Effect of a Glucosyltransferase Inhibitor on Glucan Synthesis and Cellular Adherence of Streptococcus mutans

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The effects of mutastein, a glucosyltransferase inhibitor derived from an Aspergillus terreus strain, on the glucan synthesis by glucosyltransferases of Streptococcus mutans and sucrose-dependent adherence of S. mutans cells were examined in vitro. The synthesis of insoluble glucan by crude glucosyltransferase of S. mutans strain B13 was markedly inhibited by mutastein at a final concentration of 10 μg of protein/ml. The synthesis of insoluble glucan adherent to glass surfaces was almost completely inhibited by mutastein at this concentration. In addition, mutastein inhibited the sucrose-dependent adherence to glass surfaces of resting and growing cells of various S. mutans strains. These results suggest that mutastein could be useful for controlling dental plaque and dental caries in vivo.

Streptococcus mutans has been strongly implicated as a causative organism of dental caries (2, 3). This organism synthesizes water-insoluble glucan (IG) from sucrose with its cell-free and cell-bound glucosyltransferases (GTase) (11). De novo synthesis of IG is essential for the adherence of S. mutans to tooth surfaces (4). In addition, IG firmly coats the tooth surface, forming a barrier which prevents the diffusion of acids produced by S. mutans from various sugars. The acids accumulated in situ will decalcify minerals of the enamel.

Many chemical and enzymatic procedures for eliminating S. mutans from tooth surfaces have been explored (3, 14, 15). However, there are few reports concerning substances that inhibit GTase activity (1, 12, 13). Recently, a GTase inhibitor was found in a culture filtrate of an Aspergillus terreus strain and named mutastein (A. Endo, N. Inoue, O. Hayashida, and S. Murakawa, Abstr. Annu. Meet. Agric. Chem. Soc. Jpn. 1981, no. 4N-7, p. 533). This report describes the effects of this inhibitor on the glucan synthesis from sucrose by cell-free S. mutans GTase and the sucrose-dependent adherence of S. mutans cells to glass surfaces.

MATERIALS AND METHODS

Bacterial strains. S. mutans strain B13 (serotype d) was mainly used. In some experiments, S. mutans strains E49 (serotype a), FA1 (b), MT8148 (c), OMZ176 (d), MT703R (e), OMZ175 (f), and 6715 (g) were used. These strains were selected from the stock culture collection in the Department of Dental Research, the National Institute of Health, Tokyo.

Mutastein. Mutastein was partially purified by successive chromatography on charcoal and hydroxyapatite columns and by subsequent gel filtration of a culture filtrate of Aspergillus terreus strain M3328 (S. Murakawa, A. Endo, T. Koga, and S. Hamada, Abstr. Annu. Meet. Agric. Chem. Soc. Jpn. 1982, no. 2M-3, p. 423). This preparation was found to contain 85% protein and 6.5% carbohydrate on a weight basis.

Preparation of cell-free GTases. Crude GTase of S. mutans B13 was obtained from the culture supernatant. Organisms were grown in dialyze broth of TTY medium (5) at 37°C for 18 h. Centrifuged supernatant was combined with ammonium sulfate, resulting in 50% saturation. The precipitate was collected by centrifugation, dissolved in 5 mM potassium phosphate buffer (KPB), pH 6.0, to 1/40 of the original volume and dialyzed extensively against KPB. No fructosyltransferase activity was detected in this preparation.

S. mutans OMZ176 GTases-S and -I, synthesizing water-soluble glucan and water-insoluble glucan, respectively, were kindly provided by M. Inoue (Kagoshima University, Kagoshima, Japan). These enzymes were separated by the chromatofocusing method and subsequent hydroxyapatite column chromatography of crude GTase of serotype d S. mutans OMZ176 (T. Koga, S. Sato, T. Yakushiji, and M. Inoue, FEMS Microbiol. Lett., in press). Cell-free dextranucrase (DSase) of Leuconostoc mesenteroides strain NRRL B512F was prepared from a culture supernatant by ammonium sulfate precipitation and was kindly supplied by Y. Nakajima (Mitsui Seito Co., Tokyo).

One unit of GTase activity was defined as the amount of enzyme that transformed 1 μmol of sucrose to glucan per min under the conditions previously described (9). Protein content was determined by the method of Lowry et al. (10). The specific activities of the crude GTases of S. mutans strains B13 and...
OMZ176, and of GTase-S and GTase-I were 0.21, 0.77, 3.7, and 1.78 U/mg, respectively. The DSase activity of *L. mesenteroides* NRRL B512F was 14 mU/mg of protein.

Preparation of resting cells. *S. mutans* B13 was grown at 37°C for 18 h in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) supplemented with sucrose at a final concentration of 0.5% (wt/vol). Cells were harvested by centrifugation, washed with distilled water three times, and lyophilized. The cells thus obtained were shown to bind extracellular GTases, synthesizing cell-associated glucan upon addition of exogenous sucrose (5, 7).

Assays for the glucan synthesis by cell-free GTases. The reaction mixture consisted of sucrose (30 mg), crude strain B13 GTase (0 to 80 μl), and mutastein (0 to 60 μg of protein) in a total volume of 3 ml of 0.1 M KPB containing 0.02% merthiolate as a preservative. The mixture was incubated in a glass test tube at 37°C for 16 h at an angle of 30° to the horizontal. After incubation, the reaction mixture was spotted on a filter paper square (15 by 15 mm, no. 514, Toyo-Roshi, Tokyo). The squares were washed three times with methanol and dried. Radioactivity retained on the filter paper was measured by a scintillation spectrophotometer to determine the total glucan synthesized (9).

DSase (20 μl) from *L. mesenteroides* was reacted with sucrose (10 mg) in the presence of mutastein (0 to 20 μg of protein/ml) in 1 ml of 0.1 M KPB containing 0.02% merthiolate. After incubation at 37°C for 18 h, the synthesized water-soluble glucan was precipitated by the addition of 2.5 volumes of ethanol, and the resulting precipitate was collected by centrifugation (8). The glucan was washed with 70% ethanol, and the precipitate was dissolved again in 1 ml of distilled water. Amounts of glucan thus obtained were determined by the anthrone method (16), using glucose as a standard.

Adherence of *S. mutans* cells to a glass surface. An assay mixture consisted of sucrose (30 mg), strain B13 resting cells (1 mg dry weight), and mutastein (0 to 60 μg of protein) in 3 ml of 0.1 M KPB containing 0.02% merthiolate. After incubation at 37°C for 18 h in a test tube (13 by 100 mm), the quantity of adherent cells was determined turbidimetrically and expressed as percent adherence, as described previously (7).

To test the adherence of growing cells to a glass surface, strains of *S. mutans* were grown at 37°C for 18 h at a 30° angle in 3 ml of brain heart infusion broth (Difco) containing sucrose (1%) and filter-sterilized the absence or presence of mutastein (10 μg of protein/ml) in 20 μl of 0.1 M KPB. After incubation at 37°C for 60 min, the reaction mixture was spotted on a filter paper square (15 by 15 mm, no. 514, Toyo-Roshi, Tokyo). The squares were washed three times with methanol and dried. Radioactivity retained on the paper squares was measured by a scintillation spectrophotometer to determine the total glucan synthesized (9).

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FIG. 1. Effects of mutastein on the synthesis and adherence of IG by crude GTase of *S. mutans* B13. Reaction mixtures consisted of sucrose (30 mg), crude strain B13 GTase (40 μl), and mutastein (0 to 60 μg protein) in 3 ml of 0.1 M KPB containing 0.02% merthiolate. After the mixture was incubated in a glass test tube kept at an angle of 30° to the horizontal at 37°C for 16 h, the amount of total IG (●) and adherent IG (○) synthesized was determined colorimetrically by measuring optical density at 550 nm (OD550) as described in the text.

![Graph showing the effects of mutastein on the synthesis and adherence of IG by crude GTase of *S. mutans* B13.](http://iai.asm.org/)

FIG. 2. Effects of mutastein on the synthesis and adherence of IG by crude GTase of *S. mutans* B13. Reaction mixtures consisted of sucrose (30 mg), various quantities of crude strain B13 GTase (0 to 80 μl), and mutastein at concentrations of 0 (●) and 5 (○) μg of protein/ml in 3 ml of 0.1 M KPB containing 0.02% merthiolate. After incubation for 16 h, total IG (-----) and adherent IG (----) were quantitated as described in the legend to Fig. 1.
mutastein (0 or 50 μg of protein/ml). The percent adherence was then determined as described above.

RESULTS

Figure 1 shows the effects of mutastein on total and adherent IG synthesis by crude strain B13 GTase (21 mU). Total IG production was repressed by more than 80% by mutastein at a mutastein concentration of 10 μg/ml. Adherent IG production was also repressed by more than 90% in the presence of mutastein at a 5 μg/ml concentration, and almost complete inhibition was observed at concentrations higher than 10 μg/ml.

Effects of mutastein on total and adherent IG synthesis were examined by increasing amounts of crude strain B13 GTase (Fig. 2). Mutastein at a constant concentration of 5 μg/ml inhibited total IG production by 50 to 70%, and adherent IG production by 70 to 90% in a wide range of GTase concentrations. On the other hand, synthesis of total glucans by crude strain B13 GTase was not significantly inhibited by mutastein even at a concentration of 10 μg/ml (Table 1). These results indicate that the components which cause the production of water-insoluble glucan are not functioning in the presence of mutastein.

Crude cell-free GTase of S. mutans OMZ176 was separated into GTase-I and GTase-S. GTase-I was a primer-dependent enzyme, whereas GTase-S was primer independent (Table 2). Mutastein at a concentration of 10 μg/ml slightly inhibited the total glucan synthesis by crude strain OMZ176 GTase. On the other hand, mutastein caused approximately 80% inhibition of GTase-I in the absence of primer dextran, and 30% inhibition in the presence of primer. However, it should be noted that the amount of glucan synthesized by GTase-I in the absence of primer dextran was very low. The synthesis of glucan by GTase-S was little affected by mutastein. Water-soluble glucan production by DSase from L. mesenteroides was partially inhibited by mutastein at a concentration of 10 μg/ml (Fig. 3).

Mutastein markedly inhibited the adherence of resting S. mutans B13 cells; this adherence was mediated by glucan production through cell-bound GTases (Fig. 4). Figure 5 summarizes the percent adherence of growing cells in glucose broth of representative strains belonging to S. mutans serotypes a through g. The degree of

<table>
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<tr>
<th>Primer</th>
<th>Mutastein (μg of protein/ml)</th>
<th>Total glucan synthesized (cpm)</th>
<th>% Control</th>
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<tbody>
<tr>
<td>-</td>
<td>0</td>
<td>1,120</td>
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a Dextran T10: −, absent; +, present.

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<th>Enzyme</th>
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<th>Total glucan synthesized (cpm)</th>
<th>% Control</th>
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a Dextran T10: −, absent; +, present.

FIG. 3. Effect of mutastein on the glucan synthesis by DSase of L. mesenteroides NRRL B512-F. Reaction mixtures consisted of sucrose (10 mg), DSase of L. mesenteroides (20 μl), and mutastein (0 to 60 μg protein) in 3 ml of 0.1 M KBP containing 0.02% merthiolate. After incubation at 37°C for 16 h, glucan synthesized was collected by the addition of 2.5 volumes of ethanol. The amount of glucan was determined by the anthrone method (16).
The growth rate and acid production of *S. mutans* strains cultured in brain heart infusion broth were not affected by mutastein (results not shown).

**DISCUSSION**

*S. mutans* produces IG from sucrose by the action of GTases and thereby adheres firmly to tooth surfaces (4). It was shown by the present study that mutastein derived from an *A. terreus* strain markedly repressed the synthesis of IG and the IG-dependent adherence of *S. mutans* cells to glass surfaces.

GTase of *S. mutans* consists of several components (11). Recently, we have isolated GTase-I and GTase-S, synthesizing IG and water-soluble glucan, respectively, from a culture filtrate of *S. mutans* strain OMZ176 (Koga et al., in press). GTase-I activity was inhibited by mutastein more effectively than GTase-S activity (Table 2). This is consistent with the results that showed that mutastein markedly inhibited the synthesis of IG by crude strain B13 GTase but only slightly repressed that of total glucan (Fig. 1 and 2, Table 1).

Recently, Felgenhauer and Trautner (1) surveyed the α-glucosidase inhibitors of microbial origin, and found that 1-desoxynojirimycin, N-methyldesoxynojirimycin, and acarbose inhibited extracellular GTase of *S. mutans*. Okami et al. (13) isolated and partially purified another type of GTase inhibitor, ribocitirin, which was inhibition of the adherence by mutastein differed considerably among these strains. The greatest adherence inhibition (79%) was obtained with strain MT703R (serotype e), whereas the least inhibition (6%) was observed with strain 6715 (serotype g). The growth rate and acid production of *S. mutans* strains cultured in brain heart infusion broth were not affected by mutastein (results not shown).
found in a culture broth of a strain of *Streptomyces* sp. It consists of three D-ribose and one (+)-homocitric acid (12). However, the mutaestin preparation used in this study consisted of 85% protein and was inactivated by treatment with pronase (unpublished data), indicating that mutaestin is a different type of GTase inhibitor from those described above.

Strains of *S. mutans* are classified serologically into seven types, a through g. Among these serotypes, serotype c strains are most frequently isolated from human dental plaque, irrespective of race, age, sampling site, or isolation procedures (3). Although the degree of inhibition by mutaestin of the cellular adherence of *S. mutans* considerably differed among the serotypes (Fig. 5), mutaestin caused 54% inhibition of the adherence of growing *S. mutans* MT8148 (serotype c) cells. This result suggests that mutaestin may be useful for control of dental plaque formation and subsequent dental caries development.

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LITERATURE CITED