Role of Primers in Glucan Synthesis by Glucosyltransferases from *Streptococcus mutans* strain OMZ176

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The effects of isomaltosaccharides of various molecular weights (isomaltose to dextran T2000) on glucan synthesis by a water-soluble glucan-synthesizing glucosyltransferase enzyme (GTase-S) and a water-insoluble glucan-synthesizing enzyme (GTase-I), both from *Streptococcus mutans* OMZ176, were examined. The activity of GTase-S was not affected by the addition of the isomaltosaccharides, but GTase-I was stimulated increasingly by isomaltosaccharides with degrees of polymerization more than 10. The GTase-I activity first increased and thereafter decreased slightly with increasing amounts of a soluble dextran. Maximal stimulation occurred at concentrations in the range 0.1 to 0.2 mg ml⁻¹, when dextran T10 was used as a primer. The rate of glucan synthesis was highly enhanced by the combined action of GTase-S and GTase-I. The profile of the net activity of GTase-I in the presence of various amounts of GTase-S was similar to that of GTase-I in the presence of increasing amounts of an exogenous dextran. These results collectively suggest that soluble glucan produced by GTase-S from sucrose acts as an intrinsic primer for the glucan synthesis by GTase-I, indicating the contribution of autoprimering in glucan synthesis by crude GTase of *S. mutans*.

INTRODUCTION

Streptococcus mutans is a prime etiological agent of human dental caries (Gibbons & van Houte, 1975; Hamada & Slade, 1980). Water-insoluble and water-soluble glucans produced from sucrose by *S. mutans* play a crucial role in the formation and metabolism of dental plaque and consequently in the induction of dental caries (Hamada & Slade, 1980). These glucans are synthesized by the action of glucosyltransferases (GTase; EC 2.4.1.5).

Enzymic activity of *S. mutans* GTases is stimulated by the addition or presence of exogenous dextrans (primer effect) (Germaine *et al.*, 1974a; Kuramitsu, 1975; Germaine & Schachtele, 1976; Newbrun *et al.*, 1977; Robyt & Corrigan, 1977; Hare *et al.*, 1978; McCabe & Smith, 1978; Hamada & Torii, 1980; Sato *et al.*, 1982). The degree of stimulation varies with different GTase preparations from a single strain of *S. mutans*, but the mechanism is unknown.

Recently, we reported the separation of a water-soluble glucan-synthesizing component (GTase-S) and a water-insoluble glucan-synthesizing component (GTase-I) from crude GTase obtained from culture supernatant of a serotype *d* strain of *S. mutans* (Koga *et al.*, 1982). We now describe the effects of various isomaltosaccharides on glucan synthesis by these purified GTase preparations and discuss the role of primers in the synthesis of water-insoluble glucan by *S. mutans* GTase.

METHODS

GTase preparations. GTase-S and GTase-I were separated by the chromatofocusing method and subsequent hydroxyapatite column chromatography from crude GTase obtained by 50% ammonium sulphate precipitation of

cell free culture supernatants of *Streptococcus mutans* OMZ176 (serotype *d*) as described previously (Koga *et al.*, 1982). Specific enzyme activities of the separated GTase-S and GTase-I were 3.7 and 1.8 U (mg protein)⁻¹, respectively. One unit was defined as the amount of enzyme that transformed 1 µmol sucrose into glucan per min, under the conditions described previously (Koga *et al.*, 1982).

Assay for GTase activity. Glucan-synthesizing activity of the GTases was measured by a modification of the method described by Germaine *et al.* (1974*b*). The standard reaction mixture contained appropriate amounts (0 to 0.37 mU) of GTase and 0.04 µCi of [U-¹⁴C]sucrose (0.2 Ci mol⁻¹, 7.4 GBq; New England Nuclear) in a total volume of 20 µl 0.1 M-potassium phosphate buffer (pH 6.0). After the incubation for 0 to 2 h at 37 °C, the amount of [¹⁴C]glucose polymerized was estimated by the filter paper method (Koga & Inoue, 1981).

To examine the effects of isomaltosaccharides on glucan synthesis by the GTase components, 0 to 0.2 mg of the saccharides were added to the above mixture and incubated.

Isomaltosaccharides. Dextran T10 (mean molecular weight 10000), T20 (20000), T40 (40000), T250 (250000), T500 (500000) and T2000 (2000000) were obtained from Pharmacia.

Preparation of oligosaccharides of the isomaltose series. Dextran T40 (0.1 g ml⁻¹) was partially hydrolysed in 0.5 M-H₂SO₄ for 60 min at 100 °C. The hydrolysate was then neutralized with BaCO₃ and filtered. The hydrolysed products were precipitated from the filtrate by the addition of cold absolute ethanol to a final concentration of 80% (v/v), collected by centrifugation, and solubilized in a small volume of water. Oligosaccharides with various degrees of polymerization (DP) were separated by paper chromatography and subsequent column chromatography on Bio-Gel P2 (Bio-Rad). The DP was determined by calculating molar proportions of total hexose residues to reducing sugar residues.

Chemical assays. Protein was estimated by the Lowry method with bovine serum albumin as a standard. Reducing sugar and total hexose were measured by the method of Somogyi-Nelson (Nelson, 1944) and by the anthrone method (Trevelyan & Harrison, 1956), respectively, with glucose as a standard.

RESULTS

Effects of isomaltosaccharides on glucan synthesis by GTase-S and GTase-I

GTase-I (0.24 mU) had synthesized only trace amounts of glucan from sucrose in the absence of dextran T10 even after 2 h incubation, but the presence of the soluble dextran at a 0.2 mg ml⁻¹ concentration significantly stimulated glucan synthesis by GTase-I.

The degree of stimulation of the rate of glucan synthesis increased with increasing amounts of the exogenous dextran T10 up to 0.2 mg ml⁻¹ but decreased at dextran concentrations above 0.2 mg ml⁻¹ (Fig. 1*a*). Significant stimulation of glucan synthesis was caused when dextran was included at concentrations of 0.1 to 0.2 mg ml⁻¹. The extent of the maximum stimulation of glucan synthesis was approximately 15-fold.

The ability of a constant amount, 0.2 mg ml⁻¹, of isomaltosaccharides with various DP to prime GTase-I activity was assessed. Glucan synthesis by GTase-I was stimulated by isomaltosaccharides with DP higher than 10, the extent of stimulation increasing with DP. However, the isomaltooligosaccharides with DP of less than 8 were essentially without effect (Fig. 1*b*). In contrast, glucan synthesis by GTase-S (0.37 mU) was not influenced by the addition of dextran T10 at concentrations in the range 0.01 to 10 mg ml⁻¹ or by 0.2 mg ml⁻¹ of isomaltosaccharides (Fig. 1).

Role of primers in the glucan synthesis by the combined action of the isolated glucosyltransferase components

When a constant amount of GTase-I (0.24 mU) and varying amounts of GTase-S (0 to 0.074 mU) were incubated with 10 mM-sucrose, the quantities of glucan synthesized by the combined action of the two GTase components were far higher than the additive amounts of glucan produced by these GTases separately (Fig. 1*c*). The addition of 0.2 mg exogenous dextran T10 ml⁻¹ stimulated glucan synthesis by the combined action of the GTase components when the activity ratio of GTase-S to GTase-I was less than 0.08 (Fig. 1*c*). However, if the ratio was more than 0.08, glucan synthesis was slightly inhibited. These results were repeatedly demonstrated. Thus, the stimulatory or inhibitory effects of exogenous soluble dextrans were strongly dependent upon the relative proportions of GTase-S and GTase-I. The maximum stimulation occurred when the ratio of GTase-S to GTase-I activities was approximately 0.15

Fig. 1(a, b). Effect of dextran T10 (a) and various isomaltosaccharides (b) on glucan synthesis, measured as the uptake of $[U-^{14}\text{C}]$ sucrose, by GTase-S (●) and GTase-I (▲). (a) GTase-S (0.37 mU) or GTase-I (0.24 mU) was incubated with $[U-^{14}\text{C}]$ sucrose (0.2 μmol , 0.04 μCi) in the presence of various amounts of dextran T10. After 60 min at 37 °C, the amounts of $[^{14}\text{C}]$ glucan synthesized were estimated by the filter paper method. (b) Experimental conditions were identical to those described for (a) except that dextran T10 was replaced by isomaltosaccharides (4 μg). (c) Glucan synthesis by the combined action of GTase-S and GTase-I. GTase-I (0.24 mU) and various amounts (0 to 0.074 mU) of GTase-S were incubated together at 37 °C for 60 min with $[U-^{14}\text{C}]$ sucrose (0.2 μmol , 0.04 μCi) in the absence (▲) or presence (○) of dextran T10 (4 μg). The reaction mixture without GTase-I and dextran T10 served as controls (●). (d) Stimulation of GTase-I-dependent glucan synthesis by GTase-S. The data are taken from the experiment described in (c). The net amounts of glucan produced by GTase-I were obtained by subtracting the amounts of glucan synthesized by GTase-S alone from those of glucan synthesized by GTase-I and GTase-S together but in the absence of dextran T10.

(Fig. 1d). The profile shown in Fig. 1(d) is similar to that obtained with the stimulation of GTase-I activity by an exogenous soluble dextran (Fig. 1a).

DISCUSSION

Stimulation of the *S. mutans* GTase-I activity by oligo- and polysaccharides of the isomaltose series has been demonstrated for the extracellular enzyme of *S. mutans* GS-5 (serotype *c*) and OMZ176 (serotype *d*) (Germaine *et al.*, 1974a; Hare *et al.*, 1978). Germaine *et al.* (1974a) have reported that the smallest effective primer is the isomaltosaccharide with DP of about 8, whereas our study indicates that primer activity of isomaltosaccharides commenced with DP of 10 or more (Fig. 1b). The difference in the minimum DP for primer activity may be ascribed to

the purity of isomaltosaccharides used. Our preparations were purified by gel filtration, followed by repeated separation of individual isomaltosaccharides by paper chromatography.

The GTase-S activity was not influenced by the addition of isomaltosaccharides tested (Fig. 1*a, b*). Germaine *et al.* (1974*a*) reported that the extent of stimulation of GTase activity by primer dextran decreased with increasing amounts of carbohydrate in the GTase preparations used. However, the GTase-S preparation used in the present study has been shown to be carbohydrate free (Koga *et al.*, 1982), indicating that the activity of the GTase-S of *S. mutans* OMZ176 (serotype *d*) is essentially primer independent.

As GTase-S activity was not stimulated by exogenous dextrans (Fig. 1*a, b*), the enhanced synthesis of glucan by the concerted action of the two GTase components (Fig. 1*c*) may be ascribed to the stimulation of GTase-I activity by glucan rich in 1,6- α linkages first synthesized by GTase-S (autoprimering effect). The variation of glucan synthesis by GTase-I depended on the amounts of GTase-S included in the mixture (Fig. 1*d*), showing a profile similar to that of glucan synthesis by GTase-I in the presence of exogenous dextran (Fig. 1*a*). This supports the suggestion that autoprimering occurs during insoluble glucan synthesis by crude *S. mutans* GTases (Germaine *et al.*, 1977; Kuramitsu, 1975).

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