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What is This?
Relation Between the Presence of Supragingival Calculus and Protease Activity in Dental Plaque

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Protease activity was measured in dental plaque collected from patients with or without supragingival calculus. Plaque specimens from the calculus group showed significantly greater protease activity in the presence of 0.05% sodium thioglycollate than did those from the non-calculus group. No significant difference between the two groups was observed in the protease activity without sodium thioglycollate. There was no significant correlation between the age of the patients and the protease activity.


Introduction.

It is generally agreed that proteases play an important role in periodontal disease. One characteristic feature of periodontal disease is breakdown of the tissues, caused, in part at least, by proteolytic enzymes of leukocytes and/or bacteria (Frostell and Söder, 1970; Ishikawa et al., 1972).

Watanabe et al. (1980a) showed a significant positive correlation between the calculus index and the protease activity of human saliva. Further, it has been reported that the subgingival calculus index correlates well with the protease activity in salivary sediment, and the supragingival calculus index shows a significant correlation with the protease in salivary supernatant (Watanabe et al., 1982a). Since the number of epithelial cells, but not of leukocytes, shows a significant positive correlation with the salivary protease activity, the origin of the salivary protease might be epithelial cells. However, since there is a correlation between the calculus index and protease activity, but not between the calculus index and the number of epithelial cells (Watanabe et al., 1980b), a source other than the latter must be postulated.

Calculation formation results from the mineralization of dental plaque, and it is usually covered with metabolically active plaque. Therefore, it is reasonable to consider that the protease activity in dental plaque, rather than salivary protease activity, can be related to the calculus formation. This study was designed to reveal any correlation between protease activity in dental plaque and calculus formation.

Materials and methods.

Subjects. — For this study, we selected 109 patients with periodontal disease who visited our clinic for the first time, 40 men and 69 women (43.1±14.3 yrs, mean age ± S.D.). They were divided into two groups, one with supragingival calculus (calculus group) and the other without supragingival calculus (non-calculus group), on the basis of a clinical examination of their lower anterior teeth. It was confirmed that the subjects in the non-calculus group had not received scaling for at least three months.

Preparation of enzyme solution. — Dental plaque from the calculus group was collected only from supragingival calculus on the surfaces of the lower anterior teeth, and that from the non-calculus group was collected only from the lingual surfaces of lower anterior teeth, between 10 and 11 a.m. The samples were dispersed in a chilled tube containing 2 mL of 0.85% sterilized saline solution with or without 0.05% sodium thioglycollate (mercaptoacetate). Each plaque dispersion was sonicated for 20 sec in an ice bath.

Determination of the enzyme activity. — The protease activity was analyzed by the method of Watanabe et al. (1981), which is a modification of the method of Esumi et al. (1978). Aliquots of the sonicated plaque (0.25 mL) were incubated with 0.25 mL of 5% hemoglobin (bovine hemoglobin, Type IV; Sigma Chemical Co., St. Louis, MO) together with 0.5 mL of 0.2 mol/L phosphate buffer at 37°C for one hr. Then, 1.0 mL of 10% trichloroacetic acid (Nakarai Chemical Co., Tokyo, Japan) was added to the reaction mixture. After filtration, trichloroacetic acid-soluble products were analyzed with fluorescamine (Roche Co., Tokyo, Japan) by the method of Böhlen et al. (1973). One unit of the enzyme was defined as the amount which yielded the same fluorescence as 1 μmol of tyrosine. The specific activity of the enzyme was expressed as units per mg of enzyme protein. The protease activity was measured within five hours of sample collection.

The following buffers were used in the examination of the optimum pH: 0.2 mol/L acetate, pH 5.0; 0.2 mol/L phosphate, pH 6.0–8.0; 0.15 mol/L barbital, pH 8.5–9.0; and 0.2 mol/L carbonate-bicarbonate, pH 9.5–10.0.

Protein measurement. — Protein concentration of the enzyme solution was measured in the residual plaque sonicate by the fluorometric assay, with bovine serum albumin used as a standard (Böhlen et al., 1973).

Results.

The influence of pH. — The influence of pH on the protease activity in pooled plaque from three patients is given in Fig. 1. It shows a maximum activity at pH 8.0; therefore, the enzyme activity in the individual samples was measured using 0.1 mol/L phosphate buffer (pH 8.0).

The frequency distribution of the specific activity in the presence of sodium thioglycollate. — Fig. 2 shows the frequency distribution of the specific activity in the presence of sodium thioglycollate. A higher frequency was observed in the calculus group for specific activities of more than 12 units/mg (p<0.01 by X2-test), which is a mean value of the samples with sodium thioglycollate.

The mean age and the specific activity in the dental plaque. — The Table shows the mean age of the patients and the specific activity in the dental plaque suspension with or without sodium thioglycollate. The highest specific activity of the protease was in the calculus group with sodium thioglycollate. Without sodium thioglycollate, no significant differences were observed between the non-calculus and the calculus groups, with regard to both the age and the specific activity. However, in the presence of sodium thioglycollate, the dental plaque from the calculus group had activity significantly higher than...
did that from the non-calculus group, and also higher than that from the calculus or non-calculus group in the absence of sodium thioglycollate (p<0.01 by ANOVA).

No significant correlation was observed between the age of the subjects and the protease activity in the plaque suspension with sodium thioglycollate (r = 0.19, n = 67, Fig. 3).

Discussion.

Driessens et al. (1985) found that dental calculus of animals having a high salivary pH contains CaCO_{3} as a major component, and suggested that this pH rise is caused by proteolytic activity in which ammonia and amines can be formed. On the other hand, Watanabe et al. (1980a, 1982a) reported that the calculus index correlates well with the protease activity in saliva, and suggested that the role of the protease in calcification of plaque might be to hydrolyze the calcium precipitation inhibitors (Grind and Hay, 1976; Bennick et al., 1979).

We demonstrated correlation between the presence of calculus and the plaque protease activity with sodium thioglycollate (mercaptoacetate). Ideally, each plaque sample should be divided for protease analysis with and without sodium thioglycollate. However, it was sometimes hard to measure the enzyme activity in duplicate because of small samples, especially in the non-calculus group. Thus, the patients were divided into four groups (Table). With sodium thioglycollate, a specific activity of more than 12 units/mg was observed in the calculus group more frequently than in the non-calculus group (X^2-test). The mean value of the specific activity was also significantly higher in the calculus group than in the non-calculus group (ANOVA). Without sodium thioglycollate, no correlation was observed between the presence of calculus and the protease activity. These results suggest that the protease that correlated with calculus was a thiol protease, because sodium thioglycollate protects SH radicals, such as that in cysteine (Kunitz and Yasunobu, 1970). Precipitation of calcium phosphate salts from supersaturated saliva is inhibited by specific salivary proteins or peptides, such as statherin (Hay et al., 1984). A thiol protease in plaque might hydrolyze those proteins, and so precipitation of calcium phosphate from saliva would be accelerated in plaque. The fact that a high calcium concentration has been reported in submandibular saliva from heavy-calculus-formers (Mandel, 1974) is not in conflict with our results and hypothesis. The specific manner in which plaque proteases might operate remains to be established.

Optimum pH of the plaque protease was 8.0 in phosphate buffer with sodium thioglycollate. Whole salivary protease activity shows three peaks of pH dependence, at pH 4.5, 5.5, and 8.5 (Watanabe et al., 1981), and the main source of the enzyme might be epithelial cells and micro-organisms (Tokumoto et al., 1982). Morishita et al. (1981) reported that the optimum pH of the protease in salivary sediment is 7.0 in phosphate buffer, and that the enzyme is activated with mercaptoethanol. The plaque protease in this study seems to resemble the protease in salivary sediment from the results of optimum pH and an activator.

Clinically, it appears that aging is an important factor in the propensity for calculus formation. A triple correlation among
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Fig. 3 — Correlation between the age of the subjects and the specific 
activity of dental plaque protease in the calculus group (○) and the 
non-calculus group (●), r=0.19, n=67.