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Effect of Lysozyme on Glucose Fermentation, Cytoplasmic pH, and Intracellular Potassium Concentrations in *Streptococcus mutans* 10449

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Several previous findings have suggested that the cationic nature of lysozyme is a major factor in its bactericidal activity. Since a number of cationic proteins or peptides have been reported to cause membrane damage in bacteria, we investigated the effect of lysozyme on glucose fermentation and intracellular pH and K^+ in *Streptococcus mutans* under conditions in which lysis does not occur. Results showed that lysozyme and poly-D-lysine (PDL) cause inhibition of glucose fermentation at pH 5.5 in a dose-dependent manner. Human placental lysozyme and hen egg-white lysozyme exhibited similar inhibitory potency on glucose fermentation. Both lysozyme and PDL caused a marked acidification of the cytoplasm of *S. mutans*. However, when cytoplasmic pH was examined as a function of fermentation rate, the relationship was similar regardless of the presence or absence of lysozyme or PDL. Therefore, acidification of the cytoplasm appeared to not depend specifically on lysozyme or PDL. In contrast, the same relationship between the profound loss of intracellular K^+, when fermenting cells were exposed to either lysozyme or PDL, and the fermentation rate was not exhibited in the controls. These results indicate that lysozyme and PDL specifically affected the ability of the cells to maintain intracellular K^+. We concluded that lysozyme and PDL indeed perturb membrane function, perhaps in a selective manner. Furthermore, the similarity in action of lysozyme and the cationic homopolypeptide PDL supports the notion that the cationic property of lysozyme indeed plays a significant role in its antibacterial activity.

Lysozyme is a prominent antibacterial component of human saliva. It is also widely distributed in the tissues, exocrine secretions, and circulating cells of the human body (10, 34, 36) and therefore is considered an important component of the host nonimmune defense against bacteria (48). Lysozyme is a cationic (pI $\approx$ 10.5), low-molecular-weight (14,500) protein that has the ability to lyse certain bacteria by cleaving the β(1-4) glycosidic bond between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine in the polysaccharide backbone of the bacterial cell wall (4). Interestingly, many gram-positive oral bacteria, including *Streptococcus mutans*, are resistant to direct lysis by lysozyme (1, 3, 8, 28). Therefore, lysis need not be the primary cause of cell death by the action of lysozyme.

The nonlytic bactericidal mechanism(s) of lysozyme has been studied extensively but still remains unclear. Several findings suggest that at least part of the bactericidal activity of lysozyme is due to its cationic nature (11, 28, 32, 37, 38, 51, 64). The ability of cationic peptides or proteins, such as Pep 5 (42), nisin (41), and cationic proteins of human granulocytes (10, 18, 33, 48) and rabbit macrophages (35), to kill microorganisms has been recognized for many years. Their antibacterial activity has been attributed to action on the cell membrane that leads to an increase in membrane permeability followed by electrolyte and osmotic changes within the cell (18, 42, 48). Several colicins have a cationic-rich region that is thought to mediate interaction with bacterial membranes (24), resulting in formation of ion-permeable channels (6, 24, 62). Therefore, as a cationic protein, lysozyme may have a mode of bactericidal action similar to other cationic proteins or peptides and bacteriocins which act on the cell membrane and result in selective loss of permeability.

It is well known that bacteria conserve and transduce metabolic energy by means of an electrochemical gradient (13, 14). Bacterial membranes function as a proton barrier and are able to maintain a relatively alkaline cytoplasm in acid media by extruding protons, generally through the membrane-associated proton-translocating ATPase (13, 14, 19, 23, 31). Bacteria also accumulate K^+ and maintain large K^+ gradients across the cell membrane when external K^+ is low (19, 31, 40, 58). Therefore, the inability to maintain optimal K^+ and/or H^+ transmembrane gradients due to the presence of cationic proteins or peptides or of bacteriocins may lead to loss of bacterial viability (18, 24–26, 42, 43).

S. mutans is thought to play a major role in the initiation and progression of dental caries (12, 29) and therefore is an important test microbe in studies of oral host defense mechanisms. The organism is highly acidogenic and is able to decrease the pH below the point where enamel dissolution becomes significant (29). The aim of this study was to investigate the effect of lysozyme on cell membrane permselectivity to H^+ and K^+ in *S. mutans*. Poly-D-lysine (PDL) was used as a reference cationic polypeptide, since unlike lysozyme it does not possess an associated antimicrobial enzymatic activity. Preliminary reports of this work appeared previously (59, 60).

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MATERIALS AND METHODS

Growth and harvesting of bacteria. *S. mutans* NCTC 10449 was stored by lyophilization. Cultures were routinely grown from these lyophilized stocks on blood-agar plates and were never subcultured more than four successive times. Bacterial cells were inoculated into TSYG broth (3% trypticase soy, 0.5% yeast extract, 0.25% dextrose) from blood-agar plates. Exponential- or stationary-phase cells were harvested from anaerobic cultures by centrifugation (8,000 × *g* for 10 min at 4°C) and washed twice with one volume of 2 mM potassium phosphate buffer (pH 7.0), adjusted to an A_{540} of 30, and then held on ice until needed.

Rate of acid production. The rates of acid production by resting cell suspensions fermenting glucose were estimated from the continuously recorded volume of 20 mM KOH delivered by a Metrohn pH stat unit (Brinkmann Co., Westbury, N.Y.) to maintain a constant environmental pH of 5.5. This pH stat system consisted of a 632 pH meter, an automatically controlled titrant delivery unit (614 Impulsomat), an electronic strip chart recorder (Brinkmann BR-100), and a titration assembly with a 5-ml autoburette (665 Dosimat). The reaction mixture contained ca. 390 μg (dry weight) of cells per ml, 2 mM potassium phosphate, 18 mM KCl, and 0.4 mM $MgCl_2$. Reactions were started by the addition of 20 mM glucose and run at 37°C in air. The reaction mixtures were stirred with a magnetic stirrer. The rates of acid production were calculated from the tangents of the titration curves. Controls consisted of bacterial suspensions in the absence of cationic test substance or glucose.

Measurement of pH_i. Cytoplasmic pH (pH_i) was estimated from the distribution of [^{14}C]benzoic acid (1.5 μCi/ml; final concentration, 66 μM) between the extracellular and the intracellular fluids (21, 31, 40). Triplicate 100-μl samples of reaction mixture were filtered through polycarbonate filters (pore size, 0.4 μm; Nuclepore Corp.) to collect the bacterial cells (20). Unwashed filters containing the cells and contaminating extracellular medium were then counted in a scintillation counter (model LS1701; Beckman Instruments, Inc.). Medium trapped on the filter was also determined in each experiment by using [^{3}H]inulin, and the counts obtained were subtracted from the experimental values. The amount of extracellular medium trapped on membranes, per 100 μl of reaction mixture applied, was 0.25 ± 0.05 μl. The radioactivity of the extracellular medium trapped on filters was less than 10% of the total radioactivity in fermenting cells and about 30% in nonfermenting cells. There was little nonspecific binding (about 3%) of the [^{14}C]benzoate probe to fermenting cells as determined by treating the cells with 5% *n*-butanol for 15 min at 37°C (19) or raising the medium pH (pH_o) to >7.5 to reverse the direction of the transmembrane pH gradient (19, 20, 23). The cytoplasmic volume of *S. mutans* cells was estimated by using a silicone oil method (19). In this method, [^{14}C]taurine was used as an extracellular space probe and ^3H_2O was used as a total water space probe. After incubation for 5 min at 25°C, triplicate 1-ml samples were then centrifuged through 0.5 ml of a silicone oil-octane mixture for 3 to 5 min. The cell pellet and supernatant aqueous solution were then counted separately. Strains of *S. mutans* yielded a mean cytoplasmic volume of 1.502 ± 0.145 μl per mg (dry weight) of cells. This value is similar to that (1.6 μl/mg) reported recently for *S. mutans* Ingbritt (46). From the cytoplasmic volume, the concentration of [^{14}C]benzoic acid in the extracellular medium ([A^T out]) and in the cell cytoplasm ([A^T in]), the pH_o, and the pK of benzoic acid (4.19), the pH_i was calculated from the equation $pH_i = \log [(A^T \text{ in}/A^T \text{ out})(10^{pK} + 10^{pH_o}) - 10^{pK}]$, and the transmembrane ΔpH was determined as the difference ($pH_i - pH_o$) (31, 40).

Measurement of cytoplasmic potassium. The internal K^+ content of cells fermenting in the pH stat before and after glucose addition was estimated. Washed cells were resuspended in 2 mM potassium phosphate with 18 mM KCl and 0.4 mM $MgCl_2$. During incubation at a pH_o of 5.5 at 37°C, 0.3-ml samples of the reaction mixtures were filtered through polycarbonate filters and each filter was immersed in 2 ml of 5% trichloroacetic acid and boiled for 5 min (22). After centrifugation at 8,000 × *g* for 10 min, the K^+ content in each extract was then measured with a Jarrell Ash model 82-800 atomic absorption spectrophotometer. The internal K^+ concentration was calculated after correction for contaminating extracellular fluid. If control cells (i.e., with no cationic agents) were washed twice with 5 ml of distilled water, the estimated K^+ loss was 20% in resting cells and 10% in fermenting cells. We did not wash the cells in the experiments reported here to avoid possible major loss of cytoplasmic K^+ from lysozyme or PDL-treated cells.

Chemicals or reagents. Hen egg-white lysozyme (HEWL; crystallized three times) and poly-D-lysine (PDL; molecular weight, 26,300; degree of polymerization [DP], 126) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Human placental lysozyme (HPL) was bought from Calbiochem Corp. (Calbiochem-Behring, La Jolla, Calif.). [*methyoxyl*-^{3}H]inulin and [7-^{14}C]benzoic acid were bought from Dupont, NEN Research Products (Boston, Mass.). All other chemicals were of analytical grade and commercially available.

RESULTS

We reported earlier (28) that the concentrations of lysozyme required to cause greater than 90% loss of viability of several streptococci were in the range of 2 to 10 μg/10^7 cells. We have maintained this relationship between cell numbers and lysozyme concentration in the studies reported below. All studies reported here used cell suspensions of ≈10^9/ml. Note also that salts reduce the effectiveness of lysozyme in inhibition of fermentation, probably because of decreased interactions with the microorganisms (27).

Dose response of *S. mutans* to HPL and HEWL. We have demonstrated that the bactericidal activity of muramidase-inactive lysozyme was at least equal to that of the native molecule (28). Furthermore, HEWL and HPL, which differ about two- to fourfold in specific lytic activity against *Micrococcus luteus* (17, 28, 57, 63), exhibited equal bactericidal activities on a weight basis (28). These observations suggested that the nonlytic bactericidal activity of lysozyme was independent of its muramidase activity and, therefore, might depend on its cationic nature. This issue was further investigated by using glucose fermentation by *S. mutans* 10449 as the assay system. To compare the inhibitory activities of HPL and HEWL on glucose fermentation, lysozymes were serially added to fermenting cells in 2 mM potassium phosphate buffer (pH 7.0). After each addition of lysozyme, the rate of fermentation over a 6-min interval was estimated (Fig. 1). The results suggest equal potencies of HEWL and HPL with either exponential- or stationary-phase cells. In addition, other studies were performed using exponential- and stationary-phase cells in 2 mM potassium phosphate supplemented with 18 mM KCl and 0.4 mM $MgCl_2$. Examination of six independent experiments revealed that the ratio of the dose of HEWL to that of HPL

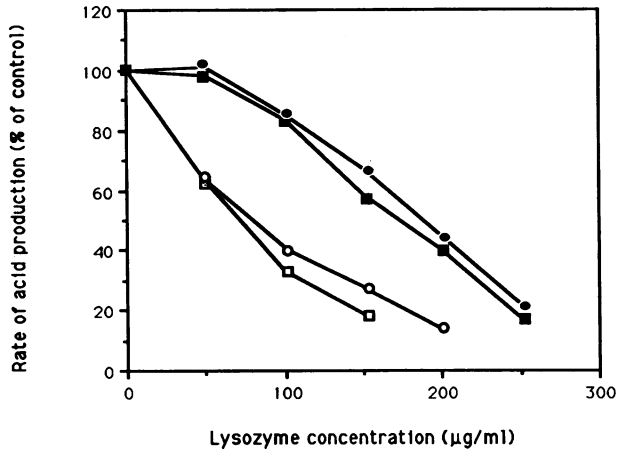

FIG. 1. Comparison of HPL (□, ■) and HEWL (○, ●) inhibition of glucose fermentation by exponential- (□, ○) and stationary- (■, ●) phase *S. mutans* 10449.

required to cause 50% inhibition of fermentation was 1.18 ± 0.18 (standard deviation). Thus, no differences were seen between HPL and HEWL. Finally, viability estimates were obtained by dilution and plating in eight independent fermentation experiments with HEWL and HPL (data not shown). A direct relationship between the percent loss of viability and percent decrease in fermentation rate was found ($y = -0.40 + 0.99x$; $n = 35$ data points; $r = 0.89$). Thus, a given decrease in fermentation rate was accompanied by a corresponding proportional decrease in viability.

Effect of lysozyme and PDL on cytoplasmic pH of *S. mutans* 10449. Fermenting cells were incubated with either lysozyme or PDL, and the cytoplasmic pH and rate of acid production were monitored (Fig. 2). Results showed that the pH_i of *S. mutans* 10449 increased rapidly from the resting value of 6.3 to a maximum of 7.3 when cells were energized with 20 mM glucose (Fig. 2A), followed by a gradual decrease over 80 min. The addition of HPL caused an immediate and rapid decrease in pH_i (Fig. 2A). Similar results were also found with HEWL- and PDL-treated cells (data not shown). The rates of acid production in control cells progressively decreased after 2 min of incubation with glucose (Fig. 2B). HPL-treated cells exhibited an accelerated reduction in fermentation rate relative to the control cells (Fig. 2B). Similar effects on fermentation rate were noted with HEWL- and PDL-treated cells. The data in Fig. 2A and B together exhibit a similar pattern; however, the reductions in fermentation rate appear to precede the de-

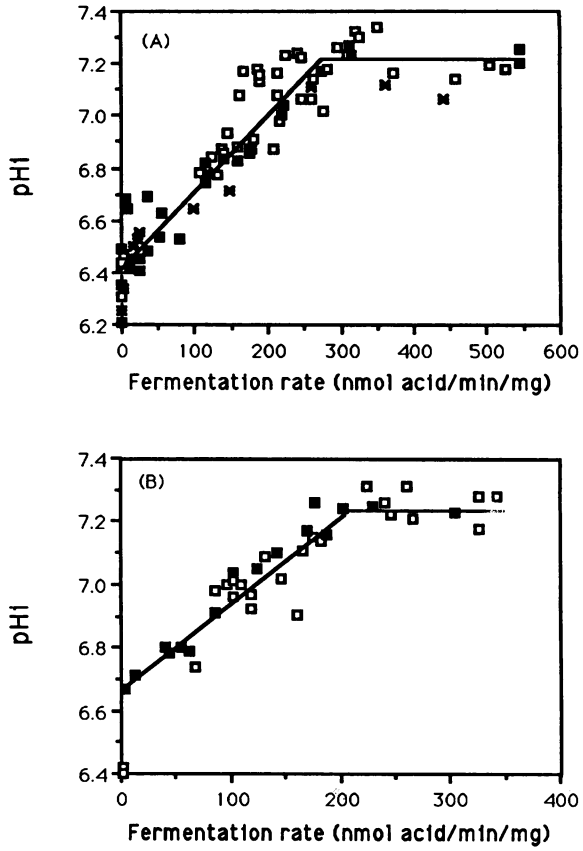

FIG. 3. Relationship between the rate of glucose fermentation and cytoplasmic pH (pH_i) in the presence and absence of lysozyme (A) or PDL (B). See text for details. Symbols: A, control (□), HEWL (■), HPL (×); B, control (□), PDL (■).

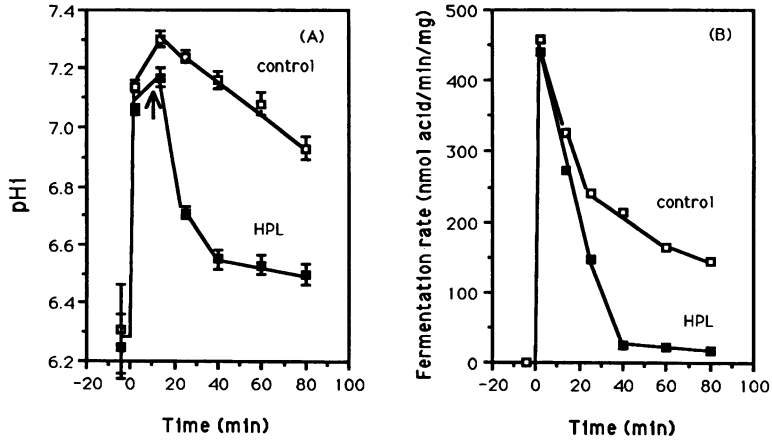

FIG. 2. Effect of HPL on cytoplasmic pH (A) and glucose fermentation (B) in *S. mutans* 10449. Exponential-phase cells were incubated in 2 mM potassium phosphate with 18 mM KCl and 0.4 mM $MgCl_2$. Glucose (20 mM) was added at time zero, and HPL (600 μg/ml) was added at 12 min (arrow). Fermentation was carried out at pH 5.5.

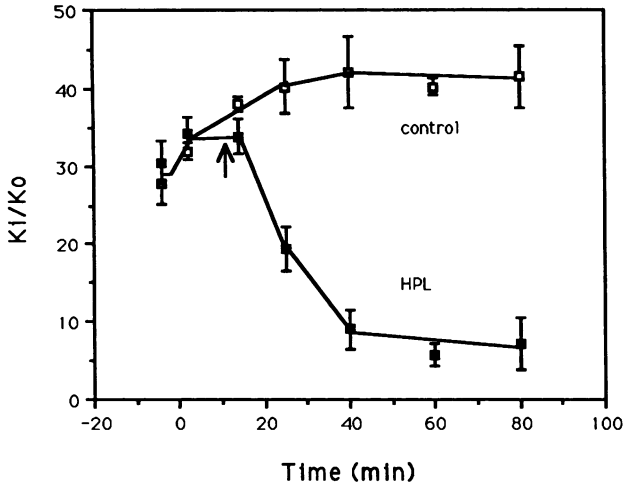

FIG. 4. Effect of HPL on the ratio of cytoplasmic (K_i) to extracellular (K_o) potassium levels in *S. mutans* 10449.

creases in pH_i. Since glycolysis can provide ATP for the proton-translocating ATPase to pump out H^+ (23), decreases in pH_i may be due to reduced amounts of ATP supplied by the fermentation of glucose. We therefore examined the relationship between fermentation rate and pH_i. The results of six lysozyme experiments (two HPL and four HEWL) and four PDL experiments are shown in Fig. 3. The results demonstrate that pH_i and fermentation rate are indeed related. Furthermore, control and lysozyme- or PDL-treated cells exhibit similar relationships. It appears that at fermentation rates below ≈200 to 300 nmol of acid produced per min per mg (dry weight) of cells, the organism is not able to maintain the pH_i near 7.2 to 7.3. Nonfermenting cells treated with HEWL or PDL did not show significant changes in pH_i. For example, control, HEWL-, and PDL-treated cells exhibited a pH_i of 6.03 ± 0.15, 6.09 ± 0.18, and 6.10 ± 0.12, respectively. Since the relationship between the rate of fermentation and pH_i was essentially identical in either the presence or absence of lysozyme or PDL, it appears that neither PDL nor lysozyme had a unique effect on pH_i. That is, neither agent dissociated pH_i from the fermentation rate as determined by a comparison with control cell responses.

Effect of lysozyme and PDL on cytoplasmic K^+ concentration of *S. mutans* 10449. Since no unique effects of lysozyme and PDL on the pH_i of the organism were noted, we examined the effects of these agents on intracellular K^+ levels as a reflection of the membrane potential component of the proton motive force. The results showed that the ratio of cytoplasmic K^+ level (K_i) to extracellular potassium level (K_o) of resting cells was ca. 28 and increased to ca. 45 after incubation with 20 mM glucose for 40 min (Fig. 4). After addition of either lysozyme or PDL, the K_i/K_o ratio dropped rapidly, eventually reaching ca. 5 (Fig. 4). In contrast to the effect on pH_i (Fig. 3), intracellular K^+ concentrations of cells exposed to either lysozyme or PDL did not exhibit the same relationship to fermentation rate as that seen in the controls (Fig. 5). At similar rates of acid production, lysozyme- or PDL-treated cells exhibited marked efflux of K^+. The K_i/K_o ratio of nonfermenting cells dropped from 22 to 5.5 after incubation with lysozyme or PDL (data not shown). Therefore, lysozyme and PDL specifically affect the capacity of the cells to maintain intracellular K^+.

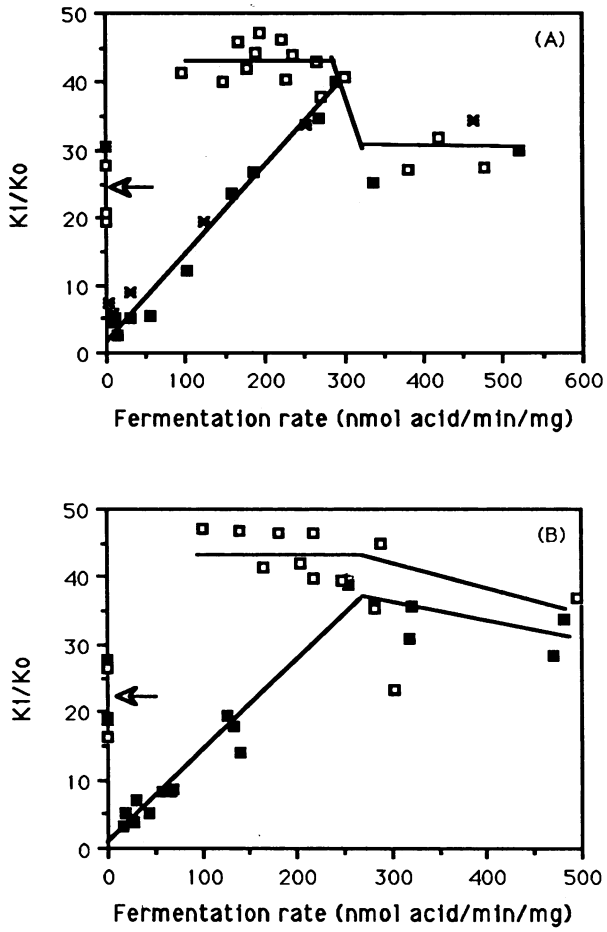

FIG. 5. Relationship between the rate of glucose fermentation and ratio of cytoplasmic (K_i) to extracellular (K_o) potassium levels of *S. mutans* 10449 in the absence and presence of lysozyme (A) or PDL (B). Arrows indicate resting cell suspensions. Symbols: A, control (□), HEWL (■), HPL (×); B, control (□), PDL (■).

DISCUSSION

Streptococci accumulate K^+ into cells against a concentration gradient when energy sources (e.g., glucose) are available (22, 39, 65). This rapid uptake of K^+ may involve an exchange of K^+ for cellular H^+ and/or active transport by K^+ transport systems (7, 58). The cytoplasmic potassium concentration of streptococci varies depending on the conditions of cell growth and preparation, the ionic compositions of washing buffers (46), the metabolic state of cells (i.e., resting or fermenting) (22), the growth phase of cells (exponential or stationary), and the pH of the medium (65). In our experimental conditions, after 5 min of incubation in 2 mM potassium phosphate (pH 5.5) with 18 mM KCl and 0.4 mM $MgCl_2$, the cellular concentration of K^+ of resting cells was 518 ± 48 mM. After incubation with glucose for 40 min, this value increased to 1,062 ± 142 mM. These values are similar to those reported by others for *S. mutans* (400 ± 80 mM) (46) and other streptococci (range of 300 to 450 mM in resting cells and 550 to 900 mM in fermenting cells) (2, 22, 65). Treatment of cells with lysozyme or PDL depleted cellular K^+ to about 100 mM.

The potassium ion is the major cytoplasmic monovalent cation of most bacteria (58). Two important functions of

potassium in bacteria are the maintenance of cell osmolarity and the activation of cytoplasmic enzymes (7, 58). In general, bacteria have the capacity to maintain internal osmolarity at a relatively fixed level above that of the medium (58). This turgor pressure, the difference in osmotic pressure, appears to be needed for bacterial growth. In our study, lysozyme caused a major loss of potassium from *S. mutans*. Loss of K^+ may result in (i) a marked drop in membrane potential since K^+ is the main monovalent cation of bacterial cells, (ii) loss of transport functions dependent on the energized state of the membrane, (iii) inactivation of potassium-dependent cellular enzymes, (iv) loss of turgor pressure, (v) cessation of growth, and (vi) cell death (18, 43, 47, 48). We observed a direct relationship between the decline in viable cells and the decrease in fermentation rate. It is uncertain from this observation, however, whether cell death was (i) a cause or (ii) a result of K^+ loss from the organism.

Several cationic proteins are able to induce a potassium or rubidium ion leak from bacterial cells (33, 45, 53). Cationic Pep 5 and nisin have been shown to render cytoplasmic membranes of *Staphylococcus cohnii* 22 cells permeable to K^+, ATP, and glutamic acid (26, 45), resulting in dissipation of the transmembrane electrochemical potential (42) and inhibition of cellular functions (44). Several colicins which have a cationic-rich C terminus (6) form ion-permeable channels in bacterial cytoplasmic membranes and collapse the membrane proton motive force (6, 26). In the case of colicin E1 (24), Ia (52), and K (62), the membrane potential but not ΔpH collapsed upon treatment of *Escherichia coli*. Our study provides strong evidence that the membrane becomes markedly permeable to K^+ ions but apparently not to protons after treatment with lysozyme or PDL. These results are similar to the colicin E1, Ia, and K studies which did not cause a significant proton flux (24, 52, 62). The basis for K^+ loss without marked changes in pH_i is not known. It has been suggested that the apparent lack of H^+ influx as judged by pH_i estimations may reflect the large cytoplasmic buffering capacity of cells (47). Thus, H^+ fluxes may occur but are of insufficient magnitude to affect the pH_i.

Stationary-phase cells exhibited a twofold-lower sensitivity than exponential-phase cells to lysozyme inhibition of fermentation. This result is consistent with the growth phase-dependent sensitivity of several microbes to Pep 5, nisin, and leukocyte cationic proteins (25, 26, 41, 42, 45, 48). The basis for growth-phase-dependent sensitivity to lysozyme inhibition of fermentation may include differences in cell surface composition (e.g., lipoteichoic acid, peptidoglycan, or capsules) and membrane potential (42, 45, 48). Further studies to elucidate the basis of growth-phase-dependent differences in sensitivity to nonlytic inhibitory effects of lysozyme are in progress.

The relationship between glycolytic rate and cytoplasmic pH observed here with *S. mutans* was also found in *Streptococcus lactis* (30). Further, a simple correlation between glycolytic rate and log(ATP/ADP) was shown with *S. lactis* (30). Fermentation in streptococci provides energy to proton-translocating ATPases and initiates an ionic circulation by coupling H^+ extrusion to ATP hydrolysis (23). As glycolysis of the cells decreases due to depletion of glucose or the presence of inhibitors such as lysozyme or PDL, the supplies of ATP become reduced. This results in decreased H^+ extrusion and an acidification of the cytoplasm. Lower pH_i can further contribute to decreased metabolism through pH-related effects on enzymes (23, 30). Other work from our laboratory (9a) has shown that whole-cell glucose uptake exhibits about a two-pH-unit-greater acid tolerance than does phosphoenolpyruvate-dependent glucose phosphorylation via the phosphotransferase sugar transport system in permeabilized cells (or whole cells treated with carbonyl cyanide *m*-chlorophenylhydrazone which collapses the ΔpH). These data demonstrate the relative pH sensitivity of the glycolytic system in *S. mutans*. Note that over a range of fermentation rates >200 to 300 nmol of acid produced per min per mg of cell (dry weight), the cytoplasmic pH was stable, whereas at rates of fermentation below this range, pH_i decreased. Thus, some threshold rate of fermentation is needed to stabilize pH_i. We (61) and others (54, 55) have observed that lysozyme interferes with glucose uptake by whole cells of *S. mutans*. It seems likely, therefore, that inhibition of glucose transport may be a principal basis for the inhibition of fermentation reported here. This possibility is currently under investigation.

Antibacterial activity of lysozyme has been suggested to be due to aggregation of microorganisms, dechaining of cells, impairment of cellular metabolism, changes of membrane permeability, and bacteriolytic phenomena (1, 4, 15, 28, 37, 38). Note that lysis of our test organism does not occur in our studies. The primary cause of bacterial death by other cationic peptides and proteins has been suggested to involve (i) abolition of the transmembrane electrochemical gradient and, secondarily, (ii) inhibition of active transport, macromolecular synthesis, and membrane function (24–26, 32, 43, 48). We reported earlier (28) that chitotriose inhibited the bactericidal activity of native and reduced (muramidase-inactive) lysozyme and of polylysine. It was postulated, on the basis of these data, that the target of chitotriose was microbe associated and participated in a sequence of events initiated by cationic proteins that resulted in bacterial death. More recently (53a) however, we have shown that micromolar quantities of Fe(III) in chitotriose preparations, and not the chitotriose itself, were responsible for inactivation of the antibacterial action of polylysine (but not of lysozyme). The above model for a generalized mechanism of cationic protein-induced bacterial death, therefore, now seems unlikely. The equivalent bactericidal activity of native and reduced lysozymes (28), however, plus two pieces of evidence from the present study support the idea (16, 27, 28, 32, 37, 38, 51, 64) that the antibacterial activity of lysozyme is mainly due to its cationic properties rather than its enzymatic activity. Firstly, on a molar basis, HPL and HEWL have similar bactericidal and glucose fermentation inhibitory potency, even though HPL has two- to four-times-higher mucolytic activity than HEWL (17, 28, 57, 63). Secondly, lysozyme and PDL have the same effect on the organism in terms of pH_i and intracellular K^+. Therefore, lysozyme functioned just like PDL and other cationic proteins, all of which act on bacterial membranes and deenergize cells (18, 25, 26, 48). The mechanism(s) of cationic protein and nonlytic lysozyme-induced loss of cell viability and function (i.e., the identification of the essential events), however, remains unknown.

Lysozyme concentrations in saliva reportedly vary widely over the range of $\approx$2 to 60 μg/ml (5, 49, 56). The fluid present in dental plaque has been reported to contain up to 15 times the salivary level of lysozyme (5). The results of the present study also indicate that the inhibition of acid production was dependent on HEWL concentration (Fig. 1). Thus, especially in plaque, large quantities of lysozyme are present and in localized areas may exceed the relative quantities (of lysozyme per microbe) used here. Direct evidence of the role of lysozyme intraorally is lacking, although in vitro studies

have demonstrated its potent bacteriolytic potential when incorporated into acquired salivary pellicle (9). Of particular importance is the ionic environment in which microbe-lysozyme interactions take place (27). As noted here, potassium and magnesium reduce the effectiveness of lysozyme as an inhibitor of fermentation. The concentrations of K^+, phosphorus, and Mg^{2+} used here are similar to those reported in saliva (K^+, 14 to 32 mM; phosphorus, 2.0 to 23 mM; and Mg^{2+}, 0.29 to 0.53 mM) and plaque fluid (K^+, ≈62 mM; phosphorus, ≈14 mM; and Mg^{2+}, ≈4 mM) (50). Thus, we anticipate that the inhibition of fermentation and promotion of K^+ loss by lysozyme seen in our studies also occur with plaque microbes in vivo.

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