thus, when only neokestose is used as the substrate for the enzyme, $1^F,6^G$-di-β-D-fructofuranosylsucrose and 6^G(1-β-D-fructofuranosyl)$_2$sucrose are expected to be produced.

The third reaction studied involved fructosyl transfer between neokestose molecules.

A mixture of 0.4M [U-^{14}C]neokestose (0.04 μCi, 10 μL), enzyme (3.7 U/mL, 20 μL), and McIlvaine buffer (pH 5.5, 10 μL) was incubated at 30°. After the reaction had been stopped by the addition of 0.1M mercuric chloride (10 μL), the mixture was subjected to p.c. and charcoal–Celite chromatography, to give radioactive $1^F,6^G$-di-β-D-fructofuranosylsucrose and 6^G(1-β-D-fructofuranosyl)$_2$sucrose. Amounts of the saccharides isolated were calculated from their radioactivities.

The tetrasaccharides $1^F,6^G$-di-β-D-fructofuranosylsucrose and 6^G(1-β-D-fructofuranosyl)$_2$sucrose were synthesised proportionally to the reaction time up to 3 h (Fig. 1) and were accompanied by the liberation of sucrose but not 6^G-β-D-fructofuranosyl-D-glucose (t.l.c., p.c.). The amount of the former tetrasaccharide produced at the beginning of the reaction was five times that of the latter, and three times after prolonged reaction. The above findings show that the enzyme catalyses the transfer of the D-fructosyl group linked to HO-6 of the D-glucosyl residue of neokestose to HO-1 of the D-fructosyl groups of another neokestose molecule; the fructosyl transfer occurs preferentially at the fructosyl group of the sucrose moiety in neokestose.

Thus, asparagus 6^G-fructosyltransferase catalyses reversible fructosyl-transfer reactions between fructo-oligosaccharides.

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