Effect of glucanases of *Bacteroides oralis* Ig4a on artificial plaque of *Streptococcus mutans*

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Extracellular water-insoluble glucan (mutan) has an important role in the colonization of *Streptococcus mutans* and in the deposition of dental plaque. Many reports have been published on the effects of glucan hydrolyzing enzymes, including dextranases and mutanases, to remove plaque from tooth surfaces. Among them, dextranases have been reported as showing little effect on removal of the plaque *in vivo*¹⁻⁵). Treatment of the plaque with purified mutanase [α -(1,3)-glucanase] extracted from several kinds of fungi also had only a slight effect on decreasing the plaque *in vivo*^{6,7)}, and *in vitro*⁸⁾, while Guggenheim et al. observed the effects of mutanase on rat caries and plaque⁹⁾. Since mutan is the glucan containing α -(1,3)- and α -(1,6)-glucoside linkage in various percentages, both dextranase and mutanase should be required for hydrolyzing it. In this connection, we also studied the crude enzymes of an oral bacteria, *Bacteroides oralis* Ig4a. This organism was isolated from human dental plaque and hydrolyzed with mutan as well as dextran. The conclusion was that it produced both α -(1,3)-glucanase as well as α -(1,6)-glucanase¹⁰⁾. In this report, we confirmed that the homogenized bacterial suspension and the enzymes of *B. oralis* were effective in removing the deposits of *Streptococcus mutans* from the glass surface.

The bacterial strains used in this experiment were *Bacteroides oralis* Ig4a and *Bac-*

teroides fragilis #4. The latter, isolated from rat dental plaque, produced dextranase but no mutanase. The organisms were cultured in Trypticase soy broth supplemented with 0.2% dextran (Pharmacia, T-2000) under anaerobic conditions. The cell suspension, obtained by centrifugation and washing with 20 mM phosphate buffer pH 6.5, was homogenized with sonication at 100 W for 10 min. The dextranase was extracted by the method reported previously¹¹⁾. Mutanase was extracted from the cells of a batch culture of *B. oralis* Ig4a as follows: The cells were collected by centrifugation at 8,000 $\times$ g for 20 min, washed twice with 20 mM phosphate buffer pH 6.5, and suspended in a small volume of the same buffer. The cell suspension was mixed with an equal volume of butanol and kept at 4°C for 30 min. The aqueous layer was obtained by centrifugation at 20,000 $\times$ g for 30 min. To the aqueous layer was added cold acetone (-20°C) to a final concentration of 50% (v/v), and kept at -10°C for 20 hr. The suspension was centrifuged at 10,000 $\times$ g for 30 min and the precipitate was washed twice with cold acetone and dissolved in 20 mM phosphate buffer. Further purification of the preparation was performed by chromatography of DEAE-cellulose followed by that of phospho-cellulose. The purity of the final preparation was checked by detecting a single band on the SDS-PAGE. (Details of the methods and

Fig. 1 Hydrolyzation of pseudonigeran by homogenized cell suspension of *B. fragilis* #4 and *B. oralis* Ig4a (left), and dextranase and mutanase of *B. oralis* Ig4a (right).

properties of the enzyme will be published.) Pseudonigeran was extracted from the mycelium of *Aspergillus niger* ATCC 16888 by the method of Hasegawa et al.¹²⁾

Trypticase soy agar supplemented with pseudonigeran at a final concentration of 0.5% was prepared, and small wells were made in the agar. Fifty μ l each of the disintegrated cell suspension (20 mg/ml) of *B. oralis* Ig4a or *B. fragilis* #4, a dextranase, or mutanase (200 U/ml*) was placed in the wells, and incubated at 37°C for 24 hr. As shown in Fig. 1, the homogenates of *B. oralis* and the mutanase hydrolyzed the pseudonigeran showing a clear zone around the well while the homogenates of *B. fragilis* and the dextranase did not.

Streptococcus mutans GS-5 was incubated in test-tubes containing Trypticase soy broth supplemented with 5% sucrose. After 48 hours' incubation, the culture medium was removed and the bacterial film adhering to the glass surface was washed with sterilized saline. A fresh medium containing the homogenized cell suspension (50 mg/ml) of either *B. oralis* or *B. fragilis*, or the enzyme(s) (100 U/ml) was poured into the test-tubes and incubated at 37°C. The effect on removal of the film from the glass surface was

Table 1 Effect of homogenized cell suspension and glucanase(s) of *B. oralis* Ig4a and *B. fragilis* #4 on removal of the film of *S. mutans* on glass surface¹⁾

	Removal of film
TSB ²⁾ + <i>B. oralis</i> Ig4a ³⁾	+
TSB + <i>B. fragilis</i> #4 ³⁾	—
TSB + Dextranase	—
TSB + Mutanase	—
TSB + Dextranase & Mutanase	+
TSB	—

¹⁾ The film of *S. mutans* GS-5 was performed by incubation in TSB supplemented with 5% sucrose.

²⁾ Trypticase soy broth.

³⁾ The bacterial cells were homogenized by sonication (see the text).

determined after 2 to 20 hours incubation. The results are shown in Table 1. The homogenized suspension of *B. oralis* Ig4a showed significant activity for removing the streptococcal film within 2 h, while the suspension of *B. fragilis* #4 showed no activity. Either the dextranase or the mutanase had no effect on the streptococcal film when used individually. However, when both enzymes were added to the medium, a significant effect on the film was observed. These results confirmed that the combined activity of dextranase and mutanase are required for decreasing the accumulation of *S. mutans* and also indicated that the activity may be effective in removing matured dental plaque *in vivo*. Since *B. oralis* is a normal inhabitant of the human oral cavity, it may have a role in the ecology of the dental plaque.

References

- 1) Bowen, W. H.: Effects of dextranase on cariogenic and non-cariogenic dextrans. *Brit. Dent. J.* 124: 347-349, 1969.
- 2) König, K. G. and Guggenheim, B.: *In vivo* effects of dextranase on plaque and caries. *Helv. Odont. Acta*, 12: 48-55, 1968.
- 3) Guggenheim, B., König, K. G., Mühlemann, H. R. and Regolati, B.: Effect of dextranase on caries in rats harbouring an indigenous

* The unit of enzyme activity was defined as the amount of a enzyme which released 1 mmol of reducing sugar per minute under standard conditions described in reference 11.

- cariogenic bacterial flora. Archs. oral Biol. 14: 555-558, 1969.
- 4) Block, P. L., Dooley, C. L. and Howe, E. E.: The retardation of spontaneous periodontal disease and the prevention of caries in hamsters with dextranase. J. Periodontol. 40: 105-110, 1969.
 - 5) Caldwell, R. C., Sandham, H. J., Mann, W. V., Finn, S. B. and Formicola, A. J.: The effect of a dextranase mouth wash on dental plaque in young adults and children. J. Amer. Dent. Assoc. 82: 124-131, 1971.
 - 6) Guggenheim, B., Regolati, B. and Mühlemann, H. R.: Caries and plaque inhibition by mutanase in rats. Caries Res. 6: 289-297, 1972.
 - 7) Kelstrup, J., Funder-Nielsen, T. D. and Møller, E. N.: Enzymatic reduction of the colonization of *Streptococcus mutans* in human dental plaque. Acta Odont. Scand. 31: 249-253, 1973.
 - 8) Takahara, T. and Incue, M.: Inhibitory effects of endo- α -1,3-glucanase on glucan film formation and glucan synthesis by the glucosyltransferase of the oral bacterium *Streptococcus mutans*. Archs. oral Biol. 26: 217-222, 1981.
 - 9) Guggenheim, B., Regolati, B., Schmid, R. and Mühlemann, H. R.: Effects of the topical application of mutanase on rat caries. Caries Res. 14: 128-135, 1980.
 - 10) Takahashi, N., Horikawa, T., Mizuno, F. and Takamori, K.: Insoluble glucan hydrolyzing enzymes from oral *Bacteroides*. Bull. Tokyo Med. Dent. Univ. 27: 79-88, 1980.
 - 11) Takahashi, N.: Isolation and properties of dextranases from *Bacteroides oralis* Ig4a. Microbiol. Immunol. 26: 375-386, 1982.
 - 12) Hasegawa, S., Nordin, J. H. and Kirkwood, S.: Enzymes that hydrolyze fungal cell wall polysaccharides. I. Purification and properties of an endo- α -D-(1 $\rightarrow$ 3)-glucanase from *Trichoderma viridae*. J. Biol. Chem. 244: 5460-5470, 1969.