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Autolysis of Microbial Cells: Salt Activation of Autolytic Enzymes in a Mutant of *Staphylococcus aureus*

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The effect of various salts on the autolysis of cell wall of a ribitol teichoic acid-deficient mutant of *Staphylococcus aureus* H (strain 52A5 carrying *tar-1*) was compared with the parent strain. In the presence of high concentrations of certain salts such as 1.0 M NaCl, the mutant undergoes autolysis with the release of osmotically sensitive spheroplasts. The parent strain is not affected by these conditions. The stimulation of lysis is related to an activation of *N*-acyl-muramyl-L-alanine amidase.

Autolysis of cell walls has been implicated in the regulation of the bacterial cell surface and cellular division. This contention is supported by studies which demonstrate that the zone of lysis is confined to the newly synthesized cell wall in the streptococcal model system (18, 31). In other organisms, such as *Bacillus subtilis*, there appears to be an interaction between teichoic acid and the autolysin(s) which modifies the enzymatic activity (2). More recently, Boylan et al. have noted a marked decrease in autolytic activity in a temperature-sensitive Rod^- mutant which is deficient in teichoic acid (1).

A difference in distribution of autolysin was noted earlier by Chatterjee et al. in a ribitol teichoic acid-deficient mutant (*tar-1*) of *Staphylococcus aureus* H (6). In this strain (52A5) there was a greater localization of autolysin in the cell walls of the mutant strain than in the wild type; however, the mutant cell walls lysed more rapidly. Because the mutant did not grow in clumps if the strain was grown in 1.0 M NaCl, we hypothesized that NaCl influenced the autolysis of whole cells. The data presented demonstrate that high concentrations of certain salts activate the *N*-acyl-muramyl-L-alanine amidase bound to the cell wall producing focal cleavage of the cell wall with the release of osmotically sensitive spheroplasts.

MATERIALS AND METHODS

Bacterial strains and growth medium. The isolation and characterization of the streptomycin-resistant parent strain *S. aureus* H (*str*) and the derived phage-resistant, teichoic acid-deficient mutant, *tar-1*, have been described previously (5). Stock cultures were maintained on Brain Heart Infusion agar (BBL) slants and transferred every 3 weeks. The growth medium (PYK broth) consisted of 0.5% Phytone (BBL), 0.5% yeast extract (Difco) and 0.3% K_2HPO_4 adjusted to *p*H 7.2 with 4 N HCl. After autoclaving, glucose (0.2% final concentration) was added. In some studies PYK medium contained 1.0 M NaCl.

Autolysis of whole cells. Cells were shaken at 100 rev/min overnight in 50 ml of PYK medium at 36 C. In the morning, an 8% inoculum of the overnight culture was placed in fresh PYK medium and grown at 230 rev/min to late logarithmic phase (absorbance of 2.40 to 2.60 at 585 nm). Growth was monitored turbidimetrically (585 nm, 1.0-cm light path) with a Beckman Acta III spectrophotometer. Samples (6 to 7 ml) from exponential-phase cultures of 1.0×10^9 to 1.7×10^9 cells/ml were harvested by centrifugation at $2,000 \times g$ for 4 min, washed twice in potassium phosphate buffer (0.05 M, *p*H 7.0), resuspended in the appropriate autolysis buffer to a cell concentration producing an absorbance ranging from 6.00 to 7.00 at 585 nm, and incubated at 37 C. Samples (0.5 ml) were removed at zero time and at 30 min intervals. Absorbance was measured after 10-fold dilution in distilled water to eliminate the osmotic protection of 1.0 M NaCl present in the autolysis buffer. Cells damaged by autolysis lysed immediately upon dilution. The zero-time absorbance of diluted cell suspensions ranged between 0.560 to 0.680 and was designated 100%. Subsequent absorbance decreases due to autolysis were calculated in terms of percent decrease from the zero-time value for absorbance. The logarithm of percent of initial turbidity was plotted versus time of incubation to determine the rate and extent of autolysis. The standard autolysis buffer was either potassium phos-

phate or tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (0.05 M, pH 7.0) containing 1.0 M NaCl. To determine viability during autolysis, samples of autolyzing cell suspensions were diluted in either distilled water or 1.0 M NaCl and appropriate dilutions were plated on nutrient agar (Difco) with and without 1.0 M NaCl. The plates were incubated for 48 hr at 37 C.

Electron microscopy of whole cells. Logarithmic-phase cells grown in PYK medium with or without 1.0 M NaCl were first fixed at 5 C for 60 min in 0.1 M cacodylate-hydrochloride buffer, pH 6.8, containing 2.5% glutaraldehyde and 0.01 M $CaCl_2$. The cells were sedimented and washed in Kellenberger Veronal-acetate buffer, pH 6.1 (22). Cells were then fixed at 5 C for 14 hr in Kellenberger buffer containing 1% osmic acid, pre-embedded in Kellenberger buffer containing 2% agar and soaked in 0.5% uranyl acetate solution for 2 hr at 25 C prior to dehydration in ethanol. After dehydration the cells were embedded in Epon 812. Examination and photography were performed with a Phillips electron microscope.

Synthesis of cell walls and proteins. Washed early-logarithmic-phase cells were suspended in PYK medium (1.5×10^8 to 2.0×10^8 cells/ml) and shaken at 37 C. After 5 min of incubation a radioactive amino acid, either ^{3}H-L-lysine (0.1 μCi/ml) or ^{14}C-glycine (0.04 μCi/ml, New England Nuclear), was added. At suitable intervals, 1.0 ml of culture was pipetted into cold 10% trichloroacetic acid, and incorporation into protein and peptidoglycan was measured after the Park and Hancock method of fractionation (29). Radioactivity was measured in a Beckman LS 230 scintillation counter by using a toluene base scintillation fluid containing Triton X-100.

Preparation and analysis of cell walls. Mutant cells were grown to logarithmic phase in 2-liter flasks containing 500 ml of PYK medium, with or without 1.0 M NaCl, and harvested by centrifugation at 10,000 × g for 10 min. Cell walls were isolated by mechanical disintegration in a Braun homogenizer as described previously (5). Walls used for autolysis experiments were prepared similarly except that the boiling and trypsin treatments were omitted (6). In some experiments the cell wall polymers released from autolyzing whole cells were also isolated. Mutant cells were grown to logarithmic phase in 800 ml of PYK medium containing 1.0 M NaCl, harvested by centrifugation at 10,000 × g for 5 min, washed in Tris-hydrochloride buffer (0.05 M, pH 7.0), split into two samples (140 ml each) and autolyzed in Tris-hydrochloride buffer, with or without 1.0 M NaCl. Samples were removed at appropriate intervals and sedimented at 10,000 × g for 10 min. The supernatant fluids were dialyzed at 4 C against distilled water, lyophilized, and stored at −20 C.

The qualitative and quantitative analyses of *N*-terminal amino acids were determined by the procedure of Ghuysen et al. (14). Reducing groups were determined by the method of Thompson and Shockman (37). Cell walls were hydrolyzed by 6 N HCl at 100 C for 12 hr for examination with a Technicon TSM amino acid analyzer. Results were computed from a standard containing the wall amino acids and amino sugars that had been heated with HCl under identical conditions.

RESULTS

Effect of NaCl on autolysis of intact cells. Preliminary experiments indicated that *tar-1* grew as individual cells rather than in clumps when the PYK medium contained 1.0 M NaCl. Therefore, we investigated the effect of NaCl on resting cells by suspending them in buffer containing NaCl and determining the rate of autolysis. A dramatic difference in both the rate and extent of autolysis resulted when *tar-1* was incubated in the presence of 1.0 M NaCl (Fig. 1), whereas no significant difference was found with wild type cells. Tris-hydrochloride and potassium phosphate buffers (0.05 M, pH 7.0) gave the same results. The rate and extent of lysis of *tar-1* was dependent on the concentration of NaCl (Fig. 2) with an optimum at 1.0 M. Greater molarities of NaCl did not produce further increases in the rate or extent of autolysis.

The enhancement of autolysis by NaCl was reproducible, but the amount of lysis varied

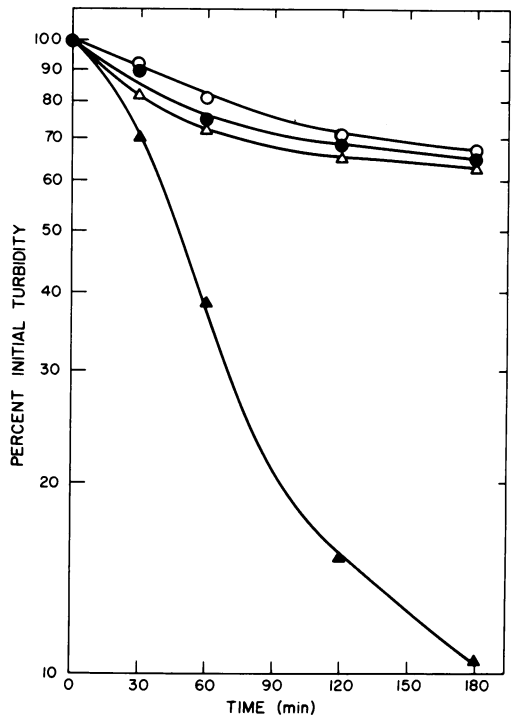


FIG. 1. *Effect of NaCl on the autolysis of Staphylococcus aureus H (str)* (○, ●) *and mutant tar-1* (△, ▲). *Cells were incubated at 37 C in Tris-hydrochloride buffer (0.05 M, pH 7.0) with* (●, ▲) *or without* (○, △) *1.0 M NaCl.*

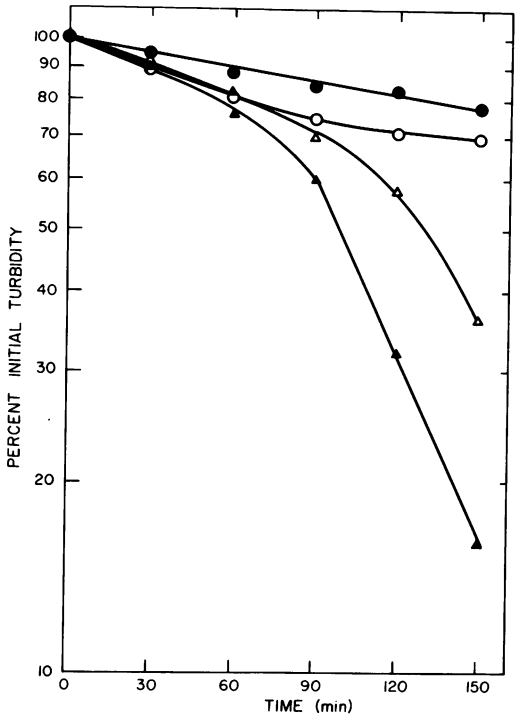


FIG. 2. *Dependence on NaCl concentration of autolysis of tar-1. Cells were incubated at 37 C in 0.05 M Tris-hydrochloride buffer, pH 7.0 (●), and in buffer containing 0.25 M (○), 0.5 M (△), or 1.0 M NaCl (▲).*

with different preparations of cells (cf. Fig. 1 and 2). Lysis was more pronounced and reproducible if *tar-1* was grown in PYK medium containing 1.0 M NaCl. The optimum *p*H and temperature for NaCl-induced lysis of wild type and *tar-1* were 6.5 to 7.0 and 37 C, respectively.

Salt specificity. There was some specificity in the type of salt which induced lysis of *tar-1*. Tris-hydrochloride buffers (0.05 M, *p*H 7.0) containing 1 M solutions of NaCl, KCl, NaBr, and NH_4Cl each stimulated autolysis to the same extent. Equivalent and higher molarities of $NaNO_3$, Na_2SO_4, $(NH_4)_2SO_4$, NH_4NO_3, LiCl, urea, sucrose, and divalent cations, such as $MgCl_2$, were inactive.

$MgCl_2$ has been reported to protect a marine bacterium from NaCl-induced lysis (9). A similar effect was noted in *tar-1* when $MgCl_2$ was added to the standard autolysis buffer (Fig. 3). The inhibition of lysis increased with increasing concentrations of $MgCl_2$ until complete inhibition at 1.0 M $MgCl_2$. To determine whether this inhibition was reversible, *tar-1* was preincubated for 30 min at 37 C in Tris-hydrochloride buffer containing 1.0 M $MgCl_2$, washed twice in buffer, and incubated at 37 C in standard autolysis buffer (0.05 M Tris-hydrochloride plus 1.0 M NaCl, *p*H 7.0). No lysis occurred during 120 min of incubation, suggesting that Mg^{2+} inhibition was irreversible.

LiCl can dissociate autolysin from cell walls of *S. faecalis* (30) and *B. subtilis* (10). Because LiCl did not enhance autolysis of *tar-1*, the possibility of removal of autolysin by the salt was investigated. LiCl (0 to 4.0 M) added to standard autolysis buffer adversely affected the lysis of *tar-1* (Fig. 4). The inhibition increased with LiCl concentration, and no autolysis was observed in the presence of 4.0 M LiCl. Because this inhibition was reversible by dilution of the LiCl or washing the cells, it is unlikely that the inhibition by LiCl was merely due to dissociation of the autolysin.

Requirement for NaCl. The requirement for the continued presence of NaCl to achieve lysis of *tar-1* was studied by incubating the cells for 60 min at 4 C (a condition which does not permit lysis), washing the cells to remove NaCl, and resuspending them in various

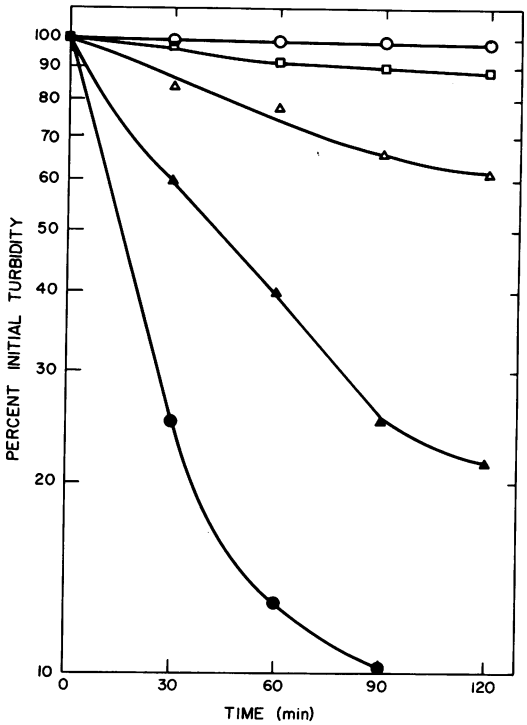


FIG. 3. *Inhibition of lysis of tar-1 by $MgCl_2$. Cells were autolyzed in Tris-hydrochloride buffer alone (○) and in buffer containing 1.0 M $MgCl_2$ (□), 1.0 M NaCl (●), 1.0 M NaCl + 0.5 M $MgCl_2$ (△), and 1.0 M NaCl + 0.12 M $MgCl_2$ (▲).*

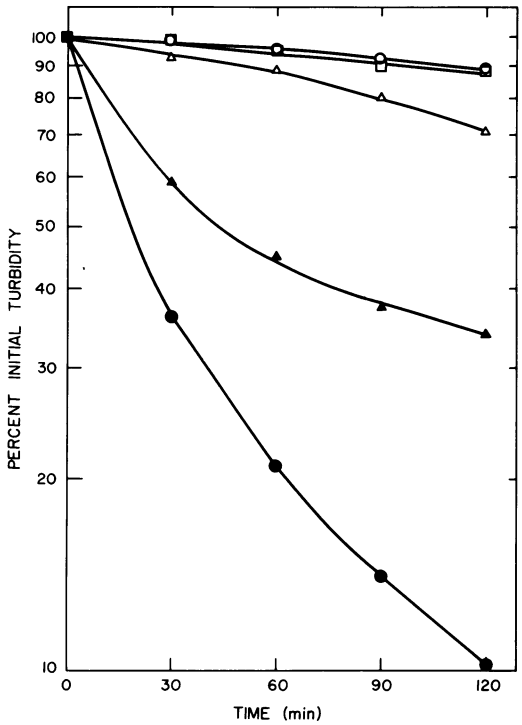


FIG. 4. *Inhibition of autolysis of tar-1 by LiCl. Autolysis was followed in Tris-hydrochloride buffer (○) and in buffer containing 4.0 M LiCl (□), 1.0 M NaCl (●), 1.0 M NaCl + 2.0 M LiCl (△), and 1.0 M NaCl + 1.0 M LiCl (▲).*

buffers at 37 C. There was a 68% decrease in turbidity after 120 min of incubation in the absence of NaCl, whereas cells incubated in standard autolysis buffer (containing 1.0 M NaCl) showed only 22% greater lysis (Fig. 5). Thus, exposure to 1.0 M NaCl can sensitize *tar-1* so that subsequent autolysis may occur in the absence of salt.

Effect of NaCl on cellular morphology. One characteristic feature of *tar-1* is its tendency to grow in clumps when grown in PYK broth (6). A phase contrast photomicrograph of *tar-1* grown in PYK medium is shown in Fig. 6. After these cells were incubated in buffer containing 1.0 M NaCl for 120 min, they appeared as well-separated cocci with little or no clumping (Fig. 7). Although the cells shown in Fig. 7 appear intact, they lysed immediately when the osmotic protection of NaCl was removed by introducing distilled water under the coverslip (Fig. 8).

The cell wall of the parent *S. aureus* H grown at 42 C displays trilaminar morphology which is noted in most gram-positive cell walls (Fig. 9a and b). In sharp contrast, the mutant (*tar-1*) does not have a visible outer electron-dense layer and contains increased amounts of ragged homogeneous cell wall material (Fig. 10a and b). The division planes are distorted and irregular (Fig. 10a). When this mutant is suspended in broth containing 1.0 M NaCl there are focal disruptions of the cell wall (Fig. 11a, arrow) with the release of vesicles. After completion of autolysis, the spheroplasts are apparently extruded from hydrolyzed fragments of cell wall (Fig. 11b, arrow). A detailed study of the morphology of this mutant and the changes observed during autolysis will be presented in a subsequent publication.

Cell viability during autolysis. The recovery of viable cells was dependent on the presence of osmotic stabilization both in the dilution medium and nutrient agar plates. When cells undergoing autolysis were diluted and plated in the presence of 1.0 M NaCl, a significant number of viable cells could be recovered (Fig. 12). No recovery was observed when these cells (diluted in 1.0 M NaCl) were plated in the absence of NaCl (data not shown). Autolyzing mutant cells underwent exponen-

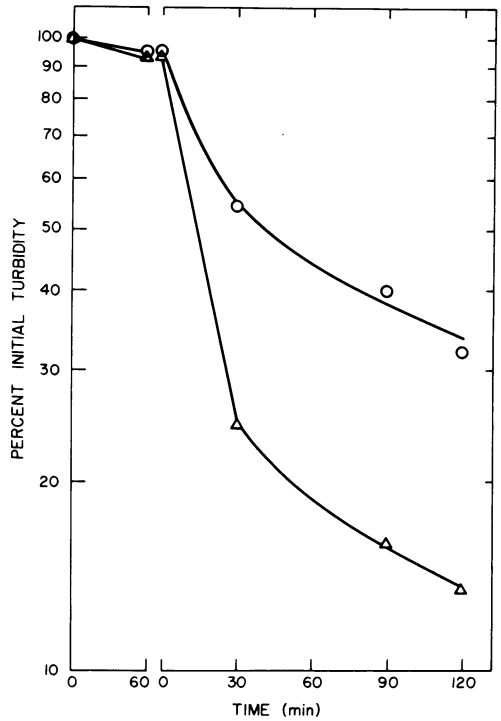
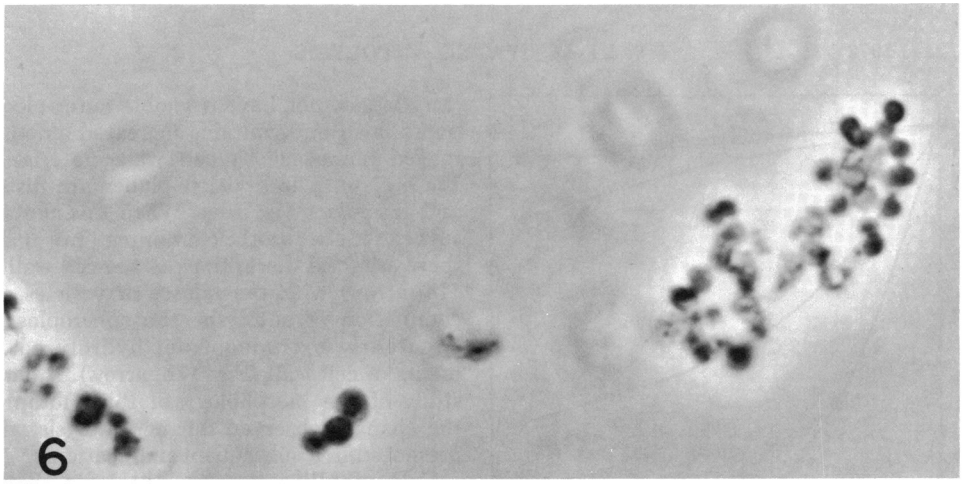
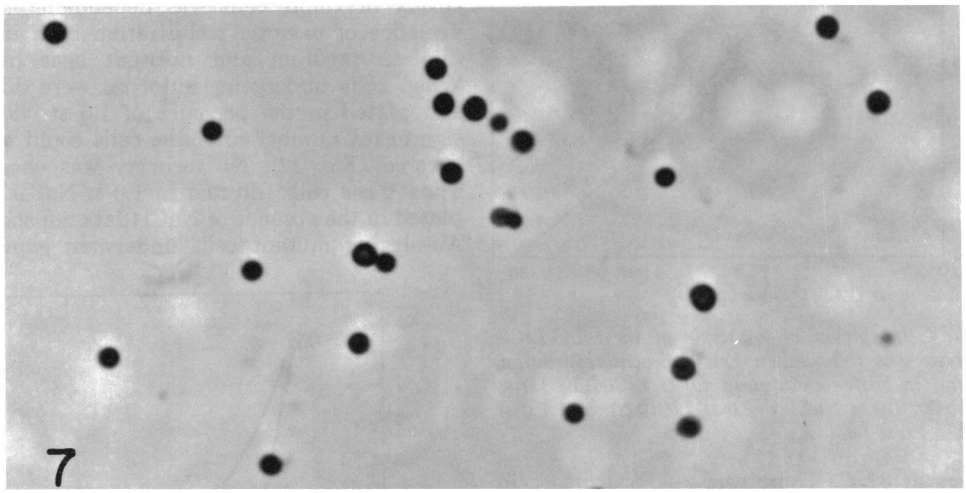


FIG. 5. *NaCl sensitization of tar-1. Cells were preincubated in buffer containing 1.0 M NaCl for 1 hr at 4 C, washed free of salt, resuspended in buffer with (△) or without (○) 1.0 M NaCl and autolyzed at 37 C.*





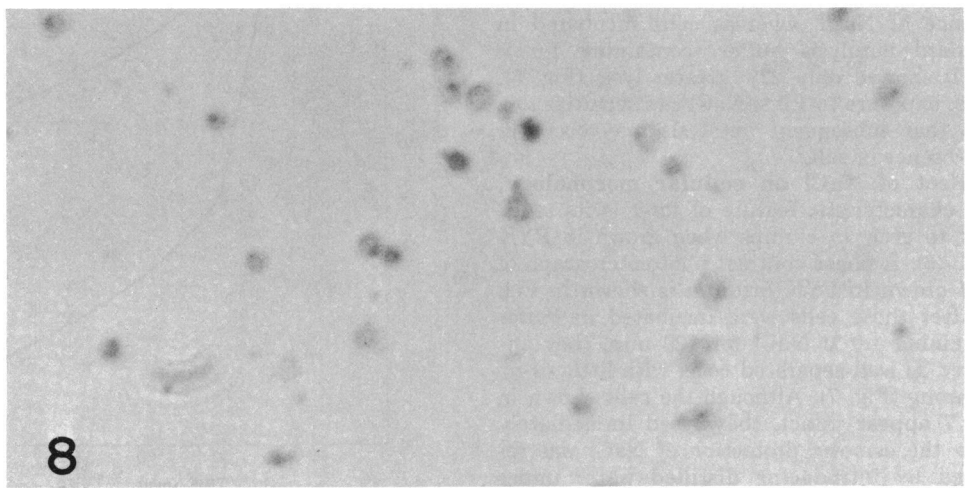
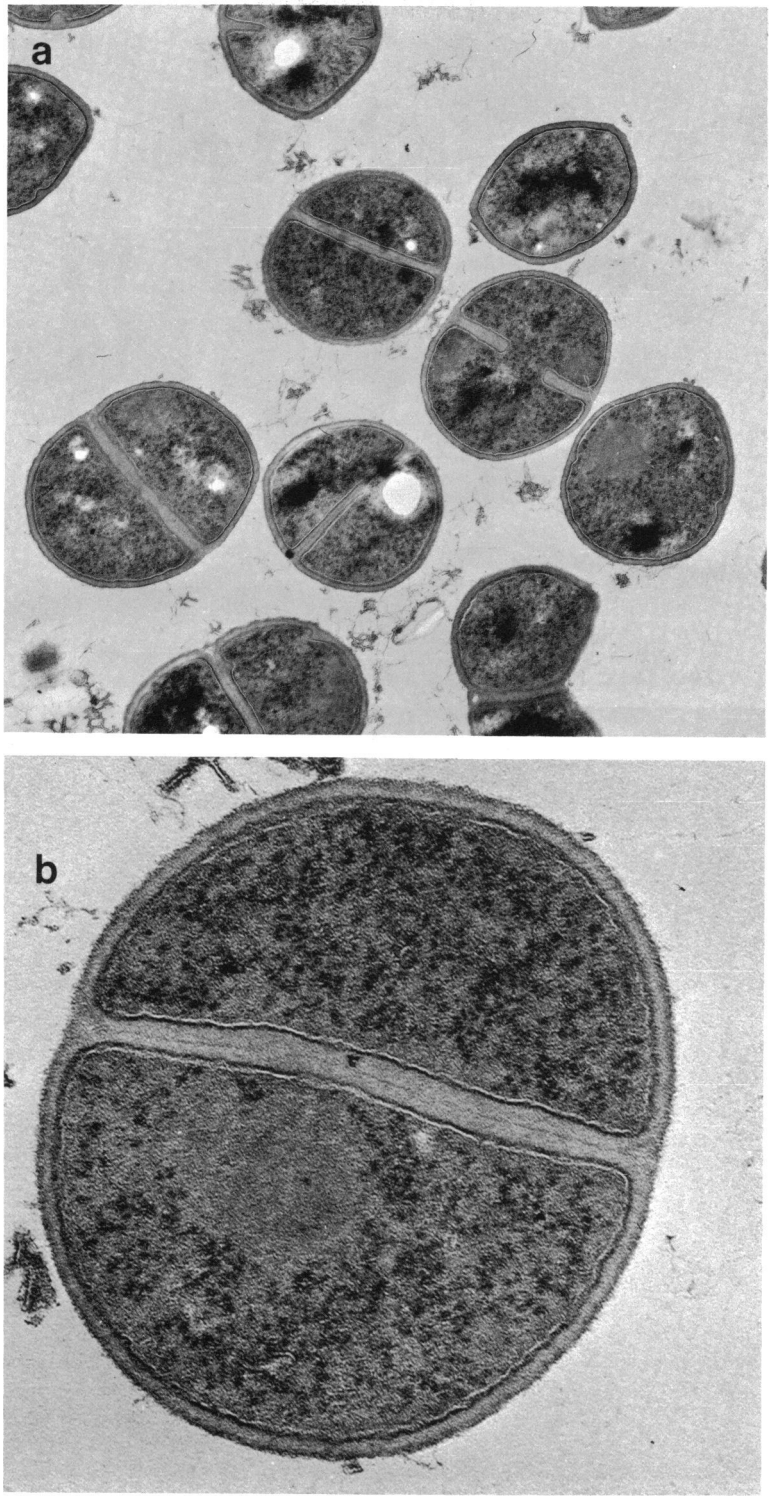


FIG. 6. *Phase contrast photomicrograph of tar-1 grown in PYK broth medium to late logarithmic phase.* ×*2,500.*

FIG. 7. *Phase contrast photomicrograph of tar-1 after 2 hr of incubation at 37 C in Tris-hydrochloride buffer (0.05 M, pH 7.0) containing 1.0 M NaCl.* ×*2,500.*

FIG. 8. *Phase contrast photomicrograph of the same field shown in Fig. 7 immediately after distilled water was introduced under the coverslip.* ×*2,500.*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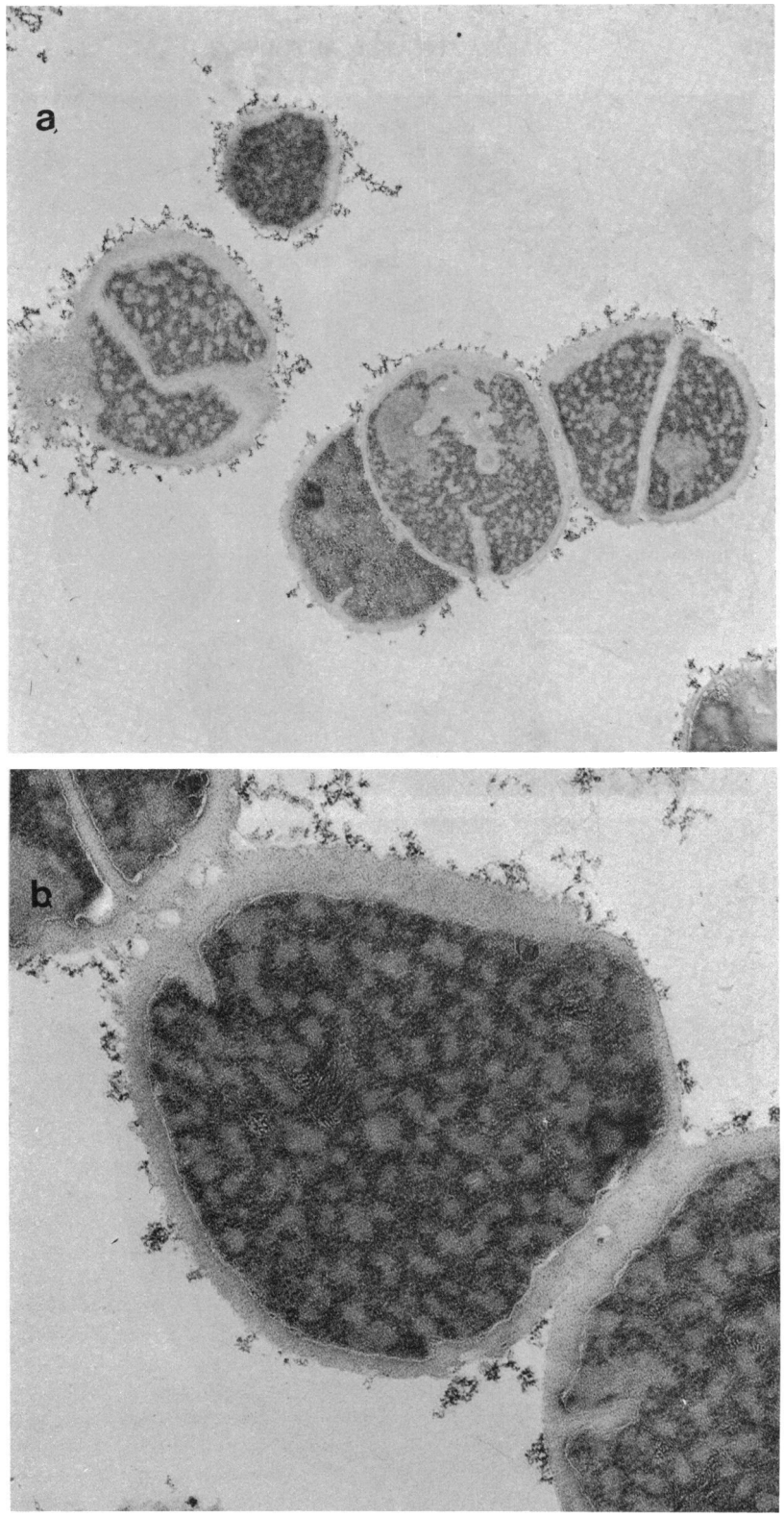

FIG. 10. *Mutant (tar-1) grown at 42 C in PYK broth medium. Note the absence of the outer electron-dense cell wall layer, increase in wall thickness with loose organization, irregular cross wall formation, and mottled cytoplasm; compare with Fig. 9. Magnification: a, ×30,000; b, ×70,000.*

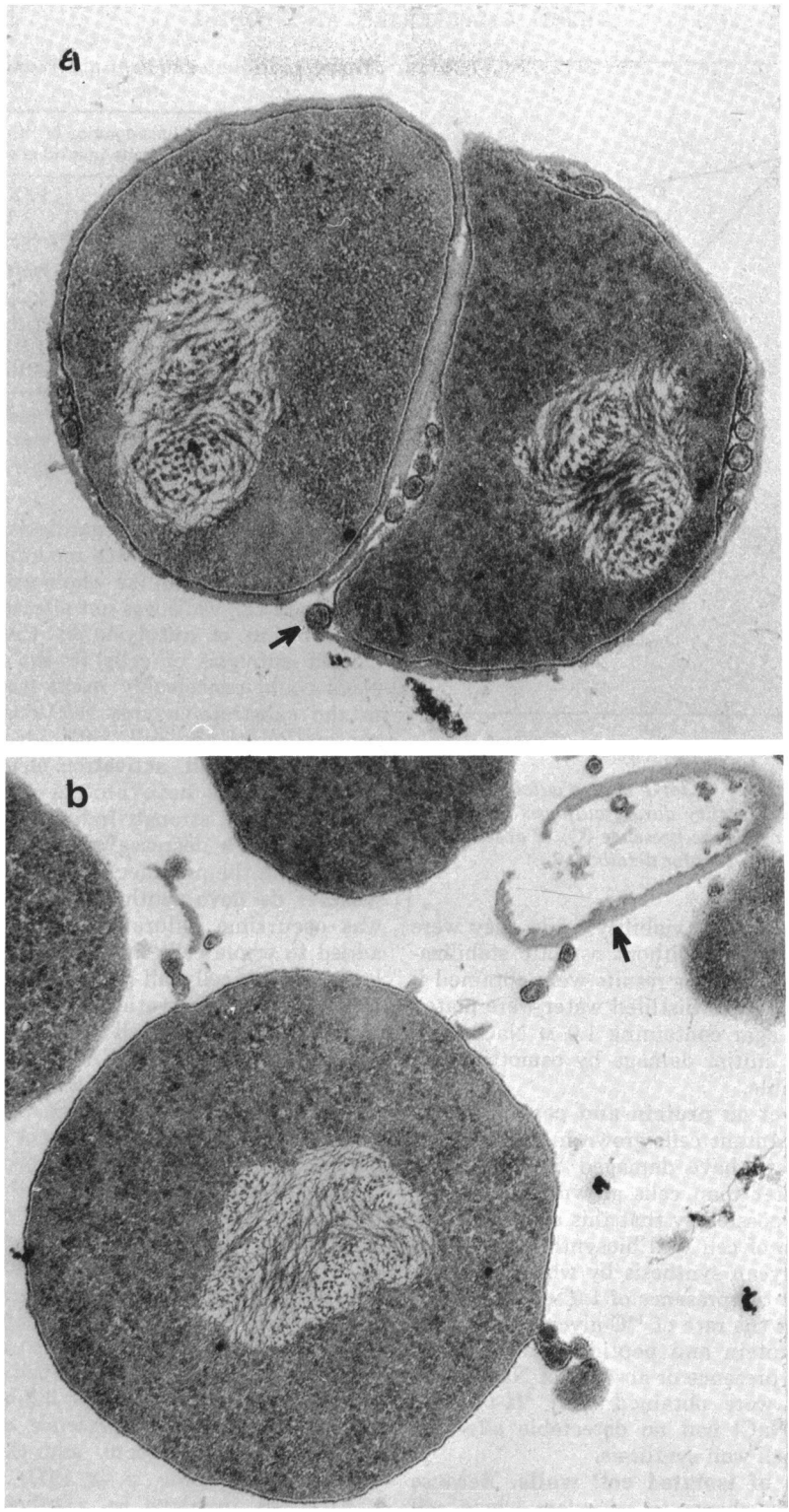

FIG. 11. *Mutant (tar-1) grown in PYK broth medium containing 1.0 M NaCl. The cell wall is more compact, and areas of focal degradation can be observed with the release of vesicles (arrow in a). Note the cytoplasmic membrane, granular cytoplasm, and large nucleoid masses. Spheroplasts are observed (b) and large fragments of cell wall are present (arrow in b). Magnification: a, ×100,000; b, ×80,000.*

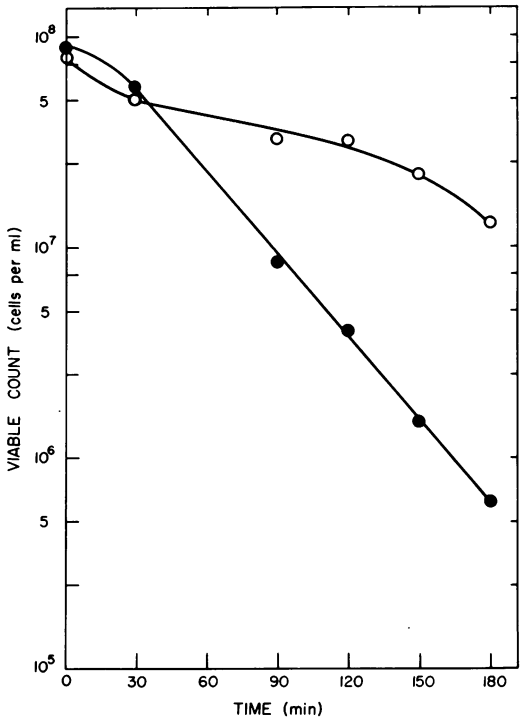

FIG. 12. *Viability of tar-1 during autolysis. Comparison of cell viability during autolysis after dilution and plating in the presence* (○) *or absence* (●) *of 1.0 M NaCl. See text for details.*

tial decrease in cell viability when they were diluted and plated without osmotic stabilization (Fig. 12). Similar results were obtained if the cells diluted in distilled water were plated on nutrient agar containing 1.0 M NaCl, suggesting that initial damage by osmotic shock was irreversible.

NaCl effect on protein and peptidoglycan synthesis. Mutant cells grown in the presence of 1.0 M NaCl have damaged cell walls and autolyze faster than cells grown without salt. To test the possibility that this effect was due to inhibition of cell wall biosynthesis, the rate of peptidoglycan synthesis by whole cells was examined in the presence of 1.0 M NaCl. Table 1 shows that the rate of ^{14}C-glycine incorporation into protein and peptidoglycan was the same in the presence or absence of NaCl. Similar results were obtained with ^{3}H-L-lysine. Therefore, NaCl had no detectable effect on the rate of cell wall synthesis.

Autolysis of isolated cell walls. Because salt has been shown to stimulate whole cell autolysis, it was important to determine whether NaCl affected autolysis of isolated mutant cell walls. We found that walls autolyzed at the same rate regardless of the presence of NaCl in the growth medium or incubation buffer. Thus, unlike whole cells, autolysis of isolated cell walls was not affected by NaCl.

TABLE 1. *Protein and peptidoglycan synthesis in tar-1*[a]

Time of incubation	Incorporation of ^{14}C-glycine (counts/min/ml of culture)			
	PYK medium		PYK medium plus 1.0 M NaCl	
	Protein	Peptidoglycan	Protein	Peptidoglycan
15 min	1,700	8,200	1,400	7,200
30 min	3,700	17,200	2,600	16,400
60 min	7,600	58,000	6,100	52,000

[a] The analysis of distribution of radioactivity between protein and peptidoglycan was performed as described in Materials and Methods.

Inhibition of autolysis by CAP. The enhanced autolysis of cells in the presence of NaCl could conceivably result from a change in the substrate-enzyme relationship due to contraction of the cell walls, decreased synthesis of cell wall, activation of autolytic enzymes, or a de novo protein synthesis. The data in Table 1 strongly indicate that autolysis is not due to a decrease in synthesis of peptidoglycan in the presence of salt. To investigate whether de novo synthesis of a lytic enzyme was occurring, chloramphenicol (CAP) was added to whole cells in the standard autolysis buffer, where cell wall and protein synthesis is minimal due to starvation for amino acids, and in PYK broth where cell wall and protein biosynthesis occur. When CAP (100 μg/ml) was added to the standard autolysis system there was no inhibition of autolysis indicating that de novo protein synthesis was not a significant factor. However, when cells growing in PYK medium were pretreated with CAP (100 μg/ml), washed and suspended in the autolysis buffer, inhibition of autolysis occurred within 10 min of treatment with CAP (approximately 25% of the cell division cycle), as shown in Fig. 13. This system, therefore, appears similar to that of *S. faecalis* where resistance to autolysis occurs either due to unbalanced cell wall biosynthesis (35) or liberation of an inhibitor of autolytic enzymes in the presence of CAP (M. Sayare, L. Daneo-Moore, and G. D. Shockman, Bacteriol. Proc., p. 48, 1971).

Enzymes involved in autolysis. Previous studies have demonstrated that the major autolysin in *tar-1* is *N*-acyl-muramyl-L-alanine amidase (6). It was of importance to determine

whether NaCl-enhanced lysis was due to this enzyme. Washed logarithmic-phase cells were incubated at 37 C in buffer, with or without 1.0 M NaCl, and samples were withdrawn after 0, 30, and 60 min of incubation. The supernatant fluids (10,000 × *g*, 10 min) were dialyzed at 4 C against distilled water to remove salt, and the nondialyzable fractions (NDF) were concentrated by lyophilization. During autolysis there was an increase in both *N*-terminal amino groups and reducing groups in the NDF fractions (Table 2). The ratio of total *N*-terminal amino groups to reducing groups in the NDF increased during autolysis when cells were incubated with 1.0 M NaCl, whereas no such increase was noted when cells were incubated without salt (Table 2). The only detectable *N*-terminal amino acid in the NDF was alanine, suggesting that NaCl-enhanced autolysis primarily involved amidase activity. The molar ratios of alanine to the wall glycans (*N*-acyl-muramic acid and *N*-acyl-glucosamine) for *tar-1* cell wall and NDF hydrolysates were 0.89 and 0.91, respectively. Therefore, autolysis did not cause extensive degradation of the cell wall with release of dialyzable fragments.

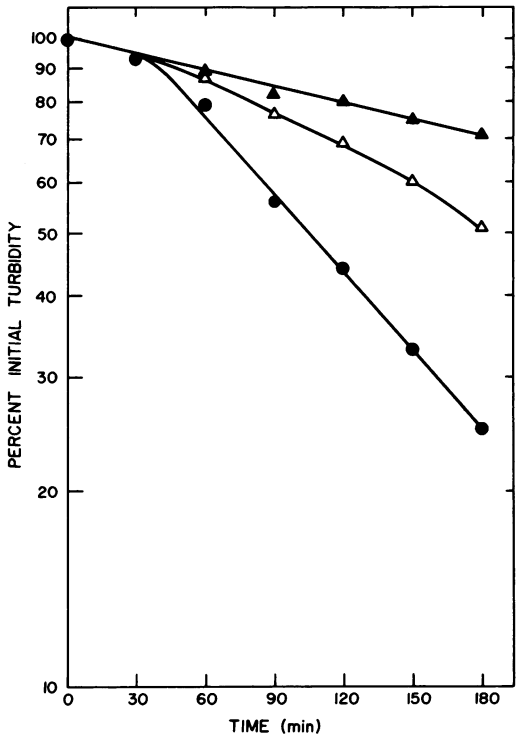

FIG. 13. *Inhibition of autolysis of tar-1 by chloramphenicol (CAP). Cells were grown in PYK broth medium without CAP (●), or with CAP (100 μg/ml) for 10 (△) and 30 min (▲), washed, and incubated at 37 C in buffer containing 1.0 M NaCl.*

DISCUSSION

Staphylococci are resistant to high concentrations of NaCl (23). This property is often used to select staphylococci from mixed cultures because many bacteria are inhibited by concentrations of salt in excess of 1.4 M (4, 24). The one-step pleiotropic mutant deficient in ribitol teichoic acid (*tar-1*) is extremely sensitive to 1.0 M NaCl and autolyzes in its presence. This observation prompted us to investigate further the effect of NaCl on this mutant. The results demonstrate that high concentrations of specific salts activate a cell wall-bound *N*-acyl-muramyl-L-alanine amidase, resulting in focal cleavage of cell wall. With osmotic stabilization reversion of some of the cells with damaged cell walls can occur. This finding is of considerable interest because clinically isolated L-forms (21), wall-defective mutants of *Bacillus pumilus* (3), *B. subtilis* (*Sal-1*) (39), *B. subtilis* TOF (15), and L-forms produced in vitro (20) are either stabilized or prevented from reverting to wild type by high concentrations of NaCl.

TABLE 2. *Release of amino acid and reducing groups during autolysis of tar-1*[a]

Incubation in autolysis buffer (min)	Total DNP amino groups and reducing groups in NDF					
	Control buffer			Buffer with 1.0 M NaCl		
	DNP-amino (nmoles)[b]	Reducing (nmoles)	Ratio[c]	DNP-amino (nmoles)[b]	Reducing (nmoles)	Ratio[c]
0	1.2	0.44	2.7	1.0	1.01	1.0
30	2.8	0.99	2.8	18.0	2.71	6.6
60	2.4	0.85	2.8	70.2	5.42	13.0

[a] Cells were incubated at 37 C in autolysis buffer. Samples were removed at 0, 30, and 60 min and the nondialyzable supernatant fluids (NDF) were isolated as described in Materials and Methods.

[b] Results are expressed as nanomoles of dinitrophenylated (DNP) amino groups or reducing groups per milligram of NDF.

[c] Ratio of nanomoles of DNP amino groups per nanomole of reducing groups.

Recent reviews (13, 33-36) have emphasized that autolytic enzymes (those which specifically hydrolyze polymers in the bacterial cell wall) occur ubiquitously in bacteria. Autolysins are postulated to play a role in bacterial growth because greatest autolytic activity has been found in the septum region of streptococci (18, 31). Mitchell and Moyle (27), and more recently Forsberg and Rogers (12), have suggested that one role of autolysin is related to separation of individual cells after completion of division. Other proposed roles include: (i) making openings in old wall so new polymer can be added (7) and (ii) modifying the wall during septum formation (32). Autolysin may also produce focal gaps in the cell wall to facilitate the passage of deoxyribonucleic acid (DNA) during DNA-mediated transformation (38).

We have shown that salt-activated autolysis in *tar-1* results from focal cleavage of cell wall. (i) Most of the peptidoglycan is recovered in the nondialyzable supernatant fluid of autolyzing cell suspensions. The molar ratio of glycan to alanine in the nondialyzable fraction is equivalent to that seen in the intact cell wall. (ii) Large fragments of cell wall are identified in electron micrographs of autolyzed cultures. (iii) The minimal release of reducing groups precludes extensive digestion of the cell wall and release of small molecular weight fragments as predicted from the cell wall structure of *S. aureus* (13). This hydrolysis is similar to that noted first by Mitchell and Moyle (27) and confirmed by Cripps and Work (8).

Autolysin in wild type cells of *S. aureus* H was previously reported to be located in the soluble fraction of cell-free extracts, whereas autolysin in *tar-1* was closely associated with isolated cell walls (6). This differential location of autolysin in *tar-1* could be due to an altered conformation or charge distribution resulting from the lack of polymerized teichoic acid. At present it is not possible to exclude binding to the cytoplasmic membrane instead of the cell wall. Mirelman et al. (26) reported that carefully washed, isolated *tar-1* walls contain membrane fragments with more C-17 branched and less C-20 fatty acids than wild type walls. Furthermore, Rogers (33) has recently proposed that connecting bridges may form between the bacterial wall and cytoplasmic membrane when intermediates in wall synthesis are bound simultaneously to membrane components and wall. We suggest that autolysin in *tar-1* may be bound to membrane fragments or cell walls by hydrophobic interactions. The effect of NaCl would be similar to the effect of chaotropic ions on hydrophobic areas (16). Thus, water is more disordered and lipophilic in the presence of ions such as Br^- and Cl^-, resulting in a weakening of the hydrophobic areas of membranes and multicomponent enzymes. Of the ions tested for ability to activate autolysis, only salts containing these anions were effective. Frank and Evans (11) state that all the alkali and halide ions, except Li^+ and F^-, disorder the structure of water, which may explain why autolysis was not enhanced by LiCl. On the other hand, DeVoe and Oginsky (9) suggested that Mg^{2+} ions may form cross-bridges within phospholipids or protein side chains in the cell envelope of a marine bacterium, preventing Na^+-induced lysis. The finding that autolysis was irreversibly inhibited by Mg^{2+} suggests that this ion may form cross-bridges and prevent Na^+ activation in *tar-1*.

Marquis (25) and Ou and Marquis (28) have demonstrated salt-induced shrinkage of isolated cell walls of gram-positive bacteria due to electrostatic contraction of wall peptidoglycan. Cell wall contraction may accelerate autolysis by bringing substrate closer to the enzyme. Teichoic acid appears to be involved in the binding of ions on the cell surface (17, 19). In the absence of this polymer, the contraction of the substrate may be enhanced in whole cells. Therefore, studies are in progress to determine the effects of deletion for cell wall polymers on the binding of ions and the porosity of cell walls.

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