Effect of lysozyme on lactate production of *Streptococcus mutans*

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Human whole saliva contains significant quantities of lysozyme, but very few species of oral bacteria, including streptococci, have been shown to be lysed directly by lysozyme¹⁾. Sugishima²⁾ has found an inhibitory factor for the lactate production of *Streptococcus salivarius* ATCC 9758 in the salivary supernatant and has reported that the factor is non-dialysable, heat labile at pH 7 (but heat stable at pH 3) and is similar to lysozyme. Koyama³⁾ has demonstrated that *Streptococcus sanguis* ATCC 10557 treated with hen egg white lysozyme (HEWL) shows a decrease in lactate production without any reduction in turbidity. On the other hand, the lysis of normally resistant streptococci has been achieved through sequential treatment with lysozyme and either detergents or high concentrations of NaCl⁴⁾. Coleman et al.⁵⁾ have shown that many cariogenic and noncariogenic oral streptococci can be lysed by lysozyme and sodium lauryl sulfate (SLS), and loss of viability (colony-forming unit) of *Streptococcus mutans* BHT can be induced by lysozyme alone. The treatment of strain BHT with lysozyme alone also results in extensive degradation of the cell wall as shown under electron microscopy⁶⁾. It is generally accepted that the importance of lysozyme in the oral cavity may not only reside in its lytic potential but also in its bactericidal effects which are not dependent upon cell lysis⁷⁾.

The purpose of this investigation was to compare the effects of HEWL on lactate production among *Streptococcus mutans* strains (serotypes a, b, c and d described by Bratt-hall⁸⁾) in the salivary supernatant (SS),

whose lysozyme was inactivated by heating.

After rinsing the mouth with deionized water, naturally occurring human whole saliva was collected in a test tube. Then the saliva was centrifuged at 12,000 g for 10 minutes. The supernatant was heated at 100°C for 30 minutes and used as a culture medium for *Streptococcus mutans* strains (AHT, BHT, FA1, JC2, PK1, Ingbritt and B13). These strains were inoculated in TEP bouillon and cultured at 37°C for 18 hours. The cells were harvested by centrifugation at 12,000 g for 10 minutes and washed three times with 10 ml of 6 mg/ml NaCl. The washed cells were suspended in the NaCl solution. The optical density of the suspension at 660 nm was adjusted to 0.60 adding the additional NaCl solution. HEWL (Seikagaku Kogyo, Tokyo) was dissolved in 6 mg/ml NaCl so as to make the concentration of 2 mg/ml immediately before use. One milliliter of 4 mg/ml glucose, 0.5 ml of 2 mg/ml lysozyme or of 6 mg/ml NaCl and 0.5 ml of the bacterial suspension (BS) were added to 2.0 ml of SS and incubated at 37°C for 4 hours under aerobic condition (unshaken). The lactic acid produced was determined by the method of Barker-Summerson⁹⁾. The results obtained are shown in Fig. 1. The open bars show the amount of lactic acid produced by each strain in SS during 4 hours of incubation. The shaded bars indicate the lactic acid produced in SS in the presence of HEWL at a final concentration of 250 µg/ml. Among these 7 strains, the lactate productions of serotype b strains, BHT and FA1, were apparently inhibited by the addition of HEWL. However, serotype a

Fig. 1 Production of lactate by a variety of *Streptococcus* mutants strains exposed to lysozyme (250 μ g/ml) in SS.

Fig. 2 Lactate production of *Streptococcus* mutants FA1 in ① control (SS 2 ml, BS 0.5 ml, 4 mg/ml glucose 1 ml, 6 mg/ml NaCl 0.5 ml), ② +HEWL (SS 2 ml, BS 0.5 ml, 4 mg/ml glucose 1 ml, 240 μ g/ml HEWL in 6 mg/ml NaCl 0.5 ml).

strain (AHT), serotype c strains (JC2, PK1, Ingbritt) and serotype d strain (B13) were not affected in their lactate production by HEWL. Fig. 2 shows the lactate production of *Streptococcus* mutants FA1 in SS containing 1 mg/ml glucose and 30 μ g/ml HEWL.

The test mixtures were incubated at 37°C for 2, 4 and 6 hours. When HEWL was added to SS, the lactate production of strain FA1 was reduced during incubation. It is conceivable that lysozyme can inhibit lactate production of some strains at a concentration of physiologic levels in saliva.

Some investigators have observed that serotype b strains are the most susceptible to lytic action of lysozyme, but other strains also lyse well^{5,6}. Our data indicate, however, that only b strains reduced the lactate production ability by lysozyme. The differences may exist in the characteristics of the outer layer, cell wall and cell membrane, among various strains.

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