Carbohydrate Research, 177 (1988) 253–258
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthesis of 6-deoxy-6-fluorosucrose, and its inhibition of *Leuconostoc* and *Streptococcus* D-glucansucrases*

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(Received June 22nd, 1987; accepted for publication in revised form, October 10th, 1987)

The D-glucansucrases of *Leuconostoc* and *Streptococcus* utilize sucrose as a D-glucosyl donor for the synthesis of D-glucans[1]. *L. mesenteroides* NRRL B-512F dextran and the soluble D-glucan produced by *S. mutans* 6715 GTF-S (soluble-D-glucan-producing D-glucosyltransferase) have main chains that consist of α-(1→6)-linked D-glucosyl residues, and the insoluble D-glucan produced by *S. mutans* GTF-I consists of α-(1→3)-linked D-glucosyl residues[1]. Several sucrose analogs modified at C-6 have been found to inhibit the enzymes of *S. mutans*[2] and *S. sanguis*[3].

The fluorine atom resembles a hydroxyl group in some ways: it has similar electronegativity and can participate as a hydrogen acceptor, but not as a hydrogen donor; it is about the same atomic size[4]; but, whereas a hydroxyl group can react to form a second covalent bond to oxygen, the fluoro group cannot react to form a second covalent bond. These properties make deoxyfluorosucrose analogs useful, active-site probes for enzymes that utilize sucrose as a substrate.

Although Hough *et al.*[5] reported a synthesis of 6-deoxy-6-fluorosucrose heptaacetate, they did not isolate the free sugar, and only reported the properties of the heptaacetate. We describe here the synthesis of 6-deoxy-6-fluorosucrose using *N,N*-diethylaminosulfur trifluoride (DAST), and report the properties of the free sugar. We also report an examination of the inhibition of D-glucansucrases by 6-deoxy-6-fluorosucrose which is a continuation of our studies of D-glucansucrase inhibition by sucrose analogs[2,6].

EXPERIMENTAL

General methods. — Thin-layer chromatography was performed by using one of two systems: system *A*, Analtech HPTLC-GHLF plates, one ascent with 2:1 (v/v) hexane–EtOAc, or system *B*, Whatman K5F plates, one ascent with 17:3

*Supported by Grant No. DE-03578 from the National Institute of Dental Research, National Institutes of Health, U.S. Public Health Service.
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0008-6215/88/$ 03.50

(v/v) acetonitrile–water. Benzoylated compounds were detected by quenching of u.v. fluorescence, and deprotected compounds by spraying with 20% sulfuric acid in methanol and heating for 10 min at 110°. Elemental analysis was performed by Galbraith Laboratories, Knoxville, TN. ^{19}F-Nuclear magnetic resonance (n.m.r.) spectroscopy was performed at 282 MHz with a Bruker WM-300 spectrometer, and proton-decoupled, ^{13}C-n.m.r. spectroscopy at 75.5 MHz with a Nicolet NT-300 spectrometer.

6-Deoxy-6-fluorosucrose. — 6-Deoxy-6-fluorosucrose was prepared as follows, 2,3,1′,3′,4′,6′-hexa-*O*-benzoylsucrose[7,8] (20 mmol) was dissolved in pyridine (120 mL) and converted into the 6-trityl ether by using chlorotriphenylmethane (50 mmol) for 60 h at 30–35°, at the end of which time, t.l.c. in system *A* showed nearly complete reaction. The free 4-hydroxy group was then benzoylated by adding BzCl (40 mmol), and stirring for 12 h at 30°. The product, hepta-*O*-benzoyl-6-*O*-trityl-sucrose, was extracted into CH_2Cl_2, and the extract washed with dilute aqueous HCl, and evaporated to a syrup. Refluxing in 4:1:1 (v/v/v) AcOH–Me_2CO–H_2O (300 mL) for 3 h removed the trityl group, as shown by t.l.c. of the reaction mixture in system *A*. The detritylated product, 2,3,4,1′,3′4′,6′-hepta-*O*-benzoylsucrose, was extracted into dichloromethane, and the extract washed with dil. aq. Na_2CO_3. Silica gel (100 g) was added to adsorb the solute, and the solvent removed by rotary evaporation. The silica gel was placed on a column (4.8 × 20 cm) of silica gel, and a step gradient of hexane (500 mL), 19:1 (v/v) hexane–EtOAc (500 mL), 4:1 (v/v) hexane–EtOAc (500 mL), and 3:2 (v/v) hexane–EtOAc (2 L) was applied. The hepta-*O*-benzoylsucrose was eluted in the last step. Fractions containing this compound (detected by t.l.c. in system *A*) were evaporated to a syrup which was dissolved in dichloromethane, and the solution dried (sodium sulfate), evaporated, and the residue dissolved in dry diglyme (100 mL). The solution was cooled to 0°, and diethylaminosulfur trifluoride (48 mmol) was added[9,10]. After 22 h at 30°, t.l.c. in system *A* showed nearly complete conversion into hepta-*O*-benzoyl-6-deoxy-6-fluorosucrose. The mixture was poured into dil. aq. Na_2CO_3, and extracted with CH_2Cl_2. The extract was dried (sodium sulfate), and evaporated to a syrup which was dissolved in methanol (100 mL). Sodium methoxide (0.5 g) was added, to remove the benzoyl groups. When t.l.c. in system *B* showed that de-esterification was complete, silica gel (25 g) was added, and the product was adsorbed thereto by evaporation. This solid was placed on a column (2.8 cm × 14 cm) of silica gel which was irrigated with acetonitrile (400 mL) followed by 19:1 (v/v) acetonitrile–water (2 L). 6-Deoxy-6-fluorosucrose was eluted pure in the last step. Yield was 2.2 g (11% from sucrose); $[\alpha]_D^{20}$ +60.5° (*c* 2.54, H_2O).

Anal. Calc. for $C_{12}H_{21}FO_{10}\cdot H_2O$: C, 39.8; H, 6.35; F, 5.2. Found: C, 39.3; H, 6.48; F, 5.12.

The ^{19}F-n.m.r. spectrum of the product showed a triplet of doublets centered on −232.2 p.p.m. relative to trichlorofluoromethane; $J_{2,H6-F6}$ 47.9 Hz, $J_{3,H5-F6}$ 27.1 Hz. Proton-decoupled ^{13}C-n.m.r. spectroscopy in methanol-d_4 showed the C-6 signal absent from its usual 61-p.p.m. position (relative to tetramethylsilane);

present instead was a doublet centered on 83.3 p.p.m.; $J_{1,C6-F6}$ 170 Hz for the two protons on C-6; C-5 gave a doublet centered on 73.1 p.p.m., $J_{2,C5-F6}$ 18 Hz.

Enzymes. — *Leuconostoc mesenteroides* NRRL B-512F dextransucrase was prepared as described previously[11] through the DEAE-cellulose chromatography step; its specific activity was 70 IU/mg of protein. The two *Streptococcus mutans* 6715 D-glucosyltransferases, GTF-S (2.8 IU/mg of protein) and GTF-I (3.0 IU/mg of protein) were prepared according to Robyt and Martin[12]. Enzyme activity was assayed radiometrically using [U-^{14}C]sucrose[13].

Kinetic studies. — All digests contained 5 to 50mM sucrose and 2 mIU glucansucrase, in a total volume of 200 μL. *L. mesenteroides* B-512F dextransucrase digests contained 6-deoxy-6-fluorosucrose (0, 1.7, or 3.3mM), 0.02% (w/v) sodium azide, 50mM sodium acetate buffer (pH 5.4), 0.05% (w/v) Tween 80, and 2mM calcium chloride[14]; triplicate digests were performed for each substrate and each inhibitor concentration. *S. mutans* D-glucosyltransferase digests contained 6-deoxy-6-fluorosucrose (0, 1, or 2mM), 0.02% (w/v) of sodium azide, 25mM imidazolium chloride buffer (pH 6.5), and 0.2% of Dextran T-2000 (Sigma Chemical Co.), and a single digestion was performed for each substrate and each inhibitor concentration. Aliquots (25 μL) were taken from each digest at 0, 10, and 20 min, and added to 1.5-mm squares of Whatman 3MM paper. After addition of the aliquot, the paper was immersed in methanol and stirred for 10 min, taken out and reimmersed in fresh methanol for a total of five times. The papers were then dried, and counted in a toluene scintillation fluid[13].

RESULTS AND DISCUSSION

6-Deoxy-6-fluorosucrose was tested as a glycosyl donor to all three enzymes, both by itself and in the presence of maltose as the acceptor. It was also tested as an acceptor in the presence of sucrose. T.l.c. in system B failed to show new products in any of the digests, indicating that 6-deoxy-6-fluorosucrose does not participate either in glycosyl donation or D-glucosyl-acceptor reactions.

Lineweaver–Burk double-reciprocal plots of 6-deoxy-6-fluorosucrose inhibition of the three D-glucansucrases are shown in Fig. 1. The kinetic inhibition constants, determined by slope replots[15], are summarized in Table I. All three enzymes are strongly and competitively inhibited by 6-deoxy-6-fluorosucrose. The inhibition constants are one-tenth or less of the Michaelis constants for sucrose, indicating that 6-deoxy-6-fluorosucrose is relatively tightly bound at the sucrose binding-site for all three D-glucansucrases.

Previous kinetic studies with the *S. mutans* glucansucrases have shown 6-deoxysucrose to be a potent, competitive inhibitor of the *S. mutans* D-glucansucrases[2]. This suggests that hydrogen-bond formation with the 6-hydroxyl group is not necessary for sucrose to bind, and that a smaller, more hydrophobic function at this carbon atom enhances binding. The affinity of the 6-fluoro analog for the sucrose site of the *S. mutans* enzymes is higher than that of sucrose, and is similar

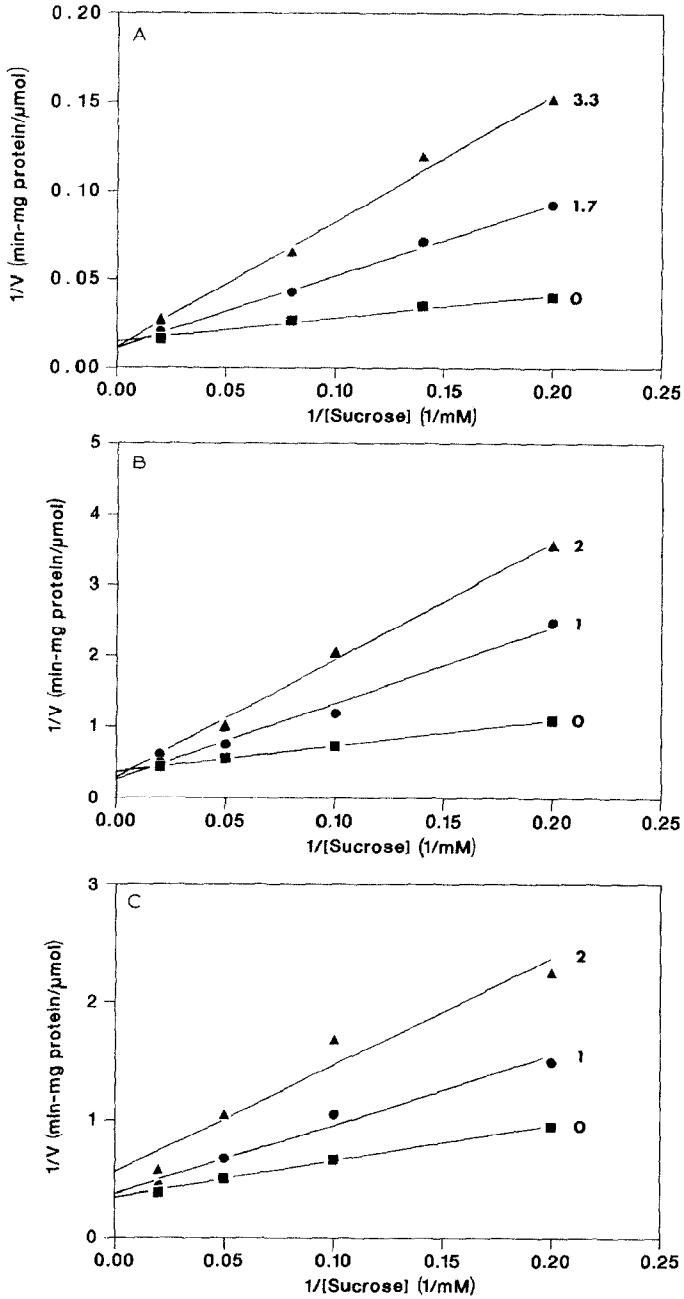

Fig. 1. Double-reciprocal plots of formation of D-glucan from sucrose by D-glucansucrase in the presence of 6-deoxy-6-fluorosucrose. (A) *Leuconostoc mesenteroides* B-512F dextransucrase; (B) *Streptococcus mutans* 6715 GTF-I; (C) *Streptococcus mutans* 6715 GTF-S. [Numbers by individual curves indicate concentrations (in mM) of 6-deoxy-6-fluorosucrose.]

TABLE I

KINETIC PARAMETERS FOR INHIBITION OF D-GLUCANSUCRASES BY 6-DEOXY-6-FLUOROSUCROSE

Enzyme	*Kinetic parameters*			*Type of inhibition*
	K_m *sucrose (mM)*	*Specific activity (IU/mg)*	K_i *(6-deoxy-6-fluoro-sucrose)*[a] *(mM)*	
L. mesenteroides dextransucrase	9	70	0.7 ±0.1	competitive
S. mutans GTF-I	10	2.8	0.6 ±0.1	competitive
S. mutans GTF-S	9	3.0	1.0 ±0.1	competitive

[a]Calculated from slope replots; error terms are one standard deviation.

to that of 6-deoxysucrose. This behavior further demonstrates the unimportance of the 6-hydroxyl group of sucrose as a hydrogen-bond donor for the binding of sucrose at the active site. The similarity of inhibition constants for the deoxy and the deoxyfluoro analogs indicates that the presence of the hydrogen-bond-accepting fluorine in the latter does not enhance binding, further demonstrating that hydrogen bonding at C-6 is not involved in sucrose binding.

6,6′-Dideoxy-6,6′-difluorosucrose is a poor inhibitor of *S. mutans* GTF-I, and has no effect[2] on the behavior of GTF-S. Likewise, 6,6′-dideoxysucrose does not inhibit the D-glucansucrases of *S. mutans*[16]. The contrasting inhibitor effectiveness of 6-monosubstituted *versus* 6,6′-disubstituted sucrose analogs with the D-glucansucrases of *S. mutans* 6715, and the strong inhibition of these enzymes by 6′-amino-6′-deoxysucrose[17], suggest that the 6′-hydroxyl group of the D-fructofuranosyl moiety plays a role in the binding of sucrose and is analogs. The role of the fructofuranosyl moiety was previously thought to be relatively unimportant, as D-glucosyl groups are transferred to these enzymes from α-D-glucopyranosyl fluoride[16,18,19] and from *p*-nitrophenyl α-D-glucopyranoside[20], both of which lack any semblance of a D-fructosyl moiety.

In summary, we find that, like 6-deoxysucrose, 6-deoxy-6-fluorosucrose is an effective competitive inhibitor and is bound to the sucrose binding-site of *L. mesenteroides* B-512F dextransucrase and *S. mutans* 6715 GTF-S and GTF-I, approximately ten times more strongly than is the normal substrate, sucrose.

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