# Enzymatic reduction of the colonization of *Streptococcus mutans* in human dental plaque

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The aim of the present study was to evaluate the significance of water-insoluble glucans, containing mainly α-1,3 glucoside linkages, in the colonization of *Streptococcus mutans* on human teeth *in vivo*, and furthermore to assess the influence of α-1,3 glucan hydrolase on the accumulation of dental plaque. Five dental students with excellent oral hygiene participated in the investigation. The experiment was divided into two sections, each consisting of an initial two week period of oral hygiene, followed by one week of rinsing either with a sucrose solution or with a solution of sucrose containing α-1,3 glucan hydrolase, synthesized by a strain of *Aspergillus nidulans*. Oral hygiene was discontinued during rinsing. The amount of plaque was estimated at intervals during the whole experiment, and it was found that the accumulation of dental plaque was roughly identical during rinsing with solutions of sucrose and sucrose containing enzyme, substantiating the expectation that numerous microorganisms colonize the teeth by mechanisms other than synthesis of α-1,3 glucan. However, after each rinsing period the dental plaque was examined bacteriologically, and the proportions of selected streptococcal species were calculated. This revealed that in all five subjects the proportion of *Strep.mutans* was lower after rinsing with the sucrose solution containing enzyme than after rinsing with sucrose only, the average being less than 2.2% compared to 10.4%. Based on this finding it may be concluded that *Strep.mutans*, at least in part, depends on glucans with mainly α-1,3 glucoside linkages for its colonization of the teeth in humans.

*Key-words:* Dental plaque; Streptococcus mutans; glucan hydrolase

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It is known that aggregation of *Streptococcus mutans* in dental plaque at least in part depends on high molecular weight glucans, synthesized from sucrose. It has been found that several types of glucan are synthesized by this organism, some being water-soluble and containing a high proportion of α-1,6 linkages, others being water-insoluble, containing mainly α-1,3 linkages (*Guggenheim*, 1970; *Ceska et al.*, 1972). While it has been suggested that water-soluble glucans may play a distinct role in cell aggregation (*Gibbons & Fitzgerald*, 1969), the significance of water-insoluble glucans has been pointed out by other authors (*Guggenheim, Regolati & Mühlemann*, 1972; *Kelstrup & Funder-Nielsen*, 1972).

On this background it may appear logical to use enzyme preparations con-

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taining glucan hydrolase in plaque and caries prevention. This has, indeed, been tried *in vitro* and *in vivo*, but only with limited success (reviewed by *Guggenheim et al.*, 1972). With a single exception, though, the preparations used have contained α-1,6 glucan hydrolase (EC 3. 2. 1. 11) without, or with only traces of α-1,3 glucan hydrolase. Recently, however, *Guggenheim et al.* (1972) reported on animal experiments with a crude enzyme preparation from *Trichoderma harzianum*, containing α-1,3 glucan hydrolase, but devoid of α-1,6 glucan hydrolase. It was noted that in rats, in which *Strep. mutans* had been implanted after antibiotically induced depression of the normal mouth flora, the amount of plaque and the degree of caries was somewhat lowered by α-1,6 glucan hydrolase, while both parameters were markedly reduced by α-1,3 glucan hydrolase. When applied concomitantly the enzymes had an additive effect, suggesting that α-1,3 as well as α-1,6 linked glucans are significant in plaque formation by *Strep. mutans*.

In the present communication studies in humans are reported, concerning the influence of an enzyme preparation containing α-1,3 glucan hydrolase on the colonization of *Strep. mutans* on the teeth and the development of dental plaque during frequent rinses with a high concentration of sucrose.

## MATERIAL AND METHODS

*Subjects.* Five dental students, aged 20—22 years, known to harbor *Strep. mutans* in the mouth participated in the study. During the six week experimental period they maintained their normal diet, but candy and excessively sucrose-sweetened food was avoided.

*Experimental procedure.* The time table may be seen in Fig. 1. Following a thorough scaling and cleansing controlled oral hygiene measures were instituted for two weeks. After this period oral hygiene was discontinued for seven days, during which the participants rinsed the mouth eight times per day with 10 ml of a 20% solution of sucrose. Rinsing was done for two minutes with two hour intervals from 7 a.m. to 9 p.m. The teeth were then scaled and cleansed again, and oral hygiene was reinstituted for two weeks, followed by a second seven day period without oral hygiene but with rinsing, this time with 10 ml of a 20% sucrose solution containing 0.5 units of α-1,3 glucan hydrolase (see below). All rinsing fluid contained 0.1% sodium methyl-p-oxybenzoate as an antimicrobial agent. After the experiment had been concluded the teeth were scaled and cleansed, 2% NaF was applied topically, and the partipants were instructed to rinse with 0.2% NaF daily for two months to avoid development of caries.

*Plaque scoring.* The amount of plaque was scored by the same examiner at intervals during the whole period according to *Silness & Löe* (1964).

*Enzyme.* The enzyme used was synthesized by a strain of *Aspergillus nidulans*, kindly supplied by Dr. B. J. M. Zonneveld. Cultures were grown for five days at 37°C in air on a special medium (*Zonneveld*, 1972b). Enzyme purification was performed by acetone precipitation after homogenization and centrifugation of the cultures, and the precipitate was dissolved in 0.05 mol/l citrate-phosphate buffer (pH 6.2), containing 0.1% sodium methyl-p-oxybenzoate, and dialyzed against the same buffer (*Zonneveld* 1972 a). The enzyme has been characterized as an exosplitting α-1,3 glucan hydrolase with

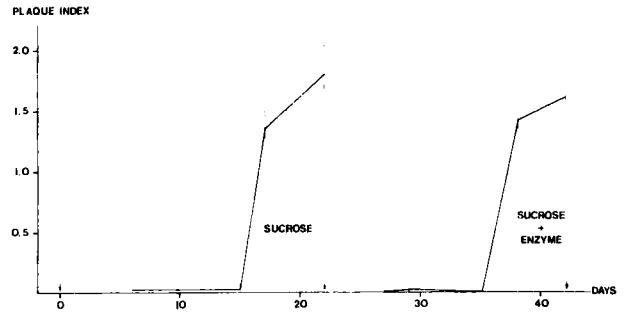


Fig. 1. Plaque index values during the whole experimental period. Rinsing with sucrose took place at day 15—22, with sucrose containing enzyme at day 35—42. The mean estimates are shown, the ranges being represented by vertical bars. Scalings are indicated by arrows.

activity over a rather broad pH range and an optimum at pH 5.0—6.2 (*Zonneveld*, 1972a). It was tested quantitatively against water-insoluble polysaccharides formed by *Strep. mutans* strain GS5 (*Kelstrup & Funder-Nielsen*, 1972), the reaction mixtures containing equal volumes of enzyme and polysaccharide suspensions (2 mg/ml) in 0.05 mol/l citrate-phosphate buffer (pH 6.2). The amount of reducing sugar liberated after 60 min at 37°C was measured as glucose, and one unit was defined as the amount of enzyme capable of liberating 1 μmol/ml of glucose per min in the standard /assay.

*In vitro* plaque (*Gibbons & Nygaard*, 1968) from *Strep. mutans* strains GS5 and Ingbritt was shown to be degraded by the enzyme preparation over a period of 24 h at 37°C. The α-1,3 glucan hydrolase concentration in these experiment was 0.01 unit per ml in 0.05 mol/l citrate-phosphate buffer (pH 6.2). Starch and casein were found also to be degraded by the preparation, but no activity against Dextran T 40 (Pharmacia), containing mainly α-1,6 glucoside linkages (*Lindberg & Svensson*, 1968), could be detected with qualitative methods detailed by *Guggenheim* (1970).

*Bacteriological procedures.* After each period of rinsing dental plaque was collected from all teeth in the same side of both jaws with sterile scalers. The plaque was immersed in 0.1% Bacto-Peptone (Difco) and immediately brought to the laboratory. Homogenization, serial dilution and triplicate plating of $10^{-4}$, $-5$, and $-6$ dilutions were done as described previously (*Kelstrup et al.*, 1970). Tryptone -menadione-blood agar was used to assess total viable counts (*Jensen et al.*, 1968), and the total number of streptococci and *Strep. mutans*, *Strep. sanguis* and *Strep. salivarius* was estimated on Mitis-Salivarious Agar (Difco) and Mitis-Salivarius Agar containing Elkosin® (*Carlsson*, 1968). Colony morphology was utilized to discern the selected streptococcal species (for references to colony descriptions see *Kelstrup et al.*, 1970), and representative colonies were isolated and tested for synthesis of extracellular polysaccharide from sucrose and for fermentation of sorbitol and mannitol.

## RESULTS

*Dental plaque.* From Fig. 1 it is seen that while virtually no plaque could be detected during the hygiene periods, dental plaque accumulated rapidly in the absence of hygiene measures during rinses with sucrose as well as with sucrose containing enzyme. No appreciable difference between the two periods of rinsing was observed.

Table I. *The proportion of selected streptococcal species in human dental plaque subsequent to rinsing with solutions of sucrose or sucrose containing enzyme. Mean percentages of total viable counts are given with ranges in parentheses*

| Rinse | *Strep. mutans* | *Strep. sanguis* | Streptococci (total) |
|---|---|---|---|
| Sucrose | 10.4 (2.9—32.7) | 11.0 (2.0—28.1) | 38.5 (15.8—60.0) |
| Sucrose containing enzyme | <2.2 (<0.3—8.8) | 9.3 (1.2—16.8) | 19.6 (3.9—31.6) |

*Bacteriological findings.* The proportion of *Strep. mutans* of the total viable plaque flora was found to be lower in all subjects after the period of rinsing with a sucrose solution containing enzyme than after the period of rinsing with sucrose only. After the sucrose period the mean proportion of *Strep. mutans* was 10.4% compared to less than 2.2% after the sucrose-enzyme period, an average reduction of more than 75% (Table I). The mean proportion of *Strep. sanguis* was not significantly changed. *Strep. salivarius* was encountered in one subject only, comprizing 0.3% of the total viable plaque flora.

## DISCUSSION

The present data suggest that α-1,3 glucan hydrolase impairs the colonization of *Strep. mutans* in dental plaque, even during frequent rinsing with a concentrated solution of sucrose. In all subjects tested the proportion of *Strep. mutans* was lower after seven days of rinsing with sucrose containing enzyme than after rinsing with sucrose only. Two subjects failed to show *Strep. mutans* in the dilutions used, indicating proportions of <0.8% and <0.3%, respectively, and on the average the proportion was reduced by more than 75 per cent. From this finding it appears likely that water-insoluble glucans, containing a high proportion of α-1,3 linkages, are indeed significant in the colonization of *Strep. mutans* on tooth surfaces, as expected from earlier *in vitro* studies on the aggregation of this organism (*Kelstrup & Funder-Nielsen*, 1972).

In spite of the reduced colonization of *Strep. mutans* dental plaque accumulated rapidly. This was to be expected. Tooth surfaces are colonized by many different microorganisms which aggregate in different ways, the aggregation due to water-insoluble glucans being only one. Incidentally, the plaque scoring method used would not reveal minor reductions of the amount of plaque.

It may be noted that *Strep. mutans* was not completely eliminated from the plaque. This may be due to several factors. The administration of sucrose was frequent and the sucrose concentration was high, both favoring colonization. Furthermore, the enzyme is exo-splitting and its concentration may not have been optimal. The substrate, moreover, is water-insoluble, and under such circumstances the enzyme velocity is a function of the total surface area of the particulate substrate and the area occupied by the enzyme (*Mosbach*, 1972). This means that the

shape of the substrate is highly important, and since α-1,3 glucan is believed to be quite irregular (*Sundaralingam*, 1968; *Rees & Scott*, 1971), it may well be adverse to the activity of the enzyme. Finally, the enzyme is not active towards α-1,6 glucoside linkages, and glucans with this type of bonds predominating have been shown to agglutinate cells of *Strep. mutans* and might induce their colonization on the teeth (*Gibbons & Fitzgerald*, 1969).

It may be concluded from this study that α-1,3 linked glucans appear to be significant for the colonization of *Strep. mutans* in human dental plaque, although they may not be the only polysaccharides leading to colonization of this organism. This corresponds well with previous animal studies (*Guggenheim et al.*, 1972). Under the experimental conditions employed the overall accumulation of plaque was not appreciably altered, indicating the importance of other microorganisms in the formation of human dental plaque.

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