Acceleration of Dextran sucrase Activity of *Streptococcus mutans* by Secretory Immunoglobulin A

KAZUHIRO FUKUI, YOSHIO FUKUI, AND TAKAFUMI MORIYAMA

Department of Microbiology, Hiroshima University School of Dentistry, Hiroshima 734, Japan

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The effect of immunoglobulins on the activity of dextran sucrase purified from *Streptococcus mutans* strain HS-6 is described. When human salivary immunoglobulin A (IgA) or colostral IgA, either nature or denatured, was incubated with dextran sucrase, the rate of the dextran synthesis was markedly accelerated, whereas human serum IgA or IgG neither accelerated nor inhibited the enzyme activity. The results suggest that a portion unique for secretory IgA, the secretory component, might be related to the enzyme acceleration. On the other hand, specific rabbit antisera against the dextran sucrase inhibited completely dextran synthesis by the enzyme.

The cariogenicity of *Streptococcus mutans* is considered to be related to its capacity for synthesizing extracellular glucans from sucrose (8, 12). In view of the importance of glucans in adhesion of dental plaque to tooth surfaces and their synthesis by glycosyltransferases, several investigators have examined the possible effect of antibodies, which were directed against bacteria or partially purified glycosyltransferase preparations, on plaque formation and caries incidence, but have obtained variable results (3, 4, 7, 13). The reason for the discrepancy among investigations may be due to the impurity of enzyme preparations employed and the variety of immunoglobulin class in oral cavities.

In the oral cavity, immunoglobulins may originate from serum, gingival fluid, or salivary glands (1, 14, 17), and we have reported that secretory immunoglobulin A (IgA) obtained from saliva effectively inhibited various enzymes of oral bacteria (10). The contribution of each immunoglobulin to plaque formation and caries immunity, however, is not yet clear.

For these reasons, we first attempted to purify and characterize glycosyltransferases from the culture fluids of *S. mutans* HS-6. The successful isolation of one of the enzymes, dextran sucrase (EC 2.4.1.5), has enabled us to elucidate the interaction of the enzyme with each immunoglobulin in vitro.

This report describes the effect of various immunoglobulins on dextran sucrase.

**MATERIALS AND METHODS**

**Preparation of dextran sucrase.** Dextran sucrase was purified from culture fluids of *S. mutans* HS-6 by treatment with Sepharose 6B, diethylaminoethyl (DEAE)-cellulose, and hydroxypatite as described previously (11). The purified enzyme used throughout this study was homogeneous, as judged by polyacrylamide gel electrophoresis and immunodiffusion.

Polyacrylamide gel electrophoresis, immunodiffusion, and immunoelectrophoresis were carried out by the methods of Davis (5) and Campbell et al. (2).

**Collection of saliva and preparation of salivary IgA.** Whole saliva was collected from 40 healthy adults and concentrated with ammonium sulfate as described previously (9). Secretory IgA was purified from the concentrated saliva by DEAE-cellulose and Sephadex G-200 treatment, as described by Tomasi and Bienenstock (17). Salivary IgA was homogeneous as judged by polyacrylamide gel electrophoresis and immunoelectrophoresis (Fig. 1).

**Serum, IgA, IgG, and colostralm IgA.** Human serum was obtained from a healthy adult 45 years of age. Human serum IgA, IgG, and secretory IgA purified from human colostrum were kindly provided by K. Inoue, Institute for Microbial Diseases, Osaka University, Osaka, Japan. These preparations were homogeneous as judged by polyacrylamide gel electrophoresis and immunoelectrophoresis.

**Antiserum against purified dextran sucrase.** Rabbits were immunized three times by an intramuscular injection, at 3-week intervals, with 100 μg of purified dextran sucrase in Freund incomplete adjuvant. After these injections, the rabbits were bled. The gamma globulin fraction containing the antibodies was purified from the serum with ammonium sulfate fractionation and dialyzed against buffered saline by the method of Campbell et al. (2).

**Chemical manipulations of salivary IgA.** Reduction-alkylylation treatment of salivary IgA was carried out with 0.2 M mercaptoethanol and 0.3 M iodoacetamide by the method of Edelman and Marchalonis (6).

**Enzyme assay.** The activity of dextran sucrase was measured as described previously (11). The standard reaction mixture of 80 μlitters containing 0.063 M succinic acid-0.125 M K2HPO4, buffer (pH 6.0),
IgA, the upper salivary IgA. The center well contains 20 μg of salivary IgA, the upper trough contains anti-whole saliva, and the lower trough contains anti-secretory IgA sera.

[^1]C]sucrose (0.125 mCi/mmol), and 1 μg of the purified dextran sucrase was incubated at 37 C. To test the effect of immunoglobulin, various concentrations of immunoglobulins were added to the assay system prior to the addition of [^1]C]sucrose. The mixture was incubated at 4 C overnight. After the preincubation, [^1]C]sucrose was added to the system and the reaction mixture was incubated at 37 C. A sample (5 μl) of the reaction mixture was withdrawn at various times of reaction and applied directly to a sheet of Toyo chromatography no. 50 paper (40 by 40 cm). The reaction was stopped when the paper was rapidly dried with warm air, and the paper was developed by ascending chromatography in the solvent system n-butanol-pyridine-water (6:4:3, vol/vol). The radioactivity of the reaction products on the chromatogram was measured by removing the radioactive material from the paper, mixing it in 10 ml of scintillation fluid in a vial, and counting in an Aloka type LSC-601 liquid scintillation counter. The locations of glucose, fructose, sucrose, and dextran were identified by comparing their migration with those of known sugars (Fig. 2). Glucose was also identified by visualizing its location with glucose oxidase as described by Pazur and Klepe (16). Dextran was identified from analysis of the products after acid hydrolysis and periodate oxidation as described previously (11).

**Protein determination.** Protein was measured by the method of Lowry et al., with bovine serum albumin as standard (15).

**RESULTS**

Acceleration of dextran sucrase activity by secretory IgA. In our previous report (10), it was shown that human secretory IgA inhibited various enzymes produced by oral bacteria. Accordingly, the effect of secretory IgA on dextran sucrase purified from *S. mutans* HS-6 was investigated. Increasing amounts of secretory IgA purified from human colostrum were added to the enzyme assay system and incubated at 4 C overnight. After the preincubation, [^1]C] sucrose was added to the mixture as substrate, and the reaction mixtures were incubated at 37 C for 10 min. Five-microliter volumes of the reaction mixtures were applied to a sheet of chromatography paper and dried immediately with warm air. The applied samples were developed and separated into glucose, fructose, sucrose, and dextran as described above. The formation of dextran and the release of glucose and fructose by the enzyme were considerably accelerated with the addition of increasing amounts of the secretory IgA (Fig. 3). The radioactivity in released fructose corresponded to the total activity of both formed glucose and dextran, and no formation of saccharides except for glucose, fructose, and dextran was observed by paper chromatography of the final reaction products.

### Effects of various immunoglobulins, serum and, denatured secretory IgA on dextran sucrase.

To determine whether the dextran sucrase was activated by other immunoglobulins, human serum, IgA, IgG, whole saliva, secretory IgA purified from both human saliva and colostrum, and heat-denatured and chemically treated secretory IgA were added to the enzyme assay system and preincubated at 4 C overnight. After the addition of [^1]C]sucrose, the reaction mixtures were incubated at 37 C for 10 min and the enzyme activities were checked as described above. The results indicated that the

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**FIG. 1.** Immunoelectrophoretic pattern of purified salivary IgA. The center well contains 20 μg of salivary IgA, the upper trough contains anti-whole saliva, and the lower trough contains anti-secretory IgA sera.

**FIG. 2.** Paper chromatographic identification of products formed by dextran sucrase from sucrose. The chromatogram was developed as described in the text, the reducing sugars were stained with silver nitrate reagent, and the radioactivity was measured with an Aloka type JTC-201 radio thin-layer chromatogram scanner. (1) 5 μg of fructose (F); (2) 5 μg of glucose (G); (3) 5 μl of the reaction mixture; sucrose (S); dextran (D).
secretory IgA obtained from saliva and colostrum enhanced dextran formation by the enzyme (Table 1). The values for salivary and colostral IgA were 3.0 and 7.3 times that of control, respectively, but serum and serum immunoglobulins did not increase enzymatic activity. Release of glucose and fructose upon addition of secretory IgA also increased, and the radioactivity of released fructose corresponded to the total activity of both glucose and dextran. Furthermore, heat-denatured and chemically treated secretory IgA increased the enzymatic activity, as did non-treated secretory IgA. The results show that the activation of the dextranase may be unique for secretory IgA but may not be due to antibody function of secretory IgA.

Inhibition of dextranase by anti-dextranase. Human serum, IgA, and IgG neither inhibited nor accelerated the dextranase obtained from S. mutans HS-6. Accordingly, we tested the inhibitory activity by specific antibody obtained from rabbit immunized with the purified dextranase of S. mutans HS-6. Increasing amounts of anti-dextranase rabbit globulin fraction were added to the enzyme assay system, and after 30 min of incubation at 37 C the amount of synthesized dextran was determined by removing the formed dextran from the paper chromatogram and measuring the radioactivity by liquid scintillation counting. Percent inhibition of dextran formation by addition of anti-dextranase is shown in Table 2. The kinetics of dextran formation in the presence of the antibody and collostral IgA is shown in Fig. 4. These results indicate that dextranase activity was inhibited almost completely by addition of appropriate amounts of the antibody.

Our previous study on the dextranase showed that the highly purified enzyme synthesized dextran that contained α-(1 → 6) linkages and also simultaneously released considerable amounts of glucose and fructose from sucrose (11). Therefore, the formation of glucose, fructose, and dextran by the enzyme in the presence of anti-dextranase was determined. The results indicate that dextran formation by the enzyme was completely inhibited by the specific antibody, but release of glucose and fructose was not inhibited completely (Fig. 5). On the other hand, both dextran synthesis and release of glucose and fructose were increased by the addition of small amounts of the antibody (0.25 μg of protein).

Table 1. Effect of saliva, serum, serum globulins, and secretory IgA on dextran synthesis

<table>
<thead>
<tr>
<th>Addition</th>
<th>Protein added to reaction mixture (μg)</th>
<th>Dextran formation (%)</th>
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<tbody>
<tr>
<td>No addition</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Human serum</td>
<td>212</td>
<td>122</td>
</tr>
<tr>
<td>Human serum IgG</td>
<td>50.0</td>
<td>97</td>
</tr>
<tr>
<td>Human serum IgA</td>
<td>64.4</td>
<td>119</td>
</tr>
<tr>
<td>Human colostral IgA</td>
<td>50.0</td>
<td>730</td>
</tr>
<tr>
<td>Denatured human colostral IgA*</td>
<td>50.0</td>
<td>735</td>
</tr>
<tr>
<td>Human whole saliva</td>
<td>176</td>
<td>275</td>
</tr>
<tr>
<td>Human salivary IgA</td>
<td>46.4</td>
<td>298</td>
</tr>
<tr>
<td>Chemically treated human salivary IgA</td>
<td>46.4</td>
<td>300</td>
</tr>
</tbody>
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* Human collostral IgA was heated at 100 C for 10 min.

Table 2. Inhibition of dextranase by anti-dextranase rabbit serum

<table>
<thead>
<tr>
<th>Amounts of added antibody (μg)</th>
<th>Inhibition of dextran formation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.25</td>
<td>-7</td>
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<tr>
<td>2.48</td>
<td>76</td>
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<td>12.4</td>
<td>96</td>
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<td>24.8</td>
<td>99</td>
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stage, however, it is difficult to rule out the possibility that a portion of secretory IgA other than the secretory component is responsible for its activation. Therefore, it is evident that further investigation is required to solve this problem, and studies on the mechanism of the enzyme activation by secretory IgA are being carried out. The activation of dextran formation by salivary IgA was less than that by colostral IgA (Table 1). It seems possible that salivary IgA contains antibody specific to dextran release of cariogenic streptococci living in oral cavity. However, this appears unlikely in colostral IgA. The action of the specific antibody might have resulted in the reduction of activa-

DISCUSSION

Our previous report (10) demonstrated that secretory IgA derived from human saliva effectively inhibited various enzymes from oral bacteria. Our attention was thus focused on the problem of inhibitory effects of secretory IgA on enzymes obtained from cariogenic streptococci. Contrary to our expectations, the results presented here clearly showed that not only naturally but also denatured secretory IgA markedly increased the activity of dextranase obtained from S. mutans strain HS-6. However, serum IgA and IgG obtained from human adults neither inhibited nor accelerated the enzyme activity. Therefore, it seems probable that a factor responsible for the enzyme activation may be unique for secretory IgA and might reside in a secretory component. At the present

![Graph](http://jb.asm.org/)
tion by salivary IgA. Investigation on the activity of each salivary IgA obtained from individuals immunized with the enzyme will be needed before we can reach any conclusion.

Several investigators reported that antisera against cells or partially purified glucosyltransferases from \textit{S. mutans} inhibited enzyme activity (3, 4, 7, 13), but the results were variable. This suggests that this area needs precise investigation with pure enzyme and its antibody. Thus, our successful isolation of a purified enzyme, dextransucrase, from \textit{S. mutans} HS-6 and preparation of its antibody have permitted us to carry out an investigation on the effect of the antibody on the enzyme. Our results clearly demonstrated that the antibody inhibited dextran formation by the enzyme up to 99\% in the absence of dextran and about 82\% in the presence of dextran (Table 2 and Fig. 5).

In whole saliva, various immunoglobulins such as IgG, IgA, and IgM are derived from gingival fluid, and secretory IgA is derived from salivary glands (1, 14, 17). The role of these immunoglobulins on dental plaque formation or caries in the oral cavity has attracted attention in recent years. Before attempting these investigation in vivo, however, we consider it of importance to elucidate the interactions of various immunoglobulins in the oral cavity with purified enzymes responsible for dental plaque formation in vitro. Thus, isolation and characterization of each enzyme from \textit{S. mutans} has been extensively carried out in our laboratory, and we obtained the controversial evidence that secretory IgA inhibits neuraminidase and hyaluronidase from oral streptococci (10) but increases dextransucrase activity. These investigations may provide fundamental information on the profile of antigens and antibodies involved in caries immunity.

**LITERATURE CITED**