Mechanism of chain initiation by dextransucrase

Absence of terminal 'sucrose' linkage in newly synthesized dextran chains

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Dextran was synthesized using dextransucrase from *Streptococus sanguis* 10558 and (F)-[^{14}C]sucrose as substrate to test the possibility that sucrose may be the initial acceptor for glucose. If sucrose is the initial acceptor, then dextran chains should have [^{14}C]fructose in a terminal 'sucrose' linkage which can be cleaved under mild conditions. Although incorporation of [^{14}C]fructose into dextran was observed, the label was not released by mild hydrolysis, indicating that sucrose is not the initiator for dextran synthesis. Incorporation of [^{14}C]fructose into dextran might represent its ability to act as an acceptor, as suggested by the isolation of leucrose as a by-product in the reaction.

Dextransucrase *Polysaccharide synthesis* *Chain initiation*

1. INTRODUCTION

The enzyme dextransucrase catalyzes the synthesis of dextran, a homopolymer of glucose linked principally through α-1,6-bonds, from sucrose. This reaction appears to play a central role in the development of dental caries by oral bacteria [1,2]. The enzymatic synthesis of dextran may be viewed as a two substrate reaction, in which a glucose residue is transferred from a donor substrate to an acceptor substrate. Several studies have demonstrated that sucrose is the only naturally-occurring donor substrate for dextransucrase; however, the enzyme displays a broad specificity with respect to added acceptors [3,4]. In addition, the enzyme appears to be able to catalyse dextran synthesis in the absence of added acceptors ('unprimed' synthesis) [5,6]. This might be explained by the presence of small quantities of endogenous acceptors in the enzyme preparations but our recent studies appear to rule this out [7]. On the other hand, the enzyme may initially transfer a glucosyl residue to a molecule of sucrose itself, leading to the synthesis of dextran chains tagged at one end with a 'sucrose' residue, as suggested by studies on low-M_r dextrans [8,9]. In addition, a third possibility is that polymer growth might occur on the enzyme with the formation of a covalently linked glucosyl enzyme intermediate during the reaction. This would be consistent with models for chain growth proposed [10,11], where the enzyme could serve as the initial acceptor for a glucose residue.

In this report we have tested the possibility that sucrose might be the initial acceptor for glucose residues by a careful analysis of the reaction products using (F)-[^{14}C]sucrose as substrate, but find no evidence for sucrose as initial acceptor for dextran synthesis.

2. EXPERIMENTAL

Dextransucrase was purified from culture fluids of *Streptococcus sanguis* ATCC 10558 as in [12]. The enzyme was treated with dextranase to remove

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endogenous dextran [7] and then separated from dextranase by chromatography on hydroxylapatite. Optimal conditions for the acid hydrolysis of sucrose which did not cause degradation of dextran chains were determined to be pH 2.0 at 100°C for 15 min. The (F)-[^{14}C]sucrose used in these studies was radiochemically pure since only 0.03% of the total counts were detected in glucose after acid hydrolysis of (F)-[^{14}C]sucrose.

Dextran T-10 was obtained from Pharmacia Laboratories; (F)-[^{14}C]sucrose was from New England Nuclear Corp. Dextranase (from *Penicillium* sp.) was purchased from Worthington Biochemicals. Invertase and glucose oxidase were obtained from Sigma Chemical Co.

3. RESULTS

The ability of dextransucrase to synthesize a dextran containing fructose was determined by employing (F)-[^{14}C]sucrose as substrate. Incorporation of labeled fructose into dextran chains would subsequently facilitate further analysis according to the following scheme. If chain initiation proceeds by the initial transfer of a glucose residue to a molecule of sucrose, then each new chain of dextran should contain one labeled fructosyl residue in a terminal 'sucrose' as shown below:

$-\alpha$-D-Glc$p$-(1 → 6)-α-D-Glc$p$-(1 → 2)-β-D-Fru$f$

This 'sucrose' linkage should be hydrolyzed to release labeled fructose under conditions sufficient for splitting the glycosidic bond in sucrose as shown in other studies [8,9].

Dextranase-treated and purified dextransucrase was incubated with (F)-[^{14}C]sucrose and the products separated on a Bio-Gel P-100 column (as in table 1). In different experiments 0.4–0.7% of the eluted counts, corresponding to an average of 2.8×10^{-2} μmol fructose incorporated, appeared in higher M_r material. When various fractions of this material were analyzed by paper chromatography in *n*-butanol/pyridine/0.01 M borate (2:2:1), radioactivity was predominantly present at the origin (30–60%), representing oligosaccharides >6 units in length and in a faster moving peak (13–15%) close to the isomaltose standard and tentatively assumed to be a disaccharide. The amount of fructose incorporated into higher M_r oligosaccharides would be sufficient to account for 1 fructose molecule/dextran chain of 150 glucosyl residues on the average, with an 80% efficiency of dextran synthesis by the enzyme. The material at the origin and in the disaccharide region of the paper chromatogram was eluted separately and subjected to strong hydrolytic conditions (1 M HCl, 100°C for 2 h) to verify that the radiolabel was still in fructose. Over 75% of the counts were recovered in fructose for both samples. The remainder of the counts were found in degradation products of fructose (mainly furan derivatives), also found after hydrolysis of standard fructose solutions. To determine if the labeled fructose was present in a terminal 'sucrose' linkage in the oligosaccharides, material from the origin of the chromatograms was eluted and subjected to hydrolysis at pH 2.0, 100°C for 15 min as described in section 2. Since the label was initially present only in fructose, all the counts would be expected to be releas-

Table 1

End-group analysis of ^{14}C-(F)-labeled disaccharide

Material	% Total cpm
Sorbitol	52.8
Mannitol	39.2
Fructose	0
Unhydrolyzed disaccharide	5.0

Dextranase-treated and purified dextran sucrase (1.67 units) was incubated with 0.05 M (F)-[^{14}C]sucrose (7.5×10^7 cpm) and 0.01 M phosphate buffer, (pH 6.0) in a 0.1 ml total vol. After 5 min at 37°C, the reaction mixture was heated at 100°C for 3 min (sucrose is not hydrolyzed under these conditions). The sample was applied to a Bio-Gel P-100 column (1.1 × 46 cm) and eluted with water. Fractions of 1 ml were collected and after concentration, were chromatographed on Whatman 1 MM paper in *n*-butanol/pyridine/0.01 M borate (2:2:1). Paper strips containing the ^{14}C-(F)-labeled disaccharide were eluted with distilled water. The eluate, containing 26400 cpm in 0.6 ml, was reduced in 1 M KBH_4 (pH 11) for 16 h at room temperature. After acidification to destroy excess borohydride, followed by deionization and borate removal, the sample was hydrolyzed in 1 M HCl for 2 h at 100°C. After removal of acid by evaporation, the sample was chromatographed in nitromethane/acetic acid/ethanol/water-saturated boric acid (8:1:1:1) and radioactivity was determined.

ed on hydrolysis if a terminal 'sucrose' were present. However, hydrolysis of material from several fractions released only 1–10% of the total counts. This suggests that a terminal 'sucrose' is not present in these oligosaccharides. To further verify this conclusion, the (F)-[^{14}C]oligosaccharides fraction was treated with yeast invertase, which is a β-fructosidase, and will hydrolyze sucrose to glucose and fructose. However, this treatment also failed to release any [^{14}C]fructose.

The ^{14}C-(F)-labeled material which migrated at a position tentatively assigned to a disaccharide was found to be chromatographically identical to leucrose (O-α-D-glucopyranosyl-1–5-D-fructopyranose) [13]. As expected, it was not susceptible to hydrolysis under mild acid conditions or on treatment with invertase. On strong acid hydrolysis (1 M HCl, 100°C for 2 h), [^{14}C]fructose was the only labeled sugar released. The presence of fructose in a reducing position was confirmed by borohydride reduction and acid hydrolysis, which gave approximately equivalent amounts of label in [^{14}C]mannitol and [^{14}C]sorbitol (table 1). The ability of dextransucrase to transfer a glucosyl residue to fructose to produce the reducing disaccharide, leucrose, has been well-documented [14–16].

4. DISCUSSION

Here, we have addressed the possibility that sucrose might serve as the initial acceptor for dextran synthesis. Using homogeneous dextransucrase we have clearly shown that although [^{14}C]fructose is incorporated into newly synthesized dextran chains, the [^{14}C]fructose is not present in the form of a 'sucrose' linkage. Our highly sensitive radiolabeling techniques would have detected 1 fructose molecule (in a sucrose linkage) incorporated/dextran chain of 150 glucosyl residues. The incorporation of fructose into polymeric material might be a result of transfer of glucosyl residues to fructose itself acting as an acceptor in a side reaction. This is suggested by the formation of leucrose during dextran synthesis. However, since leucrose is the predominant single product of the reaction, it appears that it is not a good acceptor for the enzyme, as observed in [17], and is highly unlikely to be a normal intermediate for dextran synthesis. It was not possible to carry out a complete structural analysis of the (F)-[^{14}C]oligosaccharides due to the small quantities of labeled material available.

Studies on low M_r dextrans have suggested that sucrose may act as the initial acceptor in these cases [8]. However, this has not been documented for native high-M_r dextrans. Our studies have clearly shown that sucrose is not the initiator for native dextran synthesis by the *S. sanguis* enzyme. This leaves open the possibility that polymer growth may occur on the enzyme as via a covalently linked enzyme–glucose intermediate [10,11].

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