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Note

Amino sugars: a new class of inhibitors of dextransucrase

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The initiation and propagation of carious lesions is a complex process. Several factors are involved in cariogenesis, including diet, heredity, bacteria, and time. It is well established that sucrose and the bacterium *Streptococcus mutans* are involved in promoting dental caries. *S. mutans* produces an enzyme, dextransucrase (D-glucosyltransferase, EC 2.4.1.5), which catalyzes the formation of extracellular dextrans (or D-glucans) from sucrose. Dextrans may play a significant role in localizing the site(s) for caries development[1]. It follows that the inhibition of D-glucan formation may be one means of preventing dental caries. Results from several laboratories show that, when used at relatively high concentrations, such compounds as D-fructose, maltose, melibiose, isomaltose, methyl α-D-glucopyranoside, a trichlorotrideoxysucrose, and 6-*O*-α-D-glucosylsucrose can partially inhibit dextransucrases[2–7]. Inoue and Smith[8] reported that periodate-oxidized dextrans are efficient inhibitors of the dextransucrase of *S. mutans*. We have begun studies to design, synthesize, and test several other carbohydrates, including sucrose analogs, as dextransucrase inhibitors. In the present study, we show that certain carbohydrates containing an amino group are potent inhibitors of dextransucrase.

EXPERIMENTAL

The amino sugars were synthesized according to established procedures. In each case, the structures of the derivatives were confirmed by melting point, optical rotation, elemental analysis, and infrared and ^{1}H- or ^{13}C-n.m.r. spectra. References for the syntheses are given in Table I. Several sugars were purchased commercially; these included 2-acetamido-2-deoxy-D-glucopyranose, 2-acetamido-2-deoxy-D-mannopyranose, 2-amino-2-deoxy-D-glucopyranose, 2-amino-2-deoxy-D-mannopyranose, 2-amino-2-deoxy-D-galactopyranose, methyl α-D-glucopyranoside, methyl α-D-

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TABLE I

INHIBITION[a] OF THE DEXTRANSUCRASE OF *Streptococcus mutans* 6715 BY CARBOHYDRATES AND SUBSTITUTED CARBOHYDRATES

Inhibitor	*Inhibition (%)*	*References*
Methyl 6-amino-6-deoxy-α-D-glucopyranoside	100	12
Methyl 6-amino-6-deoxy-α-D-mannopyranoside	95	13
Methyl 6-amino-6-deoxy-β-D-glucopyranoside	80	12
Methyl 6-acetamido-6-deoxy-α-D-glucopyranoside	8 9	14
6,6′-Diamino-6,6′-dideoxysucrose	96	15
6′-Amino-6′-deoxysucrose	98	16
Maltose	37	
Isomaltose	37	
Maltotriose	7	
Isomaltotriose	29	
D-Fructose	13	

[a]Inhibition was computed from the rates of synthesis of methanol-insoluble D-glucan in the presence and absence of inhibitor. Linear rates only were measured. The substrate was 1 7 mM [U-^{14}C]sucrose. Inhibitor concentrations were 11.8mM. K_m for the enzyme was 1 28mM, as determined by separate experiments. Compounds tested but failing to give any detectable inhibition were D-glucose, D-mannose, D-galactose, D-glucuronic acid, D-mannitol, D-glucitol, 2-acetamido-2-deoxy-D-glucopyranose, 2-acetamido-2-deoxy-D-mannopyranose, 2-acetamido-2-deoxy-D-galactopyranose, 2-amino-2-deoxy-D-glucopyranose, 2-amino-2-deoxy-D-mannopyranose, 2-amino-2-deoxy-D-galactopyranose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, and methyl β-D-glucopyranoside.

mannopyranoside, maltose, and D-fructose (which were products of Sigma Chemical Co., St. Louis, MO). Maltotriose, isomaltose, isomaltotriose, sucrose, D-glucuronic acid, D-mannose, D-mannitol, and D-glucitol were obtained from Calbiochem (La Jolla, CA). ICN (Irvine, CA) provided [U-^{14}C]sucrose.

The dextransucrase preparations were obtained from supernatant liquors of cultures of *Streptococcus mutans* 6715 grown anaerobically in the defined medium of Terleckyj *et al.*[9], supplemented with a final concentration of 20 mg of sucrose per mL. The culture-supernatant liquors were precipitated with 60%-saturated ammonium sulfate. The precipitate was collected by centrifugation and dialyzed against several changes of 50mM sodium acetate (pH 5.5). The dialyzate was then chromatographed on a column of Bio-Gel A-1.5 (Bio-Rad Laboratories, Richmond, CA). Fractions were assayed for absorbance at 280 nm and dextransucrase activity[10,11]. Levansucrase activity was not present in the preparations.

The assay for dextransucrase was adapted from the procedure described by Germaine *et al.*[11]. Routinely, enzyme (100 μL) was mixed with the inhibitor (or buffer) (250 μL), and incubated for 15 min at 37°. Substrate {100 μL of a solution containing [^{14}C]sucrose (2.5 μCi), unlabeled sucrose (170 μg), dextran primer (25 mg), and sodium fluoride (70 μg)} was added, and aliquots (20 μL) were removed at intervals, and applied to Whatman filter-discs (3 MM). The discs were then sub-

merged in absolute methanol for 15 min. After repeating the last step twice, the discs were air-dried, and the radioactivity was determined in a scintillation counter. All solutions were prepared in 50mM acetate (pH 5.5). At this pH, the dextransucrase is optimally active.

RESULTS AND DISCUSSION

In control experiments, several carbohydrates were tested for their ability to inhibit the dextransucrase from *S. mutans*. These were chosen because other investigators had determined that they possessed the capacity to inhibit the enzyme partially. The carbohydrates tested were maltose, D-glucose, D-fructose, maltotriose, isomaltose, and isomaltotriose. At the concentrations employed in the present study, only maltose, isomaltose, and isomaltotriose were effective inhibitors (see Table I). Maltotriose and D-fructose gave low levels of inhibition (7 and 13%, respectively; see Table I). In contrast, several other carbohydrates gave little or no inhibition of the enzyme. These included D-glucose, D-mannose, D-mannitol, D-glucitol, D-glucuronic acid, methyl α-D-glucopyranoside, and methyl α-D-mannopyranoside. These results are in general agreement with those reported by other workers, who found that several carbohydrates of low molecular weight are only marginal inhibitors of dextransucrase[2-7].

The significant findings in our studies were that several carbohydrates containing an amino group are highly effective in preventing the dextransucrase-catalyzed formation of alcohol-insoluble D-glucan from sucrose. For example, the 6-amino-6-deoxy derivatives of methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, and methyl α-D-mannopyranoside are potent inhibitors of the dextransucrase (see Table I). When an acetamido group replaced the hydroxyl group on C-6 of methyl α-D-glucopyranoside, no inhibition occurred, revealing that the free amino group is essential for inhibition. Furthermore, specificity was observed for the C-6 position, because 2-amino-deoxy sugars were ineffective in preventing D-glucan synthesis. Two sucrose analogs, 6,6′-diamino-6,6′-dideoxysucrose and 6′-amino-6′-deoxysucrose, were also found to be highly effective inhibitors of the dextransucrase. In preliminary studies, we have determined that 6,6′-diamino-6,6′-dideoxysucrose is an "uncompetitive" inhibitor of dextransucrase (unpublished results). At present, we do not know whether the amino sugar inhibitors bind to the catalytic acceptor or allosteric sites on the enzyme. The results suggest that certain amino sugars may have important roles in preventing sucrose-dependent synthesis of D-glucan by oral micro-organisms. Such sucrose derivatives may ultimately prove valuable in preventing the development of carious lesions. The derivatives offer a significant advantage, because of their low molecular weights and, therefore, presumed greater ability to diffuse into fissures, plaque, and interstitial spaces.

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