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Some Properties of the Autolytic *N*-Acetylmuramidase of *Lactobacillus acidophilus*

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The autolytic *N*-acetylmuramidase present in *Lactobacillus acidophilus* strain 63 AM Gasser has an optimal pH between 5 and 6 when lysing intact cells or isolated cell walls. Cellular lysis at pH 5 is two to four times more rapid in citrate buffer of 0.01 M and 0.5 M or higher than in 0.1 M acetate buffer. It seems that sulfhydryl groups are required for both cell and wall autolysis. Heavy metal ions and *p*-chloro-mercuribenzoate, at low concentrations, are powerful inhibitors. Ethylenediaminetetraacetic acid stimulates cellular but not wall autolysis in acetate buffer to the level obtained in citrate buffer. The possible involvement of sulfhydryl groups in a mechanism of control of cellular autolytic activity is discussed. The autolytic enzyme, although unstable in solution at 37 C, can be extracted from walls by the use of solutions of bovine serum albumin (100 μg/ml) in 0.01 N NaOH. Soluble enzyme extracted from walls rebinds on to sodium decylsulfate-treated walls, but three times as much of the wall material is required to completely re-adsorb the activity.

A number of bacterial species have been reported to contain autolytic enzyme systems which, under appropriate conditions, are able to cause bacteriolysis by the hydrolysis of various susceptible bonds in their cell wall peptidoglycan (3, 8, 15). The presence of such potentially dangerous enzymatic activities in rapidly growing and dividing bacterial cultures has prompted considerable speculation concerning their potential role in cell surface growth and cell division (3, 8, 15). However, thus far little specific information concerning the role of autolysins in these complicated processes is available. In part, this may be due to the fact that most bacterial species examined appear to have associated with their walls autolytic enzyme activities of more than one specificity. Only three bacterial species, *Streptococcus faecalis* ATCC 9790 (10), *Lactobacillus acidophilus* strain 63 AM Gasser (1), and *Arthrobacter crystallopoietes* (5), have been reported to contain autolytic enzymes of only one specificity, and in each case this has been found to be an *N*-acetylmuramidase. Only for the autolytic *N*-acetylmuramidase system of *S. faecalis* is considerable information available. We have therefore undertaken a study of the autolytic enzyme system in the rod-shaped *L. acidophilus* so that the properties of this system can be compared with those of the coccal-shaped *S. faecalis* and as a preliminary for determination of its role in growth and division.

MATERIALS AND METHODS

Organism and medium. *Lactobacillus acidophilus* 63 AM Gasser was grown in a chemically defined medium adapted from three different media described previously for the growth of *Lactobacillus* strains (4, 13, 14) and *Streptococcus faecalis* ATCC 9790 (9). (For composition and preparation of medium, see Table 1.)

Bacterial growth. The growth at 37 C was followed by turbidimetry at 675 nm with a Coleman model 14 spectrophotometer. The cells used in all experiments were harvested before the end of the exponential growth phase (log cells; doubling time, 50 to 60 min) at an optical density (adjusted to agree with Beer's law [9, 16]) of 0.6 to 0.8. Growth was stopped by rapid chilling of the culture at the desired time. Maximal growth in the above medium was reached at an optical density of 2.5.

Cell wall isolation. For this purpose, *L. acidophilus* 63 AM Gasser was grown, in a New

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TABLE 1. *Composition of growth medium*[a]

Ingredients	Amt (per 100 ml of final medium)	Ingredients	Amt (per 100 ml of final medium)
Sodium citrate	22.5 mg	L-Lysine-hydrochloride	11 mg
Glucose	2 g	L-Glutamine	0.5 mg
L-Aspartic acid	10 mg	L-Cystine	20 mg
L-Phenylalanine	10 mg	L-Tyrosine	20 mg
L-Serine	10 mg	L-Asparagine	0.5 mg
L-Proline	20 mg	L-Riboflavine	20 μg
L-Hydroxyproline	20 mg	Vitamin mixture	
Glycine	20 mg	Pantothenic acid	40 μg
L-Leucine	10 mg	*p*-Aminobenzoic acid	4 μg
L-Glutamic acid	30 mg	Thiamine-hydrochloride	20 μg
DL-Alanine	20 mg	Nicotinamide	100 μg
L-Isoleucine	10 mg	D-Biotin	0.5 μg
L-Methionine	10 mg	Folic acid	5 μg
L-Threonine	10 mg	Pyridoxamine	40 μg
L-Arginine	20 mg	Adenylic acid	1.6 mg
L-Histidine	20 mg	Cytidylic acid	5 mg
L-Tryptophane	20 mg	Spermidine-$PO_4 \cdot 3H_2O$	0.5 mg
L-Valine	10 mg	Thymine	0.4 mg
		Deoxyguanosine	0.8 mg
		Adenine	3.5 mg
		Guanine	2.7 mg
		Uracil	3.0 mg
		Sodium acetate	600 mg
		Salt B	
		$MgSO_4 \cdot 7H_2O$	20 mg
		NaCl	1 mg
		$FeSO_4 \cdot 7H_2O$	1 mg
		$MnSO_4 \cdot 4H_2O$	1 mg
		$(NH_4)_2SO_4$	60 mg
		KH_2PO_4	14.75 mg
		K_2HPO_4	30.5 mg
		Tween 80	100 mg
		pH	7.0 (adjusted with KOH)

[a] Most of the ingredients used in the medium were kept as stock solutions in the cold (9). Two reagents (glucose and sodium acetate) were weighed out before mixing the solutions. To avoid precipitation, citrate was added at the beginning, and the purines and pyrimidines and salt B were added last. Tween 80 was added after pH adjustment and sterilization by filtration through a membrane filter (0.45 μm pore size; Millipore Corp.).

Brunswick fermentor, in 10-liter batches of the organic medium previously described (1). The cells were collected before the end of the exponential phase of growth and immediately chilled by pouring onto ice made with distilled water. They were harvested by continuous-flow (300 ml/min) centrifugation in a Lourdes centrifuge, washed twice with cold, distilled water, and lyophilized.

The cells (1 g dry weight per 50 ml of water) were disrupted in a cell fractionator (U. S. Technical Development Co., New York, N.Y.) operated at 14,000 to 15,000 lbs/in^2. The cell walls were isolated by differential centrifugation. They were then washed three times in distilled water, twice in 0.1 M phosphate buffer at pH 7.8, and twice in distilled water. The entire procedure was done at 4 C. Walls were lyophilized and stored in a desiccator at 4 C.

Measurements of lysis of cells and walls. In both cases, measurements were made turbidimetrically. For cellular lysis, log cells were harvested and washed rapidly on a membrane filter (0.45 μm pore size; Millipore Corp.). The cell suspensions were then transferred to tubes containing the appropriate incubation mixtures so that the starting turbidity was at an optical density of 0.4 to 0.5. At appropriate intervals, the turbidities of the tubes were read at 675 nm in the Coleman spectrophotometer.

Cell wall dissolution in 0.05 M citrate buffer (pH 5; reference 1) was measured turbidimetrically at 450 nm in a Bausch and Lomb Spectronic 20 equipped with an A. H. Thomas absorbance digital readout. Absorbance readings were corrected to agree with Beer's law (16). This method was used (i) to measure and compare the rate of dissolution of walls from exponential-phase cells (log walls) under a variety of conditions and (ii) to measure and compare enzymatic activites by using sodium decylsulfate (SDS)-treated log walls as substrate. The SDS treatment

was done by the method of Shockman et al. (12) and resulted in complete inactivation of the wall substrate. Most wall lysis measurements were done starting with an optical density (OD) of 0.5. One OD unit of wall suspension is equivalent to 1 mg (dry weight) per ml. One unit of enzyme activity was defined as the amount causing a loss of 0.001 OD unit of SDS wall suspension per h.

RESULTS

Autolysis of log cells. As previously described for *S. faecalis* (7), *Lactobacillus* cell autolysis curves do not have a constant slope initially (Fig. 1). Before the cellular turbidity began to decrease at a constant rate, a lag period of 60 to 100 min was observed. The length of this lag appeared to depend on the exact stage of growth of the cells and the nature of the buffer in which the lysis was done.

The optimal pH for cellular autolysis was between pH 5.0 and 6.0 when measured in 0.025 M sodium citrate and tris(hydroxymethyl)aminomethane-maleate buffers (Fig. 2). At pH 5.0 it was found that not only was the rate of cellular lysis dependent on buffer concentration, but it also varied with the buffer used (Fig. 3). In sodium citrate buffer, two optimal buffer concentrations were seen: 0.5 M or higher and 0.01 M. On the other hand, in sodium acetate or sodium succinate buffers only one ionic optimum at 0.2 to 0.5 M was seen, and the maximal rate of cellular autolysis (20 to 25% decrease in turbidity per h) was one-half to one-fourth that seen in citrate (53 to 90% decrease per h). All subsequent experiments dealing with cellular autolysis were performed in either 0.01 M citrate or 0.1 M acetate, both at pH 5.0.

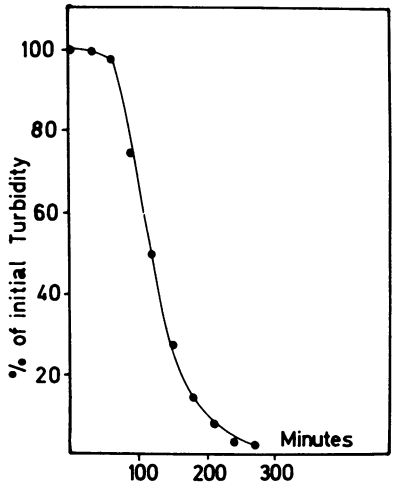

FIG. 1. *Typical lysis curve of log cells in 0.01 M sodium citrate, pH 5. The rate of lysis is measured on the portion of the curve where the rate of loss in turbidity is constant.*

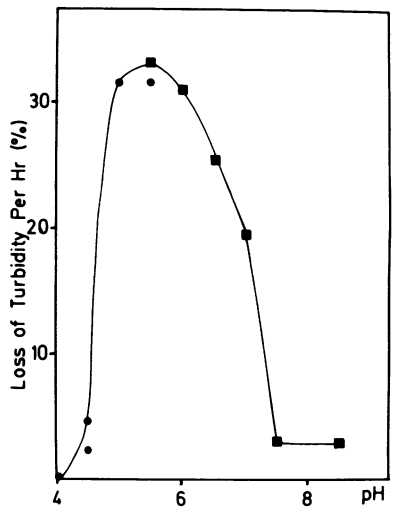

FIG. 2. *Effect of pH on the lysis rate of log cells in sodium citrate (●) and tris(hydroxymethyl)aminomethane-maleate (■), both at 0.025 M.*

It was found that very low concentrations of heavy metal ions inhibited cellular autolysis in both citrate and acetate buffers. Ferric chloride, at 10^{-3} M, reduced the rate of cellular autolysis by 50% in both buffers. A similar inhibitory effect of 10^{-5} M silver nitrate was observed. With copper sulfate, 10^{-3} M was required to inhibit the rate of autolysis by 50%. On the other hand, the divalent cations, Mg^{2+} or Ca^{2+}, failed to affect the rate of cellular autolysis in citrate buffer at concentrations of up to 5×10^{-3} M. Higher concentrations (10^{-2} M) of either ion inhibited the rate of autolysis by 80%.

In citrate buffer, ethylenediaminetetraacetic acid (EDTA) did not affect the rate of cellular autolysis at concentrations up to 10^{-3} M (Fig. 4). Concentrations higher than 5×10^{-3} M sharply inhibited cellular autolysis in citrate. However, in acetate buffer, EDTA even at low concentrations (e.g., 10^{-5} M) increased the autolysis rate by a factor of three, in fact to the rate observed in citrate buffer (Fig. 4). At the highest concentration used, 10^{-2} M, EDTA was again inhibitory.

Reagents which are known to react with sulfhydryl (SH) groups were also tested for their effect on cellular autolysis. Among them, *p*-chloromercuribenzoate (*p*CMB) was the most active inhibitor (Fig. 5). At a 5×10^{-6} M concentration, the rate of cellular lysis was reduced by 50% of its normal value either in acetate or in citrate buffer. Two other reagents, *N*-ethylmaleimide (NEM) and iodoacetate,

were much less powerful inhibitors. The 50% inhibitory doses for cellular lysis were 5×10^{-3} M and 10^{-2} M, respectively. Preincubation of log cells in buffer containing one of these SH group reagents, followed by lysis of the cells in citrate buffer without SH reagents, showed that the autolytic enzyme was inactivated. Cells were incubated for 30 min in 0.01 M ammonium acetate buffer (pH 7, 37 C) in the presence of either 10^{-2} M iodoacetate or 5×10^{-3} M NEM or for 15 min in 0.01 M citrate buffer (pH 5) in the presence of 10^{-4} M *p*CMB. The cells were then sedimented by centrifugation and suspended in 0.01 M citrate buffer, pH 5. Cellular autolysis was inhibited by 0, 50, and 80%, respectively. Moreover, the inactivation of the autolytic activity was reversible. When, after 15 min of preincubation with 10^{-4} M *p*CMB, cells were lysed in citrate buffer in the presence of 10^{-3} M 2-mercaptoethanol, about 50% of the original activity was restored.

Autolysis of log walls. In contrast to log cell autolysis, the slope of the lysis curve for log walls was constant until about 60 to 70% of the wall was solubilized.

The various substances tested for their effect on cellular lysis were also tested for their effect on autolysis of cell walls. Some striking differences in behavior were noted.

In contrast to cellular autolysis, the rate of autolysis of isolated cell walls was the same in both 10^{-1} M citrate and 10^{-2} M acetate buffer at pH 5. High concentrations (10^{-2} M) of Mg^{2+} or Ca^{2+} failed to alter the rate of wall autolysis in citrate. Also, EDTA at 10^{-4} M failed to increased the rate of wall dissolution in acetate buffer. In contrast, both heavy metals (e.g., silver) and *p*CMB inhibited wall autolysis. In

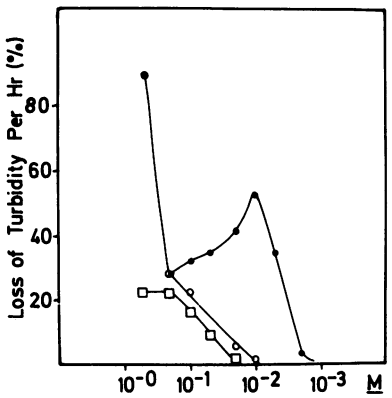

FIG. 3. *Effect of buffer concentration on the lysis rate of log cells in three different buffers at pH 5: in sodium citrate (●); in sodium succinate (○); and sodium acetate (□).*

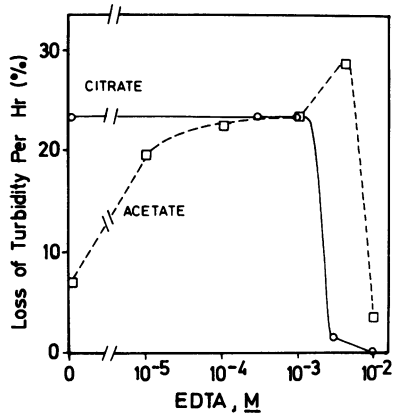

FIG. 4. *Effect of EDTA concentration on the lysis rate of log cells in 0.01 M sodium citrate buffer, pH 5 (○), and 0.1 M sodium acetate buffer, pH 5 (□).*

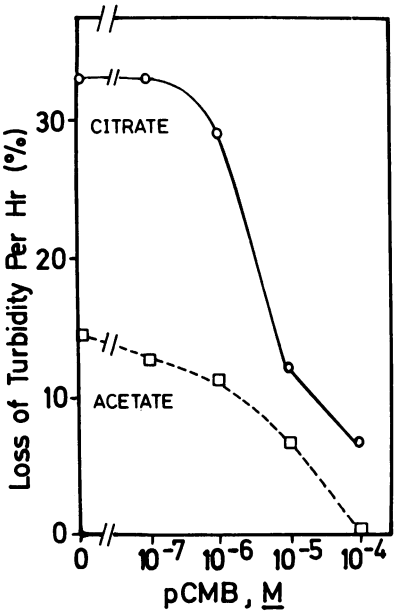

FIG. 5. *Effect of pCMB concentration on the lysis rate of log cells in 0.01 M sodium citrate buffer, pH 5 (○), and 0.1 M sodium acetate buffer, pH 5 (□).*

fact, similar concentrations of both compounds showed the same degree of inhibition of wall autolysis as they did for cellular autolysis. For example, silver at 10^{-4} M completely inhibited wall autolysis in citrate, *p*CMB at 10^{-5} M inhibited wall autolysis by 75% in citrate and 85% in acetate. However, the presence of 2-mercaptoethanol (10^{-3} M) partially neutralized the inhibitory effect of 5×10^{-5} M *p*CMB.

Removal of soluble enzyme from walls. As reported previously (1), autolytic enzyme activity is released into solution as walls undergo dissolution. This differs from the case of

the autolysin of *S. faecalis* which is not released from walls until wall dissolution is virtually complete (12). At any rate, soluble enzyme activity can be removed from the supernatant fluid after wall dissolution. However, when walls were allowed to autolyze for 16 h at 37 C in 0.05 M citrate (pH 5.0), only 20% of the original wall lytic activity could be recovered in the supernatant fluid. It was found that when wall autolysis was performed in the presence of bovine serum albumin (BSA; 100 μg/ml), 80% of the wall lytic activity was recovered. Also demonstrating the protective action of BSA, wall autolysis in the presence of 100 μg of BSA/ml resulted in a 40% increase in the rate of wall dissolution.

Attempts were then made to remove the enzyme from intact walls by extraction with high concentrations of lithium chloride. Such a procedure had been used successfully to recover the *S. faecalis* autolysin (6) and was later used to remove autolytic enzyme activity from walls of *Bacillus subtilis* (2). When walls of *L. acidophilus* were extracted with various concentrations of LiCl (Fig. 6) more than 90% of the autolytic enzyme activity remained with the walls until concentrations in excess of 4 M were used. Higher concentrations inactivated wall autolysis, but wall lytic activity could not be recovered in the supernatant fluids, even when the LiCl extractions were done in the presence of 100 μg of BSA/ml.

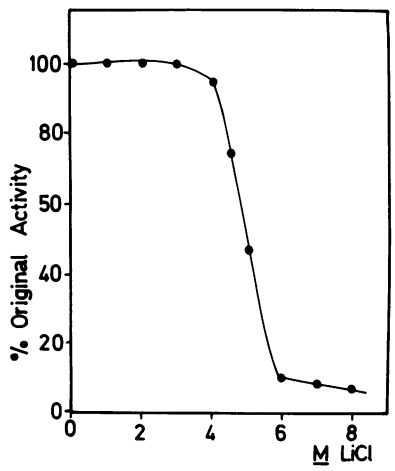

FIG. 6. *Effect of LiCl on the autolytic activity of log cells. Samples of log walls (3 mg) were treated with 3 ml of the various concentrations of LiCl indicated in the figure. After 1 h at 0 C, the tubes were centrifuged (12,000 × g; 10 min). The sedimented walls were washed with water, resuspended in 0.05 M sodium citrate (pH 5), and tested for autolytic activity. The results shown are the average of two experiments.*

Experiments with walls of *S. faecalis* showed that the autolysin could be removed from log walls and activity could be recovered in the supernatant fluid by extraction at pH 12 (A. Wallenstein and G. D. Shockman, unpublished observations). Suspension of log walls of *L. acidophilus* in 0.025 M sodium carbonate (pH 11.5 to 12, 0 C) resulted in a loss of 90% of the lytic activity from the walls, but activity could not be recovered from the supernatant fluids. Extraction with 0.01 M sodium hydroxide at 0 C also resulted in a 90% inactivation of the walls, but in this case 40% of the original activity was recovered in the supernatant fraction (12,000 × *g*; 10 min). Several substances were tested for their ability to increase recovery of enzymatic activity. *N*-acetylglucosamine (2.5×10^{-2} M), glycerol (5%), EDTA (2.5×10^{-3} M), and iodoacetate (2.5×10^{-3} M) all failed to increase recovery of the enzyme. In fact, alkaline extraction of the enzyme was inhibited by 2-mercaptoethanol and $MgSO_4$. When the extraction with 0.01 N NaOH was done in the presence of 2.5×10^{-3} M 2-mercaptoethanol or 5×10^{-3} M $MgSO_4$, 100 and 70%, respectively, of the original autolytic activity remained on the walls. However, extraction of log walls at 0 C in the presence of BSA (100 μg/ml) permitted the recovery of 90% of the original activity in the 12,000 × *g* supernatant fluid. The removal of autolysin from the walls was extremely rapid and complete well within 5 min at 0 C. In every case, to avoid possible denaturation of the enzyme, the alkaline extracts were tested immediately after extraction.

The binding properties of soluble autolysin. Both enzyme recovered from supernatant fractions after alkaline extraction of walls and enzyme recovered from supernatant fractions of wall autolysates were examined for their ability to bind to SDS-inactivated walls. SDS-treated walls were mixed with enzyme in 0.05 M citrate buffer (pH 5), and then after an incubation period at 4 C the mixture was centrifuged at 12,000 × *g* for 10 min. The activity of the enzyme (free or bound to the walls) was determined by rate of wall lysis in 0.05 M citrate buffer, pH 5.

Very little of either type of enzyme extract bound to SDS walls when the mixture was incubated in water for 15 min. In 0.05 to 0.1 M citrate, pH 5 to 6, binding appeared to be rapid. Quantitation of the speed and quanitity of enzyme bound was complicated by the observation that, once removed from the walls by alkaline extraction or wall autolysis, a much larger quantity of wall was required to bind all of the released enzyme. To bind all of the

enzyme activity extracted from 1.5 mg of log walls with 0.01 N NaOH, in the presence of BSA (100 μg/ml), 4.5 mg of SDS walls were required (Fig. 7). This effect does not seem to be related to the alkaline extraction procedure since enzyme activity recovered from an autolysate of 1.5 mg of wall also required an equivalent excess of 4 to 5 mg of walls for complete binding. The effect is also not related to the SDS treatment of the wall substrate since again a similar excess ratio was required to bind alkaline-extracted enzyme to sodium hydroxide-extracted walls. Also, treatment of SDS-inactivated walls with Pronase (500 μg/ml, 16 h) failed to affect the excess of wall required to bind the enzyme.

The binding of autolysin to SDS-treated walls does not seem to be easily reversible. Washing 1.5 mg of walls with 1.5 ml of 0.1 M citrate buffer, pH 5, failed to remove the activity. Water was not used for the washings in these experiments since log walls washed in water lost activity, and enzyme was not recovered in the washings.

Cellular localization of autolysin activity. With mechanically broken cells of *S. faecalis*, virtually all of the autolysin was found in the cell wall fraction (12), and the *S. faecalis* walls have been shown to have an excess of autolysin binding sites (10). Since *L. acidophilus* walls have an apparently limited number of binding sites, it was of interest to obtain some information concerning the cellular localization of its autolysin. In this regard, it was found that when cells were broken in the presence of 0.1 M sodium phosphate, pH 7.8, an increase in autolytic activity of the isolated walls of four- to fivefold that of walls isolated from cells broken in distilled water was observed. For example, walls obtained from disruption in phosphate autolyzed at a rate of 33%/h compared with 8.3%/h for walls isolated from cells disrupted in distilled water. This could be due to the presence in the cells of an excess of autolytic activity, not located in the wall fraction, which can bind the walls during cell disruption in phosphate buffer or to a protection of autolytic activity by the buffer. The first premise seems more likely since exposure of SDS walls to the 12,000 × *g* soluble fraction of the cell resulted in the lysis of SDS-treated walls. The activity found represented about 25% of the total activity recovered in both soluble and insoluble fractions.

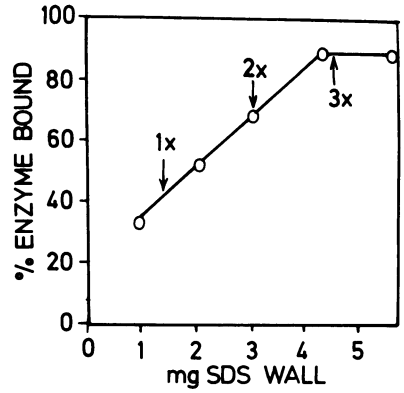

FIG. 7. *Effect of wall concentration on the binding of autolytic activity. Autolytic activity was extracted from 1.5 mg of log walls by 150 μliters of a solution of BSA (100 μg/ml) in 0.01 N NaOH. The activity bound is expressed as percent total activity present in the extract. The binding was done in 1.5 ml of 0.05 M sodium citrate buffer, pH 5, for 15 min at 0 C. The mixture was then centrifuged, and both the activity bound to the wall pellet and that left in the supernatant fluid were measured. The arrows (1×, 2×, 3×) indicate wall concentration relative to the 1.5 mg of wall initially extracted.*

DISCUSSION

Similar to *S. faecalis*, which has an optimal pH for cellular autolysis at pH 6.5, *L. acidophilus* undergoes cellular autolysis most rapidly at pH 5 to 6, where it grows well. Again similar to *S. faecalis* but in a different buffer (citrate rather than phosphate) and at different buffer concentrations, cells of *L. acidophilus* autolyze most rapidly at two different buffer concentrations, 0.01 M and 0.5 M or higher.

It seems that SH group(s) are important for autolysis of both cells and walls of *L. acidophilus*. This does not seem to be the case for *S. faecalis* (12), and similar information does not seem to be available for other autolytic systems. Several types of experiments are consistent with this interpretation. Heavy metal ions and *p*CMB at low concentrations strongly inhibited autolysis of both cells and isolated walls. Also, the *p*CMB inhibition of either cellular or wall autolysis was at least partially reversed by the presence of 2-mercaptoethanol during autolysis. The stimulation of cellular autolysis, but not wall autolysis, by EDTA in acetate buffer, as well as the stimulating effect of citrate buffer itself could also be related to the proposed requirement for free SH groups for activity of the autolytic enzyme. Moreover, cellular autolysis is a complex phenomenon dependent not only on the rate of hydrolysis of susceptible bonds but also on a multitude of factors which may be related to the osmotic stability of the cells. Thus, the stimulating effects of EDTA or citrate on cellular autolysis might not be due to a direct

effect on the activity of the autolysin. On the other hand, in cells, chelation could easily remove organic or inorganic cations bound to SH groups of the enzyme. Such cations would tend to be lost from the wall-bound enzyme during washing of the isolated walls.

Attempts to demonstrate the presence of a latent form of the *L. acidophilus* autolysin activated by a proteinase as that found in *S. faecalis* (7) have been unsuccessful (1). The presence of one or more sulfhydryl groups, which can combine with a small molecule to form an enzymatically inactive complex, would provide a mechanism for the regulation of cellular autolytic enzyme activity. Such a type of regulatory mechanism could be responsible for the earlier observation that, although stationary phase cells are resistant to autolysis, walls isolated from such cells readily autolyze (1).

The *L. acidophilus* autolysin seems to be less stable to heat and to high salt concentrations than is the *S. faecalis* enzyme. Whereas the *S. faecalis* enzyme appears to be stable at 37 C for 17 h (12) and can be recovered from 5 M LiCl extracts of walls (6), the *L. acidophilus* enzyme is much more sensitive to both treatments. In fact, since 6 M LiCl inactivates walls but enzyme cannot be recovered from the supernatant fluid, it is not possible to tell whether the enzyme is released from the walls under these conditions or is simply inactivated when still bound to the walls. BSA (100 μg/ml) protects the *L. acidophilus* autolysin from inactivation at 37 C and brief exposure to sodium hydroxide (0.01 N) but not from exposure to LiCl. In this respect, the survival of the enzyme at high pH is particularly remarkable.

A considerable fraction (perhaps more than 25% of the total activity) of the autolysin of *L. acidophilus* does not seem to be wall-bound in vivo. More activity (90 units of enzyme/mg) was found on the wall fraction of cells broken in the presence of 0.1 M sodium phosphate, pH 7.8, than found on walls of cells broken in water (20 to 28 units/mg). It appears that either the enzyme was more efficiently bound to walls during cell disruption in phosphate or that the activity was more stable under these conditions. The former seems more likely since over 25% of the total autolysin activity was found in the non-wall fraction after cell disruption in phosphate.

The requirement for a threefold excess of wall to rebind either alkaline-extracted or autolysate enzyme is difficult to explain. One can postulate some sort of conformational change to the enzyme protein which allows it to rebind to only some sites on the wall or only with a lower efficiency. Extending the time period of binding does not appear to increase the binding efficiency. Alternately, it is possible that both alkaline extraction or autolysis of walls also release some compound which interferes with the binding of the enzyme to the substrate. This problem will require further study particularly with more purified enzyme preparations.

Thus it seems that, although both *S. faecalis* and *L. acidophilus* possess autolysins of the same enzymatic specificities, several properties of the two systems differ significantly. The results suggest that the two autolytic systems have entirely different systems of regulation.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Coyette, J., and J. M. Ghuysen. 1970. Wall autolysin of *Lactobacillus acidophilus* strain 63 AM Gasser. Biochemistry **9**:2952–2955.
2. Fan, D. P. 1970. Cell wall binding properties of the *Bacillus subtilis* autolysin(s). J. Bacteriol. **103**:488–493.
3. Higgins, M. L., and G. D. Shockman. 1971. Procaryotic cell division with respect to wall and membranes. CRC Crit. Rev. Microbiol. **1**:29–72.
4. Holden, J. T., and N. M. Utech. 1967. Effect of biotin, pantothenic acid and nicotinic acid deficiencies on amino acid transport in *Lactobacillus plantarum*. Biochim. Biophys. Acta **135**:517–531.
5. Krulwich, T. A., and J. C. Ensign. 1968. Activity of an autolytic *N*-acetylmuramidase during sphere-rod morphogenesis in *Arthrobacter crystallopoietes*. J. Bacteriol. **96**:857–859.
6. Pooley, H. M., J. M. Porres-Juan, and G. D. Shockman. 1970. Dissociation of an autolytic enzyme-cell wall complex by treatment with unusually high concentrations of salt. Biochem. Biophys. Res. Commun. **38**:1134–1140.
7. Pooley, H. M., and G. D. Shockman. 1969. Relationship between the latent form and the active form of the autolytic enzyme of *Streptococcus faecalis*. J. Bacteriol. **100**:617–624.
8. Rogers, H. J. 1970. Bacterial growth and the cell envelope. Bacteriol. Rev. **34**:194–214.
9. Shockman, G. D. 1963. Amino acids, p. 567–673. *In* F. Kavanagh (ed.), Analytical microbiology. Academic Press Inc., New York.
10. Shockman, G. D., and M. C. Cheney. 1969. Autolytic enzyme system of *Streptococcus faecalis*. V. Nature of the autolysin-cell wall complex and its relationship to properties of the autolytic enzyme of *Streptococcus faecalis*. J. Bacteriol. **98**:1199–1207.
11. Shockman, G. D., M. J. Conover, J. J. Kolb, P. M. Phillips, L. S. Riley, and G. Toennies. 1961. Lysis of *Streptococcus faecalis*. J. Bacteriol. **81**:36–43.
12. Shockman, G. D., J. S. Thompson, and M. J. Conover. 1967. The autolytic enzyme system of *Streptococcus faecalis*. II. Partial characterization of the autolysin and its substrate. Biochemistry **6**:1054–1064.
13. Soska, J. 1966. Growth of *Lactobacillus acidophilus* in

the absence of folic acid. J. Bacteriol. **91**:1840–1847.
14. Soska, J., and K. G. Lark. 1966. Regulation of nucleic acid synthesis in *Lactobacillus acidophilus* R-26. Biochim. Biophys. Acta **119**:526–539.
15. Thompson, J. S. 1971. Role of autolytic enzymes in the growth and morphogenesis of bacterial cell walls. J. Theoret. Biol. **33**:63–75.
16. Toennies, G., and D. L. Gallant. 1949. The relation between photometric turbidity and bacterial concentration. Growth **13**:7–20.