# Synthesis of adherent insoluble glucan by the concerted action of the two glucosyltransferase components of *Streptococcus mutans*

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## 1. INTRODUCTION

*Streptococcus mutans*, a prime pathogen of human dental caries, produces extracellularly water-insoluble (IG) and water-soluble (SG) glucans from sucrose by the action of glucosyltransferase (GTase, EC 2.4.1.5). The IG is highly adherent and promotes the firm adherence and accumulation of this organism on tooth surface (reviewed in [1]).

It has been suggested that GTase complexes participate in the formation of IG (reviewed in [2]), which consists of α-1,3-linked backbone with α-1,6-linked branches [3–5]; however, disaggregated components of the GTases were isolated in [6–8]. This has impaired the progress in the elucidation of the detailed mechanisms of synthesis of adhesive IG.

We have isolated two components from extracellular GTase elaborated by *S. mutans* OMZ176 (serotype *d*). One was a primer-dependent IG-synthesizing component (GTase-I) and the other was primer-independent SG-synthesizing component (GTase-S) [9]. These GTase components enabled us to examine the mechanism of synthesis of adherent IG. Here we present evidence that adhesive IG is synthesized by the concerted action of the two GTase components, resulting in adherence of IG to a glass surface.

## 2. MATERIALS AND METHODS

### 2.1. *GTase preparations*

GTase-I and GTase-S were separated from crude GTase obtained by 50% ammonium sulfate precipitation of culture liquor of *S. mutans* OMZ176 by the chromatofocusing method and subsequent hydroxyapatite column chromatography as in [9]. The GTase-I produced IG consisting almost exclusively of α-1,3-linked glucose residues (90 mol%) after prolonged incubation without primer dextran, whereas the GTase-S produced SG consisting of mostly α-1,6-linked glucose residues (~ 60 mol%) as well as a significant amount of α-1,3 (28 mol%) and α-1,3,6 (5 mol%) linkages [9]. Specific activities of the crude GTase, GTase-I and GTase-S were 0.8, 1.8 and 3.7 U/mg protein, respectively. In this connection, GTase activities were assayed in the presence of 0.02% Dextran T10.

### 2.2. *Glucan preparations*

Crude GTase (15 mU/ml) was allowed to react with 10% sucrose in 0.1 M potassium phosphate buffer (pH 6.0, KPB) containing 0.02% merthiolate. After incubation at 37°C for 16 h, the mixture was heated at 100°C for 20 min to inactivate enzyme, then IG produced was collected by centrifugation at 20 000 × *g* for 15 min and washed 3 times with distilled water.

A portion (30 mg) of the native IG was subjected to the digestion with $32 \times 10^3$ IU of a dextranase (EC 3.2.1.11) preparation derived from a *Cheatomium gracile* strain (Sankyo Pharmaceuticals, Tokyo) at 37°C for 16 h at pH 6.0. The digested IG was heated and washed as described above.

Dextran T10 was purchased from Pharmacia Fine Chemicals (Uppsala).

### 2.3. *Assays for the synthesis and/or adherence of IG*

The mixtures contained 5 mU GTase-I,

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0–20 mU GTase-S and 30 mg sucrose in 3 ml KPB in test tubes (13 × 100 mm). Mixtures containing no GTase-I were served as controls. Duplicate sets were prepared for each test. After the test tubes were incubated at 37°C for 16 h at an angle of 30°, the tubes were rotated gently by hand and unattached glucans were transferred into a second tube. The first tube was washed with 3 ml KPB again and released glucans were poured into a third tube. The glucans remaining in the first tube were suspended in 3 ml KPB. The glucan suspensions in all tubes were homogenized ultrasonically at 150 W for 5 s, then the $A_{550\,nm}$ of each tube was measured with a Shimadzu-Bausch and Lomb Spectronic 88 photometer (Shimadzu Works, Kyoto). Total amount of IG formed was defined as $A_{550}$ (tubes 1+2+3) and the percentage adherence was obtained as 100 × $A_{550}$ (tube 1/$A_{550}$ (tubes 1+2+3).

GTase-I (5 mU) was also incubated at 37°C for 16 h with 30 mg sucrose in the presence of 0–30 mg dextran T10 in 3 ml KPB in pairs of tubes kept at 30° angle. Amounts of IG synthesized and adhered on glass were estimated as above.

The native IG (0.6 mg) or the dextranase-modified IG (1 mg), which gave the same density when suspended in the same volume of water, were incubated together with 0–20 mU GTase-S and 30 mg sucrose in 3 ml KPB as above. Amounts of adherent IG as well as the change in turbidity of whole mixture were determined as described above.

## 3. RESULTS

GTase-I (5 mU) or GTase-S (-20 mU) incubated with 30 mg sucrose did not yield detectable turbidity, indicating no significant IG synthesis (fig.1). When GTase-I and GTase-S together were incubated with sucrose, IG was produced significantly. Total amount of IG produced was first increased and thereafter decreased with the increasing activity of GTase-S. The highest production was observed at the GTase-S to GTase-I activity ratio of 1.5.

IG produced by the combined action of the two GTase components strongly adhered to a glass surface. Maximal adherence occurred at a GTase-S/GTase-I ratio of 0.6, where 70% of total IG produced adhered to glass. No detectable adherence of IG was observed at the highest GTase-S/GTase-I ratio (4.0) examined. Thus, maximal

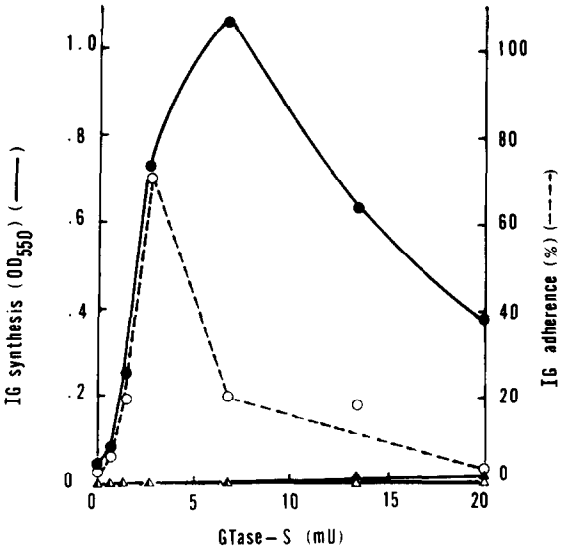


Fig.1. Synthesis and adherence of IG by the combined action of GTase-I and GTase-S: 0 mU (△,▲) or 5 mU (○,●) GTase-I, 0–20 mU GTase-S and 30 mg sucrose were allowed to react in 3 ml KPB in test tube (13 × 100 mm) kept at an angle of 30° at 37°C for 16 h. Amounts of total IG produced (▲,●) and IG adherent to glass (△,○) were determined as in section 2.

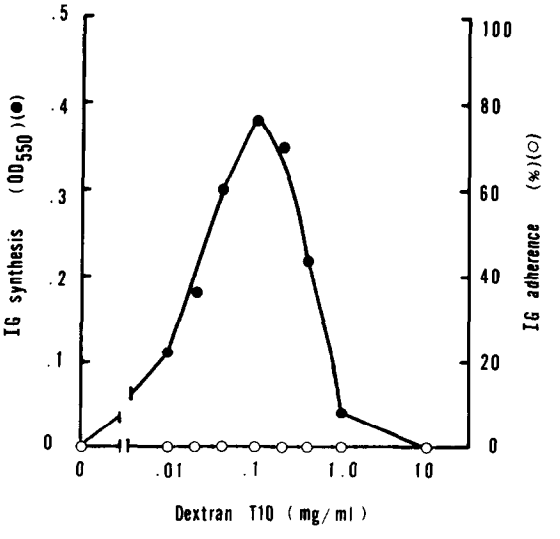


Fig.2. Synthesis and adherence of IG by GTase-I in the presence of soluble dextran: 5 mU GTase-I was incubated with 30 mg sucrose in the presence of 0–30 mg dextran T10 in 3 ml KPB as in fig.1. Amounts of total IG (●) and adherent IS (○) were quantitated as in section 2.

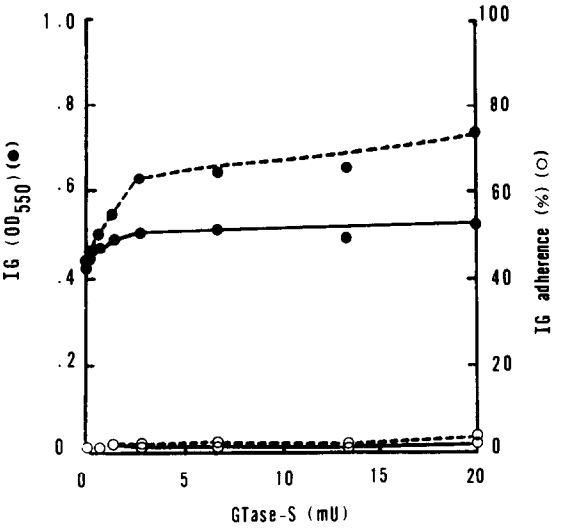


Fig.3. Adherence of the native and dextranase-modified IGs by the incubation with GTase-S and sucrose. The native IG (0.6 mg, ——) or the dextranase-modified IG (1.0 mg, ---) were incubated with 0–20 mU GTase-S and 30 mg sucrose in 3 ml KPB as in fig.1. Amounts of adherent IG (○) as well as the change of turbidity of whole mixture (●) were determined as in section 2.

synthesis of total IG and adherent IG was induced at the different proportions of the two GTase components.

GTase-I (5 mU) produced also significant amounts of IG in the presence of dextran T10 (fig.2). Maximal production occurred at 0.01% dextran, but the level attained to only ⅓rd of the highest level achieved by the combined action of the two GTase-components (see fig.1). The IGs produced by GTase-I in the presence of dextran T10 were not adherent to glass (fig.2).

Likewise, neither the native IG nor the dextranase-modified IG became adherent to glass after incubation with various activities of GTase-S and sucrose (fig.3). Apparently increased turbidity yielded in the IG suspensions containing GTase-S is probably due to aggregation of the supplemented IG molecules, which might be induced by the nascent synthesis of SG by GTase-S from sucrose.

## 4. DISCUSSION

GTase-I separated from crude GTase of *S. mutans* OMZ176 gained significant activity to rapidly synthesize IG by the presence of exogenous α-1,6-linked primer dextran (fig.2) or by the coexistence of separated GTase-S (fig.1), indicating that presence of α-1,6-linked dextran promotes rapid polymerization of glucose moiety of sucrose through α-1,3-glucosidic linkage by GTase-I. The process would certainly occur with the exogenous primer-independent synthesis of IG by crude *S. mutans* GTase, which contains both GTase activities (autopriming effect).

These studies showed the IG produced by GTase-I in the presence of commercial dextran did not adhere to glass (fig.2). Furthermore, preformed IGs, irrespective of native or dextranase-treated, did not produce adherence to a glass surface even in the presence of GTase-S (fig.3). However, combinations of GTase-I and GTase-S synthesized IG adherent to glass, which occurred at relatively restricted ratios ($\sim$ 0.6) of GTase-S to GTase-I (fig.1). These results collectively indicate that simultaneous de novo synthesis is required for the formation of adherent IG with appropriate proportions of α-1,6- to α-1,3-linkages. This idea is consistent with the previous observations that the IG produced by the action of crude *S. mutans* GTase contains smaller amounts of α-1,6-linkage, which becomes less adherent to glass with increasing concentrations of dextranase present [3,10,11].

In conclusion, α-1,3- and α-1,6-glucosidic linkage-synthesizing components of *S. mutans* GTase should concert to produce adherent IG. Our previous electron microscopic study revealed that adherent IG produced by crude *S. mutans* AHT GTase consists of the seemingly double-stranded, α-1,3-linked fibrillar glucan carrying α-1,6-linked peripheral protrusions and the α-1,6-linked single-stranded fine fibrillar glucan [12].

## ACKNOWLEDGEMENT

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