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Function of Cell Wall Teichoic Acid in Thermally Injured *Staphylococcus aureus*

DALLAS G. HOOVER AND RODNEY J. H. GRAY[1]*

Food Science and Microbiology, University of Delaware, Newark, Delaware 19711

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Thermally injured cells of *Staphylococcus aureus* lack the ability to grow on tryptic soy agar containing 7.5% NaCl. This injury phenomenon was examined in three strains of *S. aureus*: MF-31; H (Str); and, isolated from H (Str), 52A5, a mutant which lacks teichoic acid in the cell wall. Temperatures for sublethal heat treatment were selected to produce maximum injury with minimum death for each strain. Examination of isolated cell walls showed that magnesium was lost from the wall during heating, and that the degree of cell injury was accentuated when magnesium ions were either removed from or made unavailable to the cell. *S. aureus* 52A5 was more heat sensitive than its parent strain. Cells containing higher levels of wall teichoic acid generally showed less injury than normal cells. Cells with the weaker cation-binding polymer, teichuronic acid, in the cell wall generally showed greater injury. These data suggest that cell wall teichoic acid of *S. aureus* aids in the survival of the cell by the maintenance of an accessible surface pool of magnesium.

Hurst et al. (12, 13) have demonstrated the importance of the staphylococcal cell envelope during heating. Intracellular magnesium was shown to have been lost through the heat-damaged membrane (14). Immediately after heating, and even in the presence of ethylenediaminetetraacetic acid (EDTA), *Staphylococcus aureus* cell walls bound magnesium which had been lost during heat treatment. This high affinity for magnesium displayed by the cell walls was apparently due to the absence of competitive charge from the alanyl ester residues of the cell wall teichoic acids.

Comparatively little is known of the biological function of cell wall teichoic acid. Archibald et al. (2) suggested that these cell wall polymers may act as a cation-exchange resin, regulating the flow of ions, especially magnesium, through the cell envelope. Since that time, this hypothesis has been strengthened by the work of many researchers. Heptinstall et al. (10) found that cell wall teichoic acid is primarily responsible for magnesium binding in walls containing teichoic acid. Ellwood (6) demonstrated that under conditions of magnesium depletion, an increased amount of teichoic acid is synthesized by the *Bacillus* cell wall. Lambert et al. (19) found that with equilibrium dialysis, with a maximum Mg^{2+}/P ratio of approximately 0.4:1, one Mg^{2+} ion is bound to two phosphate groups, and that this mechanism is intimately involved in the assimilation of magnesium.

Ou et al. (26) reported that cell wall teichoic acid cannot serve as a unique assimilator of cations, since the mutant, *S. aureus* 52A5, which lacks cell wall teichoic acid, was able to grow as well as the wild-type parent in media containing EDTA, a high amount of sodium chloride, or an acidic pH. Lambert et al. (19) found, however, that this mutant still had adequate Mg^{2+} binding capability provided by the lipoteichoic acid and carboxyl groups of the peptidoglycan under the conditions of this examination.

The Mg^{2+}-acquiring ability of cell wall teichoic acid should be more evident under stressful conditions where acquisition of Mg^{2+} would be essential for cell survival. Sublethal heat treatment represents a stressful situation since Mg^{2+} is lost from the cell during heating (14). This study investigates thermal stress in three strains of *S. aureus*, including 52A5, in an effort to further elucidate the function of cell wall teichoic acid in heat-treated *S. aureus*.

MATERIALS AND METHODS

Test organisms. Working stocks were prepared from the reserve-stock slants by loop inoculation of 100 ml of tryptic soy broth (TSB, Difco) at 37°C. After 24 h, 0.1 ml of this culture was transferred to another 100 ml of TSB at 37°C and grown 6.5 h. The

[1] Present address: Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN 55108.

working stocks were then prepared by placing 0.1 ml of this culture in a test tube containing 100 ml of TSB, mixing it, and freezing it immediately at −10°C. The contents of these tubes were thawed as needed and decanted aseptically into flasks containing the desired growth medium.

(i) ***S. aureus* MF-31.** This strain was originally isolated from contaminated cheese. MF-31 is coagulase positive, enterotoxigenic, and heat resistant. The generation time is 30 min in TSB at 37°C.

(ii) ***S. aureus* H (Str).** Isolated from *S. aureus* H by streptomycin resistance, H (Str) is coagulase positive and mannitol fermentative, and it displays a generation time of 30 min in TSB at 37°C.

(iii) ***S. aureus* 52A5.** This strain was originally isolated by mutagenesis from *S. aureus* H (Str). 52A5 is a single-step mutant with no ribitol teichoic acid in the cell wall. The strain is coagulase positive, mannitol fermentative, and streptomycin resistant. *S. aureus* 52A5 has a generation time of 37 min in TSB at 37°C.

Media and injury menstrua. TSB was used as the general growth medium, and TSB containing 7.5% NaCl (TSBS) was prepared to examine injury of *S. aureus* growth in a high-salt environment. The phosphate-limiting and magnesium-limiting media were as described by Tempest et al. (30, 31), with the inoculum supplying the necessary amino acids and vitamins for staphylococcus growth as described by Meers and Tempest (21). Sterile, deionized, distilled water was used as a heating menstruum. Phosphate injury and wash was with 100 mM potassium phosphate buffer, pH 7.2. Except for phosphate and water heat treatments, all compounds in this injury study were dissolved in sterile 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 7.2.

Injury procedure. *S. aureus* MF-31, H (Str), and 52A5 from frozen working stocks were inoculated into TSB and grown for 12 h on a rotary shaker at 37°C to an absorbance reading of 1.1 at 620 nm. Approximately 40 ml of the culture was centrifuged at 2,100 × *g* for 10 min at 4°C to pellet the cells. (The entire procedure was carried out in the cold except as noted.) The spent medium was decanted, and the pellet was suspended and washed in sterile, deionized, distilled water. After centrifugation, the supernatant liquid was drawn off, and the cells were suspended in 5 ml of distilled water with thorough mixing. The cell suspensions were then added to 45-ml portions of the injury menstruum equilibrated to the desired temperature; the final suspensions contained approximately 10^9 bacteria per ml. Zero time was recorded upon addition of the cells to the injury menstruum. One-milliliter portions were taken at 0, 5, 15, 25, and 35 min and diluted in sterile distilled water. The temperature was maintained with constant agitation. Duplicate spread plates of Trypticase soy agar (TSA) and TSA containing 7.5% NaCl (TSAS) were inoculated and incubated at 37°C for 48 h before enumeration.

Cell wall isolation. Four liters of cell culture in TSB was collected and prepared as stated previously. The pellet was mixed with 10 ml of cold distilled water. Cells were then broken in a refrigerated French pressure cell, using a Carver press at 23,000 to 25,000 lb/in^2. This preparation was released directly into cold 0.5% sodium dodecyl sulfate, mixed, and allowed to stand in ice for 20 min. The broken cells were centrifuged three times at 2,100 × *g* for 5 min each to sediment debris and whole cells. The crude walls were recovered by centrifugation at 12,000 × *g* for 15 min at 4°C. Further purification was as described by Chatterjee (3).

Cell wall heat treatment. Cell wall preparations, isolated from 4 liters of TSB, were initially incubated 1 h in 100 mM $MgCl_2$-0.05 M Tris-hydrochloride buffer (pH 7.2) at 37°C to saturate the available binding sites of the cell wall with magnesium. The walls were then centrifuged at 12,000 × *g* for 15 min, washed with distilled water, centrifuged, and resuspended in 5 ml of water. This suspension was added to 35 ml of 100 mM potassium phosphate buffer (pH 7.2). A control was run at room temperature (25°C) along with the injury temperature for each strain. Portions of 10 ml were removed at 0 and 35 min from the time of inoculation. These samples were immediately iced, pelleted, and water washed. Dry weights were obtained by drying to a constant weight at 105°C, and the samples were then analyzed for magnesium by atomic absorption spectrometry.

Purity of cell wall preparations. Preparations of cell walls were checked for purity by examination in a phase-contrast microscope, by observing the absorption spectra from 230 to 420 nm with a Gilford 240 spectrophotometer, and by determining reactivities of the walls with Folin reagent by a modification of the Lowry method (23).

Cation extraction and analysis. This procedure was an adaptation of methods by Tempest and Strange (32) and Cutinelli and Galdiero (5).

The cell wall pellets from injured and control cells were individually weighed and mixed with 0.42 ml of cold 6.0 N HCl. This mixture was allowed to stand for 15 min in ice. After the addition of 2.5 ml of cold distilled water, the suspension was centrifuged and the clear supernatant was decanted. The pellet was then reextracted by the same procedure, and the supernatants were pooled . The final acid concentration was 1.0 N HCl. Total cation removal from the cell walls was confirmed by analysis of ashed pellets by atomic absorption spectroscopy.

Quantitation of cations. Magnesium, calcium, sodium, and potassium were quantitatively analyzed on a Perkin-Elmer 305 atomic absorption spectrophotometer. Equivalent standards were prepared with 1.0 N HCl. Appropriate sample dilutions and standard ranges were performed in accordance with the Association of Official Analytical Chemists manual (11).

Expression of data. Lethality was expressed in terms of the decimal reduction time D, as determined from colony counts on TSA. D' represented the decimal reduction time as monitored by colony counts on TSAS. The D and D' values were calculated from straight lines determined by the method of least squares (17). Linear correlation was measured for the degree of association between random variables.

RESULTS

Previous heat injury research with *S. aureus* MF-31 has involved exposure at temperatures between 52 and 55°C. At these temperatures, and over the time periods studied, MF-31 exhibits minimal death. As depicted in Fig. 1A, neither *S. aureus* H (Str) nor 52A5 tolerated 52°C. Death was extensive, as indicated by the severe drop of TSA counts.

S. aureus 52A5 was originally isolated from *S. aureus* H (Str). There is no ribitol teichoic acid in the cell wall of this single-step mutant. *S. aureus* 52A5 had a lower heat resistance than H (Str) in potassium phosphate buffer (Fig. 1B). *S. aureus* 52A5 could not tolerate exposure at 48 or 46°C without substantial death. Virtually no lethal effect was observed when *S. aureus* H (Str) was exposed to phosphate buffer at 48°C.

Since the strains tested in this study showed different temperature sensitivities, each was examined at a temperature that approached maximum injury with minimum death in 100 mM potassium phosphate buffer. The temperatures utilized were 52°C for MF-31, 48°C for H (Str), and 44°C for 52A5 (Fig. 2). The *D* values over the 35 min of heat exposure for MF-31, H (Str), and 52A5 were 106, 84, and 58 min, respectively, with the *D'* values for all strains less than 15 min. Control experiments at 25°C showed no difference between colony counts on TSA and TSAS.

The importance of the washing effect on cells before heat treatment was exemplified in a series of experiments with MF-31. Previous research with MF-31 has included washing the cells in 100 mM potassium phosphate buffer before transfer into the injury menstruum. The phosphate buffer wash rendered the cells more sensitive to the subsequent heat treatment than washing in distilled water (Fig. 3A and B). In all the injuries investigated, there was an increase in the degree of heat injury when preceded by a phosphate buffer wash, without a

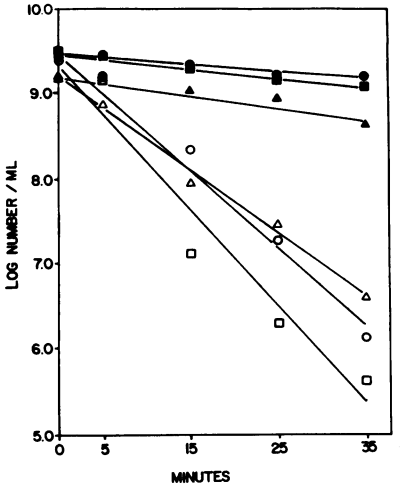

FIG. 2. *Heat treatment of three strains of S. aureus in 100 mM potassium phosphate buffer (pH 7.2). Closed symbols, TSA counts; open symbols, TSAS counts. (●, ○) MF-31 at 52°C; (■, □) H (Str) at 48°C; (▲, △) 52A5 at 44°C.*

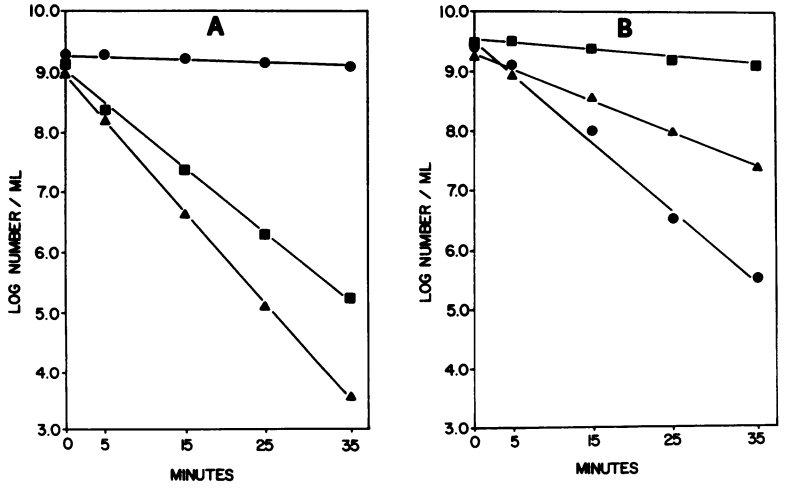

FIG. 1. *(A) Heat treatment of three strains of S. aureus at 52°C in 100 mM potassium phosphate buffer (pH 7.2), enumeration on TSA. Symbols: ●, MF-31; ■, H (Str); ▲, 52A5. (B) Heat treatment of S. aureus H (Str) and 52A5 in 100 mM potassium phosphate buffer (pH 7.2), enumeration on TSA. Symbols: ●, 52A5 at 48°C; ▲, 52A5 at 46°C; ■, H (Str) at 48°C.*

significant drop in viable cells as observed with the TSA counts. Figure 3A illustrates different concentrations of potassium chloride in the heating menstruum, with a phosphate buffer wash before heat exposure of the organism. The D'/D ratios were much lower with the phosphate wash, in comparison with the injury ratios of the water-washed cells. The average D'/D ratios were 0.05 for phosphate-washed cells and 0.25 for water-washed cells.

Chloride compounds were selected to test the effect of different cations with heat treatment, because these anions are relatively neutral to thermally stressed *S. aureus* cells (25). Comparison of the different chloride compounds at the same concentrations generally produced such similar data that the injury curves were superimposable. The data for cells heated in various concentrations of NaCl, $MgCl_2$, and $CaCl_2$ (unpublished data) resembled those of cells heated in KCl (Fig. 3A and B). The exception was that high levels of $CaCl_2$ in the heating menstruum were detrimental to the cells, as evidenced by the greatly accelerated death rate (Fig. 4A). Heat treatment in $MgCl_2$ proved it to be the most protective chloride compound at high concentrations (100 and 500 mM). Directly opposite effects were derived from the presence of $CaCl_2$ and $MgCl_2$ in the heating menstruum (Fig. 4A and B). At the 500 mM level, $CaCl_2$ was lethal to heated *S. aureus* MF-31; the D value of these phosphate buffer-washed cells was less than 6 min. The combination of 500 mM $CaCl_2$ and elevated temperature was lethal to MF-31 under the conditions tested. A lowering of the concentration of $CaCl_2$ to 5 mM decreased mortality. In contrast, increasing the $MgCl_2$ from 5 to 500 mM in the heating menstruum increased the protective effect.

Heat treatment of *S. aureus* H (Str) at 48°C in 100 mM concentrations of the chloride compounds was basically similar to that obtained with MF-31 at 52°C (data not presented). However, heat treatment in KCl was as severe as heat treatment in $CaCl_2$ for H (Str). This effect has been demonstrated previously in a comparison of the effect of heat treatment of *S. aureus* in sodium versus potassium phosphate buffer (13).

When heated at 44°C, *S. aureus* 52A5 was only mildly affected by the 100 mM chloride compounds. The D values in both 100 mM $MgCl_2$ and NaCl were 212 min. The only chloride compound causing death of 52A5 in the 35-min heating period was $CaCl_2$.

The relationship of osmotic pressure to injury was examined by duplicating the osmotic pressure of 100 mM NaCl with sucrose and glycerol (27) (data not presented). Heat treatment in solutions of these saccharides resulted in much less injury relative to that observed in NaCl for all the strains examined. Heating the cells in glycerol was more damaging than heating

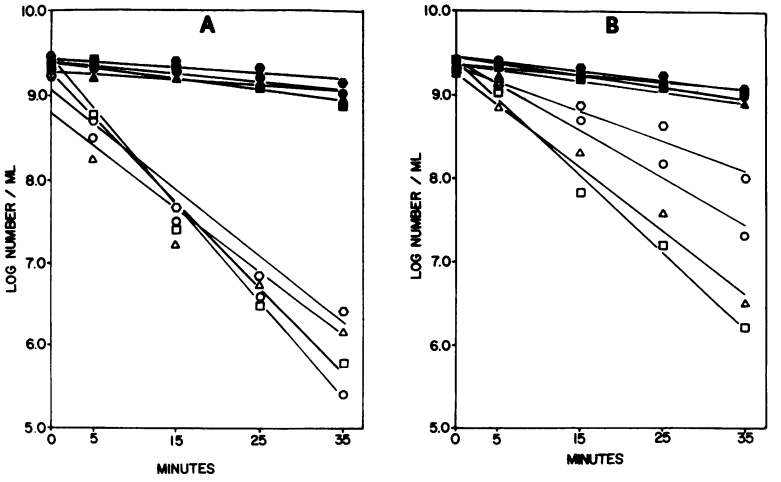

FIG. 3. *(A) Heat treatment of S. aureus MF-31 in different concentrations of potassium chloride in 0.05 M Tris buffer (pH 7.2) at 52°C with a phosphate buffer wash before the heat treatment. Closed symbols, TSA counts; open symbols, TSAS counts.* (●, ○) *5 mM KCl;* (■, □) *25 mM KCl;* (▲, △) *100 mM KCl;* (⬣, ⬭) *500 mM KCl. (B) Heat treatment of S. aureus MF-31 in different concentrations of potassium chloride in 0.05 M Tris buffer (pH 7.2) at 52°C with a distilled water wash before the heat treatment. Closed symbols, TSA counts; open symbols, TSAS counts.* (●, ○) *5 mM KCl;* (■, □) *25 mM KCl;* (▲, △) *100 mM KCl;* (⬣, ⬭) *500 mM KCl.*

them in sucrose solutions of equal osmotic pressure.

To thoroughly remove as much magnesium from the cell wall as possible, the cells were washed in 0.9% saline solution before the standard water wash. Hurst et al. (15) found 0.004 mM EDTA to bind all available free magnesium in an unsupplemented menstruum. Heat treatment in 0.004 mM EDTA proved to be one of the most injurious of the solutions tested, especially with MF-31 and H (Str) (D' values of 8.4 and 6.7 min, respectively; Fig. 5A).

Growing *S. aureus* in TSBS caused the resultant cells to be more sensitive to heat treatment (Fig. 5B). For TSBS-grown MF-31, the D' value in phosphate buffer was 12.0 min, compared with 15.0 min for MF-31 grown in TSB. *S. aureus* H (Str) and 52A5 were more sensitive

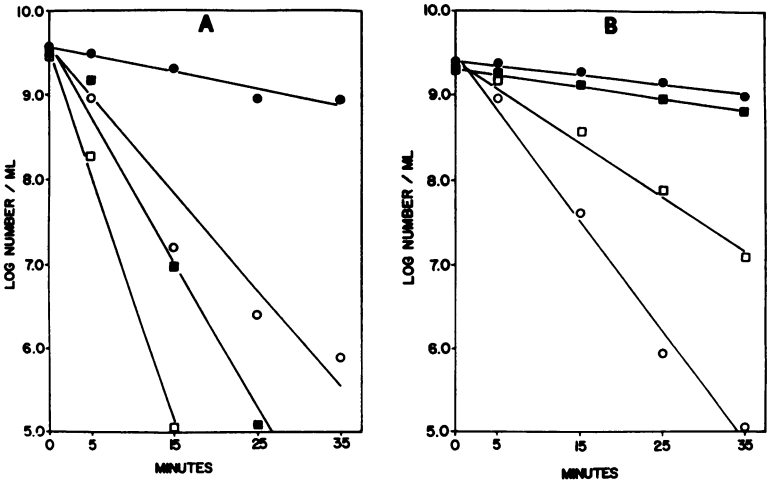

FIG. 4. *(A) Heat treatment of S. aureus MF-31 at 52°C in 0.05 M Tris buffer (pH 7.2) containing different calcium chloride concentrations. Closed symbols, TSA counts; open symbols, TSAS counts. (●, ○) 5 mM $CaCl_2$; (■, □) 500 mM $CaCl_2$. (B) Heat treatment of S. aureus MF-31 at 52°C in 0.05 M Tris buffer (pH 7.2) containing different magnesium chloride concentrations. Closed symbols, TSA counts; open symbols, TSAS counts. (●, ○) 5 mM $MgCl_2$; (■, □) 500 mM $MgCl_2$.*

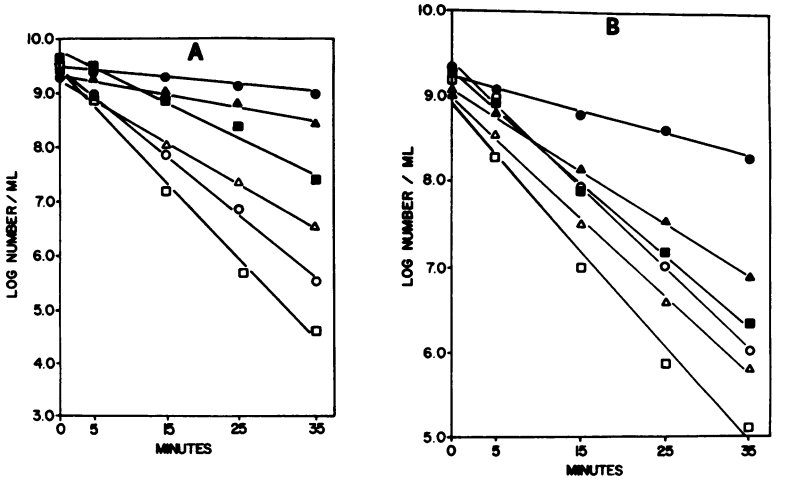

FIG. 5. *(A) Heat treatment of S. aureus MF-31, H (Str), and 52A5 in Tris buffer containing 0.004 mM EDTA (pH 7.2). Closed symbols, TSA counts; open symbols, TSAS counts. (●, ○) MF-31 at 52°C; (■, □) H (Str) at 48°C; (▲, △) 52A5 at 44°C. (B) Heat treatment of S. aureus MF-31, H (Str) and 52A5 in 100 mM potassium phosphate buffer (pH 7.2) after growth in TSBS. Closed symbols, TSA counts; open symbols, TSAS counts. (●, ○) MF-31 at 52°C; (■, □) H (Str) at 48°C; (▲, △) 52A5 at 44°C.*

than MF-31. After TSBS growth, the D' values were 7.3 and 5.4 min, respectively, in the phosphate buffer. These D' values are the opposites of those expected if the TSBS-grown cells had bound a greater amount of magnesium. Originally, this series of experiments with TSBS-grown cells was designed to determine the effect of a low amount of D-alanyl ester residues attached to the cell wall teichoic acid during heat injury. The growth of *S. aureus* in media containing a high amount of NaCl will prevent synthesis of the ester residues (3). However, due to the different effects on other areas of the cell, particularly the plasma membrane, the direct effect of the reduced D-alanyl content could not be examined exclusive of other events.

Substitution of cell wall teichoic acid with the non-phosphate polymer teichuronic acid occurs when gram-positive bacteria are grown in phosphate-limiting media (30). This replacement of wall polymers is complete within 5 h and does not represent an upgrowth of variant organisms initially present at a low concentration (7). Teichuronic acid binds magnesium less efficiently than teichoic acid. *S. aureus* strains grown in the teichuronic acid-inducing media (Fig. 6A) revealed an increase in the death rate with heat treatment. There was greater than a 90% loss in the TSA-viable population over the 35-min heating period. The D' values were similar between the two differently grown cultures with MF-31 and H (Str), whereas 52A5 exhibited substantial death and injury. This phenomenon is somewhat of a paradox, since *S. aureus* 52A5 does not have any cell wall teichoic acid to be altered.

Magnesium limitation of gram-positive bacteria causes the cell wall to thicken somewhat with the synthesis of more teichoic acid than normal (31), and, as one might expect, there is a corresponding increase in the affinity of the cell wall for divalent cations. Survival was much greater for magnesium-limited cells than for phosphate-limited cells with heat treatment (Fig. 6B) and TSB-grown cells injured in phosphate buffer (Fig. 2), although almost 90% of the exposed MF-31 cells were destroyed in the 35-min heating period. *S. aureus* 52A5 would not grow in magnesium-limiting media when tested over a pH range of media from 4.5 to 7.0.

Isolated cell walls were heat treated after saturation of the wall binding sites with magnesium. Little change was observed in magnesium content for MF-31 and H (Str) at 25°C and 52A5 at 25 or 44°C (Table 1). The strains with ribitol teichoic acid lost more magnesium than 52A5, but, as expected, MF-31 and H (Str) initially bound a higher level of magnesium and retained more after heat treatment. These data indicate that there is a loss of magnesium from the cell wall of *S. aureus* during heat injury.

DISCUSSION

Since early studies (16, 28) revealed that the recovery of thermally injured *S. aureus* could occur in the presence of penicillin, which inhibits cell wall synthesis in gram-positive organisms, any relationship of the cell wall with the sublethal phenomenon was not investigated for a time. Recently it was found that *S. aureus* did recover in the presence of penicillin, but with a 6-h lag in comparison with bacteria recovering in the absence of the inhibitor (12). An early publication (16) reported that protein synthesis was not involved in recovery and that a sugar-containing medium was necessary for complete recovery. Hurst et al. (12, 13) found good recov-

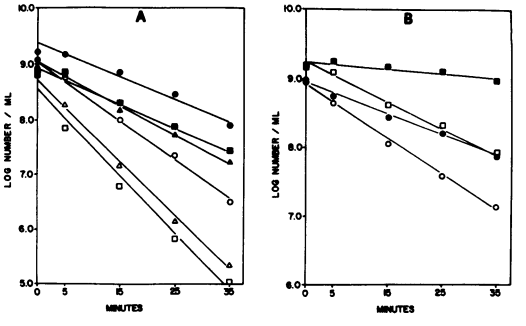

FIG. 6. *(A) Heat treatment of S. aureus MF-31, H (Str), and 52A5 in 100 mM potassium phosphate buffer (pH 7.2) after growth in phosphate-limiting medium. Closed symbols, TSA counts; open symbols, TSAS counts.* (●, ○) *MF-31 at 52°C;* (■, □) *H (Str) at 48°C;* (▲, △) *52A5 at 44°C. (B) Heat treatment of S. aureus MF-31 and H (Str) in 100 mM potassium phosphate buffer (pH 7.2) after growth in magnesium-limiting medium. Closed symbols, TSA counts; open symbols, TSAS counts.* (●, ○) *MF-31 at 52°C;* (■, □) *H (Str) at 48°C.*

TABLE 1. *Analysis for wall-bound magnesium in heat-treated cell walls of S. aureus strains*

S. aureus strains	Analysis for magnesium												
	0 min, 52°C[a]			35 min, 52°C				0 min, 48°C			35 min, 48°C			
	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Mg^{2+} (μg lost)	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Mg^{2+} (μg lost)
MF-31	6.88	0.78	0.622	6.47	0.47	0.424	0.198							
H (Str)								13.70	1.39	0.593	6.21	0.49	0.461	0.132
52A5														

S. aureus strains	Analysis for magnesium												
	0 min, 44°C			35 min, 44°C				0 min, 25°C			35 min, 25°C			
	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Mg^{2+} (μg lost)	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Cell wall dry wt (mg)	Mg^{2+} (μg/ml)	Mg^{2+} (μg/ml of wall)	Mg^{2+} (μg lost)
MF-31								12.49	1.34	0.627	9.07	0.94	0.605	0.022
H (Str)								20.76	2.16	0.608	14.19	1.43	0.589	0.019
52A5	1.66	0.14	0.493	7.00	0.49	0.409	0.084	8.51	0.78	0.480	1.66	0.13	0.457	0.023

[a] Conditions of heat treatment.

ery in a minimal medium with glucose and galactose absent, but full recovery of the cells did not occur in the presence of chloramphenicol. Such data may rule out the cell envelope as a primary area of sublethal damage, but suggest the possibility of secondary effects influencing the degree of cellular injury.

Data presented in this study agree with previous work suggesting that the presence of magnesium on the cell envelope is beneficial to the microbial cell (29). Apparently, heating in phosphate buffer or EDTA chelated and thus removed a greater amount of magnesium from the cell than did heat treatment in buffered chloride compounds, and, as a result, cell injury was greater in the presence of phosphate or EDTA. A water wash of *S. aureus* before heat treatment was less injurious than a wash with phosphate buffer.

In addition, the importance of magnesium in the heating menstruum was shown by the comparison of heat treatment in magnesium and calcium solutions. Calcium in the injury menstruum proved to be lethal to the cells, whereas magnesium was protective during heat treatment. This effect was found with *S. aureus* 52A5, which eliminated the possibility that the calcium had bound to cell wall teichoic acid and displaced magnesium.

The exact mechanism by which calcium and magnesium act on the injured cell is unclear, but it probably involves the compound effect of calcium (from the injury menstruum) and sodium (from the enumeration agar) on an internal magnesium-binding site in the cell. A leaky cell envelope permits this interaction to occur. Calcium has been found to have a somewhat higher affinity for the uninjured cell envelope than magnesium (20).

Although it has been shown that membrane damage is not directly related to the heat injury phenomenon, the degree of cellular permeability apparently affects the extent of cell injury and death. For instance, *S. aureus* heat treated in 1.0 M sucrose showed little injury or death when compared with *S. aureus* heat treated in sucrose solution of lower concentrations. Injury was even greater in solutions of equal osmotic concentrations of glycerol, which increases membrane permeability. Mitchell and Moyle (24) found that *S. aureus* had an inherent membrane permeability to glycerol, since greater leakage occurred during heat treatment in glycerol than in sucrose. Allwood and Russell (1) showed that high concentrations of sucrose prevented leakage at temperatures ranging from 37 to 50°C. Good and Pattee (9) found that sucrose decreased membrane damage; glycerol at concentrations of 2.0 M or less did not provide the same osmotic protection.

Growth in media containing NaCl may also increase leakage during heat treatment. *S. aureus* cells grown in high salt concentrations suffer an altered, more unstable membrane, with the possible expulsion of mesosomes. Cripps and Work (4) found that the presence of 4% NaCl in the growth medium caused a corresponding increase in the amount of cellular lysis of *S. aureus*, and the surviving cells displayed morphological abnormalities. Such cells manifested abnormally thick and uneven cell walls. There was often a decrease in the level of peptidoglycan synthesized. Gilpin et al. (8) found that NaCl in the growth medium altered the distribution of membrane proteins, and Kanemasa et al. (18) demonstrated that the phospholipid content of the membrane changed under these conditions.

Exposure of TSBS-grown cells to sublethal heating in this study resulted in increased injury and death of the organism *S. aureus*. The weakened osmotic barrier, induced by NaCl-supplemented growth, may permit greater leakage of magnesium and other constituents from the heated cell.

The data presented here support the hypothesis that the cell wall teichoic acid aids in the maintenance of an accessible surface pool of magnesium for the stressed cell when there is leakage of intracellular magnesium. Under favorable conditions, the cell wall may offer few significant benefits to the cell other than osmotic protection and a dispersive charge. Apparently, when the cell membrane is damaged during sublethal heat treatment, large quantities of intracellular and cell wall-bound magnesium "buffer" the cell to the initial severe effects of heating.

S. aureus 52A5, which tacks cell wall teichoic acid, could not withstand the higher temperatures tolerated by the parent, H (Str). *S. aureus* 52A5 was also incapable of growing in the magnesium-limiting medium at 37°C. These data suggest that the cell wall teichoic acid is necessary for survival of *S. aureus* under conditions of high temperature and severe magnesium depletion. Support for this theory was provided by the greater heat damage recorded in cells in which cell wall teichoic acid was replaced by teichuronic acid, and, in addition, by the greater heat resistance of *S. aureus* MF-31 and H (Str), which contain cell wall teichoic acid rather than the weaker magnesium-binding teichuronic acid.

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