Arginine Deiminase System and Bacterial Adaptation to Acid Environments

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Received 14 August 1986/Accepted 29 September 1986

The arginine deiminase system in a variety of streptococci and in Pseudomonas aeruginosa was found to be unusually acid tolerant in that arginolysis occurred at pH values well below the minima for growth and glycolysis. The acid tolerance of the system allowed bacteria to survive potentially lethal acidification through production of ammonia to raise the environmental pH value.

The arginine deiminase system provides a source of ATP derived from catabolism of arginine to ornithine, CO\(_2\), and NH\(_3\) in a variety of organisms, including many streptococci and members of the genus Pseudomonas (1). The system is generally inducible and under the control of catabolite repression. It appears to play a role in allowing the predominately aerobic Pseudomonas organisms to grow anaerobically without respiration (12).

We have recently isolated strains of Streptococcus faecium and S. sanguis that have defective catabolite repression (3, 5) and can simultaneously degrade arginine and glucose. Initial assessments of the acid sensitivity of arginine catabolism by these organisms revealed an unexpected degree of acid tolerance, which is also shown by the parent strains. In effect, it appears that arginine can be catabolized at pH values well below the minima for growth or glycolysis.

Organisms chosen for further study include S. faecium ATCC 9790, S. lactis ATCC 19435, S. rattus BHT and ATCC 19645, S. milleri ATCC 9895, S. sanguis ATCC 10556, Challis, and NCTC 10904, and Pseudomonas aeruginosa (ATCC 15442). They were maintained on tryptone-glucose-Marmite (Marmite Ltd., Montreal, Quebec, Canada) agar with weekly transfers and also as lyophilized preparations. Cultures were grown statically at 37°C in tryptone-Marmite medium (9) with added glucose or arginine.

Ammonia produced by the bacteria was assayed first by the Conway microdiffusion method (4) and subsequently with an ammonia electrode (Orion Research, Inc., Cambridge, Mass.) connected to an Orion Ionanalyzer (model 407A) by the procedures recommended by the manufacturer. A pH meter (model 45; Beckman Instruments, Inc., Fullerton, Calif.) connected to a glass electrode was used to determine pH values. Cell dry weights were determined by washing (once with deionized water) cells centrifuged from suspensions or cultures. The washed cells were suspended in deionized water, and a portion of the suspension was placed in a tared aluminum weighing pan and dried to a constant weight at 95°C.

The data presented in Fig. 1 for S. faecium ATCC 9790 indicate that the bacterium can degrade arginine to produce ammonia at nearly constant pH values as low as 3.5. In these experiments, the pH value was held nearly constant by intermittent addition of acid. This minimum pH value for arginolysis is nearly 1.5 pH units below the minimum for growth of the organism in complex medium and nearly 1 unit below the minimum for glycolysis (10). The rate of arginolysis at the low pH value of 3.5 was less than that at pH values of 4.0 or 6.0, but the bacterium could still degrade arginine in acid environments, which have been found to be damaging to the cell membrane, as reflected in loss of magnesium and metabolite pools (9).

FIG. 1. Acid sensitivity of the arginine deiminase system of S. faecium ATCC 9790. Cultures were grown to stationary phase in tryptone-Marmite broth containing 13.9 mM glucose plus 47.5 mM arginine. Cells were harvested by centrifugation, washed with water, and suspended in 20 mM potassium phosphate buffer with 1.0 mM MgCl\(_2\) at the indicated pH values to yield suspensions with 5 mg of cell dry weight per ml. Arginine was added to yield a final concentration of 47.5 mM, and ammonia was assayed by means of an ammonia electrode. HCl solution was added to maintain nearly constant pH. Incubation was at 37°C.

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### TABLE 1. Rise in pH associated with arginolysis

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<th>Time (h)</th>
<th>S. rattus BHT</th>
<th>S. rattus FA-1</th>
<th>S. sanguis NCTC 10904</th>
<th>S. sanguis ATCC 10556</th>
<th>S. faecium Challis</th>
<th>S. lactis ATCC 19435</th>
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<th>P. aeruginosa ATCC 15442</th>
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* Cultures were grown in tryptone-Marmite broth plus 5.6 mM glucose and harvested at the mid-exponential phase. After being washed, the cells were suspended at a density of 5 mg (dry weight) per ml in 20 mM potassium phosphate buffer with 1.0 mM MgCl₂ at a pH of 4.0. Arginine was added to 47.5 mM final concentration, and the suspensions were incubated at 37°C.

Arginolysis by the other bacteria we tested was somewhat less acid tolerant than that by *S. faecium* ATCC 9790. However, each of the organisms was able to degrade arginine at an initial pH of 4.0 (Table 1), or approximately the minimum pH of dental plaque (11). In these experiments, the pH was not held constant but rose to values above 7 as the bacteria produced ammonia. There was not a close correspondence between total ammonia produced and final pH, partly because of differences in buffering by the different types of cells, but mainly because some of the bacteria had storage polymers which can be degraded to produce acid end products. However, in all cases, the capacity to produce ammonia from arginine was greater than the capacity to produce acid from endogenous reserves. In addition, when the bacteria were in growth media, they could start to grow after the pH value had risen to the minimum for growth. In this sort of experiment with rising pH values, *S. faecium* ATCC 9790 could degrade arginine at an extremely low initial pH of 2.5 (Fig. 2) (however, the pH was only this low initially). At 24 h, the pH had risen to nearly 8.0 and the bacteria had increased the NH₃ level to nearly 80 mM. In similar experiments, *P. aeruginosa* could produce ammonia at initial pH values as low as 3.0. The other bacteria tested were less acid tolerant in regard to arginine catabolism, but could produce ammonia at initial pH values of 4.0 or somewhat lower.

For the experiments described in Table 1, cells were harvested from cultures grown in medium with a high level of glucose (55.6 mM) and no added arginine. These cells were repressed for the arginine deiminase system, although with this system repression is not complete (5). The responses of the cells thus included not only initial arginine degradation but also subsequent derepression.

Although extremely acidic environments can be lethal, it appeared that catabolism of arginine with a resultant rise in pH could spare the test organisms. For example, when cells of *S. faecium* ATCC 9790 were suspended in 20 mM potassium phosphate buffer with 1 mM MgCl₂ to give a suspension of 1.9 × 10⁸ cells per ml at a pH value of 2.5, the cells remained viable for 1 h at room temperature (as indicated by counts of spread plates prepared with Trypticase soy agar [BBL, Microbiology Systems, Cockeysville, Md.] at pH 7.0 and incubated at 37°C for 48 h before the colonies were counted). However, after 1 h, there was approximately a 10-fold drop in viable count per h until 6 h, when the count had declined to 2.0 × 10⁷ cells per ml; the pH remained at 2.5. In contrast, when 47.5 mM arginine was added to a companion suspension, there was essentially no decrease in viability over the 6-h period, and the pH rose to 7.9. At an initial pH value of 2.0, the population was completely eliminated after 1 h of incubation without arginine. Although addition of arginine slowed the killing (at 5 h some 1.0⁴ cells per ml remained alive), all of the cells still died. At pH values of 3.0 or 4.0, death occurred more slowly in populations without arginine, with only a 10-fold reduction in numbers after 6 h. Comparable suspensions to which arginine was added were killed under similar conditions.

*Fig. 2.* Ammonia production at low pH values by *S. faecium* ATCC 9790. Cultures were grown in tryptone-Marmite broth plus 55.6 mM glucose and harvested at the mid-exponential phase. Other experimental details are described in the legend to Fig. 1. except that HCl solution was not added to maintain a nearly constant pH.
added showed no reduction in cell numbers and an increase in pH to 7.9 at 6 h.

In conclusion, we found that the arginine deiminase system can play an important role in the acid-base physiology of each of the bacteria studied in that it could allow for recovery from acid stresses sufficiently severe to stop growth and glycolysis. For example, our past work (2, 8) indicated that the minimum pH values for glycolysis by S. faecium ATCC 9790 and S. rattus FA-1 in cultures are 4.4 and 4.8, respectively, while minimum pH values for growth are 4.8 and 5.4. However, S. sanguis NCTC 10904 proved to be less acid tolerant, with minimum pH values of about 5.2 for growth and glycolysis; yet all of these bacteria, and the others tested, were able to carry out arginolysis at a pH of 4.0 and to raise the pH value above neutrality. Kanapka and Kleinberg (7) have found that organisms in salivary sediment degrade arginine optimally at pH values between 7.0 and 8.0 and that there is low-level activity at a pH of 4.0. The mixtures they used contained 2.8 mM glucose, and the sugar may have suppressed arginolysis.

Overall, it seems that streptococci having the arginine deiminase system are adapted to reverse the adverse effects of acid environments. Even when streptococci such as S. sanguis and S. faecium are grown in media with repressive levels of glucose, there still is production of ammonia and degradation of arginine during the stationary phase when the pH value is low (3). This type of derepression is probably best interpreted in terms of reduction in the level of the glucose-specific phosphotransferase system (PTS) at low pH values as shown, for example, by Hamilton and St. Martin (6). Enzyme II of the glucose-PTS appears to be the major effector of catabolite repression, at least in oral streptococci (G. R. Bender and R. E. Marquis, J. Dent. Res. 65:242, abstr. no. 653, 1986). At low pH values, the glucose-PTS is repressed; hence, catabolite repression is reduced and ammonia is produced from arginine. If there is sufficient arginine available, the pH value will rise (Table 1), and glycolysis and growth can be reinstated. Of course, once the glucose-PTS has again started to function, if there is available glucose, ammonia production will be repressed, and arginine can be spared for possible use during another acid stress.

This work was supported by Public Health Service grant RO-DE06127 from the National Institute of Dental Research and grant PO1-DE07003 from the Rochester Cariology Center.

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