SHORT COMMUNICATION

Rare earths inhibit the adherence of *streptococcus sobrinus* B13 to glass surfaces

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Introduction

It is generally agreed that dental caries is caused by acid resulting from the action of microorganisms on carbohydrates. The concentration and amount of acid on the surface of the tooth depend on the presence of acidogenic bacteria such as *S. sobrinus*. Many mechanisms are reported to be responsible for the adherence of oral bacteria to tooth surfaces²⁾. The extracellular water-insoluble glucans synthesized by glucosyltransferase (GTase) are important factors involved in the colonization, sucrose-dependent accumulation and adhesion of oral bacteria^{1,3-5)}.

On the other hand, we reported previously that lanthanum is safer than fluorine⁶⁾.

Materials and Methods

Streptococcus sobrinus B13 from our own culture collection was grown in 10 l of sucrose-free TTY-broth medium prepared as described by Hamada and Torii⁷⁾. Cells were grown for 48h at 37°C and collected by centrifugation at 16,000 × g for 20 min (4°C). Half of the precipitate was resuspended in a 0.05M potassium phosphate buffer (pH 6.8 ; KPB) and used as viable cells. The other half of the precipitate was washed with water, followed by lyophilization and kept at 4°C. The lyophilized cells were resuspended in KPB and killed by exposure to 100°C for 10 min, and were used as killed cells.

Crude GTase was obtained from the culture

medium (TTY-broth medium) by 50% ammonium sulfate precipitation. Crude GTase was used in the adherence experiments. The crude enzyme was applied to a DEAE-cellulose column (2 × 10 cm) equilibrated with KPB and eluted with a linear gradient of 0~0.5M NaCl in KPB. Active GTase fractions were combined and used for the inhibition experiment of GTase by rare earths.

The adherence activity of viable or heat-killed *S. sobrinus* B13 was measured according to the method of Mukasa and Slade¹⁾ with the following modifications. In the experiments using viable cells, 4.7 ml of TTY-broth medium including 5% sucrose instead of 1% glucose, 0.2 ml of Ln (2.5×10^{-3} M) and 0.1 ml of cell suspension (10^7 cells/ml) were incubated in a glass tube (12 by 90 mm) for 18h at 37°C. Adherence to glass surfaces was determined by optical density at 530 nm. In other experiments using heat-killed cells, 0.4 ml of heat killed cells-suspension (2.5 mg/ml) was mixed with 0.8 ml of Ln (2.5×10^{-3} M) and 0.8 ml of 0.05M KPB in a test tube. After incubation for 1h at 37°C, Ln-treated cells were collected by centrifugation at 1,600 × g for 15 min. The treated-cells were resuspended in 1.6 ml of KPB including 0.4 ml of 5% sucrose, 50 μ l of crude GTase solution and 5 μ l of 10% thimerosal. After incubation for 18h at 37°C, adherence was determined.

Minimum inhibitory concentration (MIC) was determined by the agar dilution method essentially as described by the Association of Chemotherapy Japan⁸⁾. Inocula were prepared by dilution of 18h broth ($10^7 \sim 10^8$ cells/ml) with buffered saline to $1 \times$

Table Percentage adherence of *S. sobrinus* B13 to glass surfaces and GTase activity on the addition of Ln

	adherence (%)					GTase (%) activity
	viable cells		killed cells			
	nitrate salt		nitrate salt 2 mM	acetate salt		nitrate salt 0.575 mM
	0.1 mM	0.2 mM		1 mM	2 mM	
La	92.1	52.6	24.4	42.2	28.7	100.0
Ce	74.4	51.4	29.3	55.7	47.5	93.5
Pr	75.5	51.9	14.2	25.5	13.9	77.1
Ne	98.1	55.4	12.4	23.2	14.0	100.0
Sm	100.0	64.5	20.8	37.9	14.8	52.9
Eu	—	—	23.1	20.2	16.3	—
Ho	100.0	57.0	24.8	31.8	23.8	55.6
Er	89.0	51.5	24.8	64.7	22.8	62.4
Tm	—	—	—	75.5	24.8	—
Yb	94.4	58.0	26.0	18.2	13.1	86.9
Lu	93.9	44.9	30.0	71.9	24.9	70.7

*Represent a mean for five times experiments.

10^6 colony forming units/ml. Plates containing the brain heart infusion broth (Difco), was inoculated with a loop (i. d. 1 mm). The inoculated plates were incubated for 48h at 37°C in aerobic culture. The MIC was defined as the lowest concentration of Ln in the agar medium that resulted in complete inhibition of visible growth.

GTase activity was measured using [14 C] sucrose (New England Nuclear) as a substrate. 100 μ l of [14 C] sucrose solution (5,000 cpm), 50 μ l of Ln solution (2.3×10^{-3} M), 40 μ l of partially purified GTase and 10 μ l of 1 mM dextran (SIGMA ; average mol wt 8,800) were incubated for 18h at 37°C. After centrifugation for 10 min at $1,600 \times g$, the precipitate was washed twice with 2 ml of water. The washed precipitate was dissolved in 50 μ l of 13M H_2SO_4 ⁹⁾ and transferred to a scintillation vial containing 10 ml of Bray's solution and 1 ml of water. Radioactivity was described by subtracting the value using heat-denatured GTase.

Results and Discussion

Almost all Ln inhibited the adherence of *S. sobrinus* B13 to glass surfaces (Table). Nearly half of the viable bacteria could not adhere to glass by the addi-

tion of 0.2 mmol/l Ln. Pre-treatment of killed cells with Ln (2 mmol/l), resulted in a adherence reduction of 70~85%. At the density of 1 mmol/l of La, Yb, Eu, Ne and Pr inhibited the adherence of killed cells by over 75%. There was little difference between nitrate and acetate salts in the inhibition-effect of adherence. Bacterial growth was inhibited by acetate salts of Pr, Nd and Ho at a concentration of 3.47 mmol/l. Other Ln (nitrate or acetate salts) did not show any inhibition-effect at 6.93 mmol/l (data not shown). In any event, at the density of Ln which inhibited the adherence of bacteria, bacterial growth was not inhibited. Some Ln inhibited GTase activity at the close concentration which inhibited the adherence of bacteria. Sm and Ho inhibited in particular GTase activity (Table). Nevertheless, there was no clear correlation between the adherence inhibition and the inhibition of GTase activity. This data suggest that other inhibition mechanisms are probably involved. We are now researching the effect of Ln on bacterial-agglutination.

In conclusion, almost all Ln inhibit the adherence of *S. sobrinus* B13 to glass surfaces by the inhibition of GTase activity and other inhibition mechanisms, not by the depression of cell growth. Taking into consider-

ation its safety⁶⁾, Ln seems to be useful as anticariogenic agent.

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