IN VITRO ADSORPTION OF DEXTRANASE BY DENTAL PLAQUE AND BUCCAL MUCOSA, AND ITS ORAL RETENTION AFTER RINSING THE MOUTH

Katsuyuki FUTAKAMI*, Tatsuo KIYOSHIGE**, Yoji YAMAZAKI*** and Motoo NIWA***

Abstract: In vitro adsorption of dextranase by dental plaque and buccal mucosa was determined, and clinical oral retention of dextranase was also confirmed.

Additions of 1320, 6600 and 13200 units of dextranase to dental plaque samples resulted in adsorptions of 2.9, 10.8 and 15.1 enzyme units per 1 milligram dry weight of dental plaque, respectively. Moreover, buccal mucosa adsorbed about 6 units of dextranase per milligram dry weight of buccal mucosa when treated with 16800 units enzyme. The adsorption by buccal mucosa was considered to be weaker than that by dental plaque.

After rinsing the mouth for 30 sec. with 13200 units of dextranase, the enzyme activity in the whole saliva was reduced rapidly and disappeared completely after 90 min. In spite of this, however, a significant amount of the enzyme activity was found in the dental plaque of each subject.

I. Introduction

Extranase (α-1, 6-glucan glucanohydrolase) was investigated and its antiplaque and anticariogenic activities were confirmed, in microbiological1-6), experimental animal6-9), and clinical tests10-15). The effect of dextranase in a mouth rinse and a dentifrice was also tested. A reducing sugar was found to be released in the oral cavity after dextranase mouth rinsing16). The reduction of caries after dextranase tooth paste treatment was also noted17,18). The behavior of dextranase in the oral cavity has not been studied so far.

Chlorhexidine was proved to be the most effective antiplaque agent and its effectiveness was probably caused by the continuous oral retention through its adsorption on tooth surface, dental plaque and oral mucosa20).

In the present study, in vitro adsorption of dextranase by dental plaque and buccal mucosa, and its oral retention after mouth rinsing were determined.

II. Materials and Methods

Dextranase; Dextranase (α-1, 6-glucan glucanohydrolase) produced from Chaetomium gracile was provided by SANKYO Co. Ltd. The determination of enzyme unites was performed in the same way.
as reported previously\(^{16}\).

**Dental plaque and buccal mucosa:** Four adults, aged 27 to 32, were instructed not to brush their teeth for 1 day. Dental plaque samples were collected as much as possible from all tooth surfaces and interproximal spaces using a toothpick. They were suspended in M/10 phosphate buffer solution (pH 7.0). Samples of the 4 subjects were mixed and homogenized with a homogenizer. Buccal mucosa were collected by scraping the buccal surface with a spoon after rinsing the mouth with tap water. The scrapings were suspended in 0.02 M phosphate buffered saline (pH 7.0).

**In vitro adsorption of dextranase:** Each 1 ml of dental plaque was mixed with 1 ml of 1320, 6600 and 13200 units of dextranase and incubated for 30 min. at 37°C. The precipitate was then centrifuged (10000 r.p.m., 10 min.). The precipitate was washed 3 times with distilled water by centrifugation. For confirmation, dextranase activity in the supernatant of the 3rd washing was determined, and it was found that only 0.2 units of dextranase remained in 0.5 ml of the supernatant. The precipitate was finally resuspended in M/10 phosphate buffer solution (pH 7.0). One ml of the suspension was used for the determination of dextranase activity. Dental plaque which was not treated by any enzyme was used as a control. Dry weights of plaque sample were also measured (100°C, 24 hrs.). One half ml of buccal mucosa suspension was mixed with 16800 units of dextranase and was treated in the same manner as dental plaque. Only 0.3 units of dextranase activity was found in 0.5 ml of the 3rd washing.

**Oral retention of dextranase:** Four adults, aged 27 to 32, were asked to stop tooth brushing for 1 day and to rinse their mouth with 5 ml of 10000 units dextranase for 30 sec. One tenth ml of the whole saliva was collected as time elapsed and its dextranase activity was determined. Each subject was asked to rinse his mouth with tap water after 90 min. and the dental plaque were collected. The plaque was suspended in M/10 phosphate buffer solution (pH 7.0), and the remaining dextranase activity as well as the dry weight were determined using 1 ml of dental plaque suspension. Dry weights were about 1 milligram.

### III. Results

In vitro adsorption of dextranase by dental plaque and buccal mucosa is shown in Table 1. Treatment of dental plaque with 1320, 6600 and 13200 units of dextranase resulted in adsorption of the enzyme by dental plaque, and the enzyme activities were 2.9, 10.8 and 15.1 units per milligram dry weight of dental plaque, respectively. Untreated dental plaque showed only 0.2 units of the activity. Buccal mucosa which was treated with 16800 units of dextranase showed its adsorption. About 6 units of enzyme activity were found per milligram dry weight of buccal mucosa. However, the adsorption by buccal mucosa seemed to be weaker than by dental plaque.

As for the oral retention of dextranase in whole saliva, the results are shown in Fig. 1. After rinsing the mouth with 13200 units of dextranase, the enzyme activity in the whole saliva was rapidly reduced and disappeared completely about 90 min. later. However, in spite of this, plaque samples of the 4 subjects possessed a significant amount of dextranase activity i.e., 30.6, 14.7, 3.0 and 2.1 units of dextranase activities per milligram dry weight of dental plaque.

### IV. Discussion

Only trace amounts of the enzyme activity remained in dental plaque and in buccal mucosa after the 3rd washing. When dental plaque and buccal mucosa are treated with dextranase, their enzyme activities were significantly increased (Table 1).

After rinsing the mouth with dextranase for 30 sec. salivary enzyme activity was rapidly reduced
Fig. 1 Oral retention of dextranase in the whole saliva after the mouth rinsing with 10000 units of enzyme

Table 1. Adsorption of dextranase to dental plaque and buccal mucosa

<table>
<thead>
<tr>
<th>No.</th>
<th>Materials*</th>
<th>Treated dextranase (units)</th>
<th>Found dextranase (units/mg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>dental plaque</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>2.</td>
<td>''</td>
<td>1320</td>
<td>2.9</td>
</tr>
<tr>
<td>3.</td>
<td>''</td>
<td>6600</td>
<td>10.8</td>
</tr>
<tr>
<td>4.</td>
<td>''</td>
<td>13200</td>
<td>15.1</td>
</tr>
<tr>
<td>5.</td>
<td>buccal mucosa</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>6.</td>
<td>''</td>
<td>16800</td>
<td>6.2</td>
</tr>
<tr>
<td>7.</td>
<td>''</td>
<td>''</td>
<td>6.4</td>
</tr>
<tr>
<td>8.</td>
<td>''</td>
<td>''</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* : About 1 mg dry weight of samples were treated with dextranase

by the oral cleaning action of saliva (Fig. 1). However, the enzyme activity remained at an effective level for as long as 40 min. Moreover, the enzyme activity of dextranase which had been adsorbed clinically by the dental plaque of each subject continued more than 90 min. but the level was different from sample to sample. This tendency might be caused by the different thickness or the components of the dental plaque.

A trace amount of dextranase inhibits the polysaccharide synthesis and adherence of Streptococcus mutans<sup>6</sup>). Therefore, the adsorption of dextranase by dental plaque may influence the polysaccharide synthesis from sucrose and inhibit the development of dental plaque.

References