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Relationship Between Lysostaphin Endopeptidase Production and Cell Wall Composition in *Staphylococcus staphylolyticus*

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Mutants of *Staphylococcus staphylolyticus* incapable of producing an extracellular staphylolytic glycylglycine endopeptidase were isolated and found to have cells in the population susceptible to lysis by this enzyme, as did the wild-type organism under conditions in which the endopeptidase was not produced. These results suggest that cultures of this organism normally contain a heterogeneous population of cells with regard to cell wall composition and susceptibility to the enzyme. Production of the endopeptidase appears to act as a selective pressure which removes the susceptible cells in the population as the enzyme appears in the medium. A comparison of the peptidoglycan of the wild-type organism grown under conditions in which the endopeptidase was produced with that of this organism grown under nonproducing conditions and with those of endopeptidase-less mutants showed that in the presence of the endopeptidase the cell population had peptidoglycan with shorter peptide cross bridges and a greater percentage of serine in these cross bridges than was found in cells grown in the absence of the enzyme. The inability of the endopeptidase to hydrolyze glycylserine and serylglycine peptide bonds suggests that at least part of the resistance this organism has to the endopeptidase is due to relative amounts of serine found in the peptide cross bridges of some cells in the population.

Lysostaphin is a commercially available protein preparation obtained from the culture filtrate of the organism *Staphylococcus staphylolyticus* (12, 13). Lysostaphin contains three enzymes capable of acting on bacterial cell wall peptidoglycan (3, 7, 24). One of these enzymes is an endo-β-*N*-acetylglucosaminidase which will lyse some members of the genus *Micrococcus* by solubilization of the peptidoglycan backbone (7, 24). A second enzyme is an acetylmuramic acid-L-alanine amidase which appears not to be bacteriolytic, per se, but only capable of acting on previously solubilized peptidoglycan (24). The major component in lysostaphin is a glycylglycine endopeptidase which is capable of specifically lysing staphylococcal cells, especially those of *S. aureus* (4, 11, 12).

The lysostaphin endopeptidase is a zinc-metalloenzyme of molecular weight 25,000 (23). This enzyme lyses staphylococcal cells by hydrolyzing glycylglycine bonds in the polyglycine bridges which form cross-links between glycopeptide chains in the cell wall peptidoglycan of these organisms. The peptidoglycan of *S. aureus* consists of a backbone made up of alternating β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues. Tetrapeptide chains consisting of L-alanine, D-glutamine, L-lysine, and D-alanine are bound to the carboxyl groups of the muramic acid residues. These tetrapeptide chains are cross-linked by polyglycine cross bridges between the ϵ-amino group of the lysine residue of one chain and the D-alanyl carboxyl group of another chain. The peptidoglycan is insoluble due to this cross-linking of the polymers, and hydrolysis of any single chemical linkage in sufficient number within the cross-linked network can bring about solubilization of the cell wall (20).

Most strains of *S. aureus* are cross-linked by 5 or 6 mol of glycine per mol of tetrapeptide chain (14, 17). Infrequently, small amounts of glycine may be replaced by serine in the cross bridges. For example, *S. aureus* strain Copenhagen has been shown to contain 0.02 to 0.08 mol of serine per mol of glutamic acid (22). The peptidoglycan of *S. epidermidis* also contains polyglycine cross bridges, but a significantly higher amount of glycine is replaced by L-serine, in a range of 0.5 to 1.8 mol of serine per mol of glutamic acid (15, 16). *S. epidermidis* strains have been shown to be less susceptible to lysis by lysostaphin than are *S. aureus*, presumably because of the inability of the endopeptidase to

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hydrolyze serylglycine or glycylserine bonds (25, 26).

Changes in cell wall composition have been observed under different growth conditions in staphylococci. Schleifer (K. H. Schleifer, J. Gen. Microbiol. 57:XIV, 1969) has reported that growth of *S. aureus* strain Copenhagen in limiting amounts of glycine decreases the amount of cross-linking of the tetrapeptide chains. The addition of serine to the growth medium increased the serine content of the cross-linkages to about 0.5 mol of serine per mol of peptide subunit. Browder et al. (2) have reported that growth of *S. epidermidis* in different media affected the glycine and serine content in the peptidoglycan of this organism. Donegan and Riggs (5) have shown that growth of *S. aureus* in the presence of 2 M serine will cause these organisms to replace a fraction of the glycine residues in the cross bridges with serine. Cells with increased serine content in the peptidoglycan were resistant to lysis by 30 μg of lysostaphin per ml over a 20-min time period.

Mutants of staphylococci with altered cell wall composition and lysostaphin susceptibility also have been found. Zygmunt et al. (25) have reported isolating a mutant of *S. aureus* strain Copenhagen with reduced glycine content in the peptidoglycan and a concomitant increase in resistance to lysis by lysostaphin. Korman (9) has reported using UV irradiation to isolate pleiotropic mutants of *S. aureus* which incorporate increased amounts of serine into their cross bridges. These organisms also were more resistant to lysostaphin than the wild-type organism.

In this study we have isolated several endopeptidase-less mutants of *S. staphylolyticus* which are more sensitive to lysis by the endopeptidase than are populations of the wild-type organism grown in a medium in which the enzyme is produced. We have examined the chemical composition of the cell wall peptidoglycan in these mutants and the wild-type organism to determine if there is a quantitative relationship between cell wall composition and resistance of the organism to the staphylolytic enzyme it produces.

MATERIALS AND METHODS

Chemicals. Lysostaphin (lot no. AZ1975, 237 U/mg) was purchased from Schwarz/Mann. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was purchased from Aldrich Chemical Co. Glycylserine and serylglycine were purchased from Sigma Chemical Co. All other chemicals were reagent or analytical grade and were purchased from commercial sources.

Bacterial strains. *S. aureus* FDA 209P was obtained from the stock culture collection of The Department of Botany and Microbiology, University of Oklahoma, Norman, Okla. *S. staphylolyticus* (NRRL B-2628) was kindly provided by W. C. Haynes (Northern Regional Research Laboratories, Peoria, Ill.). All of the mutant strains used in this study were derived from a streptomycin- and rifampin-resistant mutant of *S. staphylolyticus* (*S. staphylolyticus* SR, which will be referred to as wild-type *S. staphylolyticus* for purposes of this study) to avoid the possible isolation of airborne contaminant staphylococci. All of the mutant strains isolated were capable of growing in the presence of streptomycin (0.5 mg/ml) and rifampin (0.5 mg/ml) and had biochemical characteristics identical to the wild-type organism.

Media. For the experiments in this study, *S. staphylolyticus* was grown in two types of media. Lysostaphin production medium was a modification of the medium recommended by Zygmunt and Browder (U.S. patent 3,398,056, August 1968). The medium contained (per liter): 60.0 g of enzymatic digest of casein (N-Z Amine E, Humko Sheffield Chemical), 5.0 g of soy peptone (Phytone, Baltimore Biological Laboratories), 5.0 g of NaCl, 2.5 g of K_2HPO_4, and 10.0 g of glycerol. The medium was adjusted to an initial pH of 7.0. CAA medium was a modification of the synthetic medium for staphylococci of Kloos and Pattee (8). The medium contained (per liter): 50.0 g of Casamino Acids (Difco, technical grade), 7.0 g of K_2HPO_4, 2.0 g of KH_2PO_4, 0.5 g of sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 0.1 g of $MgSO_4 \cdot 7H_2O$, and 1.0 g of $(NH_4)_2SO_4$. The medium was adjusted to an initial pH of 7.0.

Mutagenesis. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis was carried out by a modification of the method of Adelberg et al. (1). A 5-ml subculture of an overnight tryptic soy broth (Difco) culture of *S. staphylolyticus* SR was incubated for 3 h at 37°C on a New Brunswick model G-10 gyratory shaker at 250 rpm in an 18-mm culture tube. The cells were harvested during the logarithmic phase of growth, washed twice with a sterile 0.5 M NaCl solution, and suspended in 4.5 ml of sterile 0.05 M citrate buffer (pH 6.0). A 0.5-ml volume of a 1-mg/ml solution of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was added to the resuspended cells and, the mixture was incubated for 30 min at room temperature without aeration. The mutagenized cells were washed twice with sterile saline, suspended in 5.0 ml of tryptic soy broth, and then subcultured into another tube of tryptic soy broth and allowed to grow overnight at 37°C with shaking.

To detect nonproducing mutants, an overnight culture of the assay organism, *S. aureus* FDA 209P, was added to melted lysostaphin production agar to give a lawn of cells when plated. An appropriately diluted sample of the overnight culture of mutagenized cells was spread onto the plates, and cells lacking the ability to produce endopeptidase (End^-) were identified by the lack of a zone of cell lysis surrounding the colonies.

Endopeptidase assay. The assay organism, *S. aureus* strain FDA 209P, was grown in 100 ml of tryptic soy broth for 18 h at 37°C with shaking at 250 rpm in 500-ml Erlenmeyer flasks. The cells were harvested by centrifugation at 12,000 × *g* for 10 min at 4°C, and the cell pellet was washed twice with 0.05 M Tris-hydrochloride buffer (pH 7.5 at 25°C) containing 0.09 M NaCl (Tris-saline buffer). The cells were suspended in

the same buffer to an optical density of 0.250 at 620 nm in a Bausch and Lomb Spectronic 710 spectrophotometer. For the assay, 0.1 ml of an appropriate dilution of culture supernatant fluid from *S. staphylolyticus* was added to 11.9 ml of the standardized suspension of *S. aureus* cells in cuvettes (20 by 88 mm). After incubation for 10 min in a stationary water bath at 37°C, the decrease in optical density of the reaction mixture at 620 nm was recorded. One unit of activity was defined as a 50% reduction in turbidity of the standard cell suspension in 10 min. All results were corrected for autolysis of a cell control that did not contain enzyme.

Susceptibility to lysostaphin. (i) Susceptibility of End⁻ mutants. Cultures of several End⁻ mutants of *S. staphylolyticus* were grown in 50 ml of lysostaphin production broth for 18 h at 37°C with shaking at 250 rpm in 250-ml Erlenmeyer flasks. The cells were harvested by centrifugation at 12,000 × *g* for 10 min at 4°C, washed twice with Tris-saline buffer, and resuspended in the same buffer to an optical density of 1.0 at 620 nm in cuvettes (10 by 88 mm). Lysostaphin was added to 3.0 ml of cell suspension to give a final concentration of 40 μg/ml, and the mixture was incubated at 37°C. The optical density was recorded at 10-min intervals for 60 min. All results were corrected for autolysis of a cell control.

(ii) Susceptibility of the wild-type organism in nonproducing conditions. Wild-type *S. staphylolyticus* was grown in 50 ml of CAA broth for 18 h at 37°C with shaking at 250 rpm in a 250-ml Erlenmeyer flask. The cells were harvested and checked for susceptibility to lysis by lysostaphin as described above since we have found that the organism will not produce the endopeptidase in this medium (G. L. Sloan, unpublished data).

(iii) Susceptibility of the wild-type organism to lysostaphin prior to the onset of endopeptidase production. Twenty-nine 250-ml Erlenmeyer flasks which contained 50 ml of lysostaphin production medium were inoculated with a 1% inoculum of an 18-h culture of *S. staphylolyticus* and incubated at 37°C with shaking at 250 rpm. A flask was removed at zero time and every subsequent hour for 28 h. A 5-ml sample was centrifuged at 12,000 × *g* for 10 min at 4°C, and the supernatant fluid was assayed for the endopeptidase. The cells were checked for susceptibility to lysis by lysostaphin as described above except that optical density readings were made only at 0 and 30 min. The dry cell weight of the bacteria was determined essentially by the method of Stormonth and Coleman (19). In this procedure, a 5.0-ml volume of the culture was removed and centrifuged at 12,000 × *g* for 10 min at 4°C and suspended in 5.0 ml of distilled water. These cells were disrupted by sonic oscillation (Biosonik IV, Bronwill, VWR Scientific) for 1 min. A sample (0.1 to 1.0 ml) of the disrupted preparation was diluted with distilled water to 2.0 ml, and 2.0 ml of 10% (wt/vol) trichloroacetic acid was added. After thorough mixing, the optical density of the resulting turbid suspension was measured at 600 nm in 10-by-88-mm cuvettes. The dry cell weight was determined by the use of a standard curve prepared by obtaining dry weights of standard cell suspensions of the organism after drying samples at 75°C overnight in tared weighing pans.

Isolation and analysis of cell wall peptidoglycan. Isolation and analysis of the cell wall peptidoglycan of *S. staphylolyticus* was done by the rapid screening method of Schleifer and Kandler (14). Amino acid analysis was performed using a Beckman model 119 amino acid analyzer. Known concentrations of serine, *N*-acetylmuramic acid, and *N*-acetylglucosamine were hydrolyzed and analyzed in an identical manner as the peptidoglycan to correct for possible decomposition.

Action of lysostaphin on glycylserine and serylglycine. The ability of the endopeptidase to hydrolyze glycylserine and serylglycine peptide bonds was determined as previously described (18).

RESULTS AND DISCUSSION

Susceptibility of *S. staphylolyticus* to lysostaphin. The data in Fig. 1 show that cultures of End⁻ mutants of *S. staphylolyticus* had cells in the population which were more sensitive to the endopeptidase than the wild-type organism. However, these strains were not as susceptible as *S. aureus* FDA 209P, our assay organism for the endopeptidase, which gave a change in optical density of 0.5 U in 5 min under identical conditions. The most resistant nonproducer

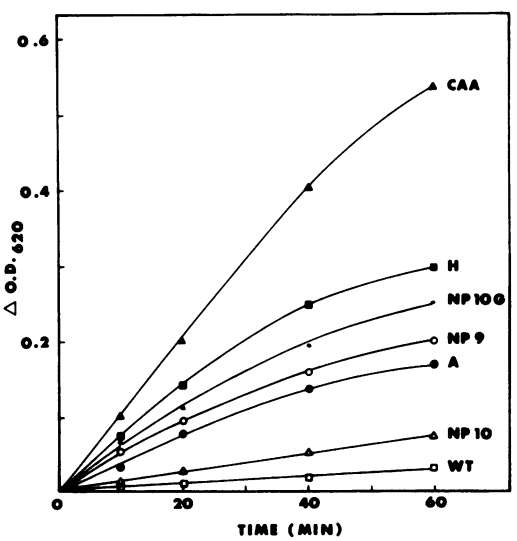

FIG. 1. *Susceptibility of various strains of S. staphylolyticus to lysis by lysostaphin. Cells were grown to stationary phase in lysostaphin production medium (except where indicated), harvested, washed and resuspended to an optical density of 1.0 (620 nm). Lysostaphin (40 μg/ml) was added, and the decrease in optical density was recorded for 60 min. Strains and conditions tested were as follows: □, wild-type; △, EndNP-10; ●, EndA; ○, EndNP-9; ⊙, EndNP-10 grown in lysostaphin production medium containing 1.5 M glycine; ■, EndH; ▲, wild type grown in CAA medium in which endopeptidase is not produced.*

(EndNP-10) became approximately four times as sensitive to lysis by the endopeptidase when grown in lysostaphin production medium which contained a high concentration of glycine (1.5 M). One possible explanation for these results might be that the mutants which had lost the ability to produce the endopeptidase also had a concomitant alteration in their cell walls which made them sensitive to the enzyme. Another possible explanation is that cultures of this organism do not contain a homogeneous population in terms of cell wall composition and/or structure, and the production of the endopeptidase is a constant selective pressure removing any susceptible cells that might arise in the population. If this pressure were removed, more sensitive cells would survive and the overall population would show some sensitivity to the endopeptidase. The sensitivity of wild-type cells, when grown in CAA broth in which the endopeptidase is not produced, seems to support the second theory. These results suggested that the endopeptidase-producing organism, even in conditions in which the enzyme would be produced, should also have susceptible cells in the population prior to the onset of endopeptidase production.

Figure 2 shows the relationship between cell growth, endopeptidase production, and susceptibility to the endopeptidase for *S. staphylolyticus*. These data show that there were endopeptidase-sensitive cells in the population prior to the onset of endopeptidase production. As endopeptidase began to appear in the culture medium the cell population became increasingly resistant to the enzyme. A similar type of adaptation has been shown to occur in *Micrococcus lysodeikticus* cells grown in the presence of lysozyme (10).

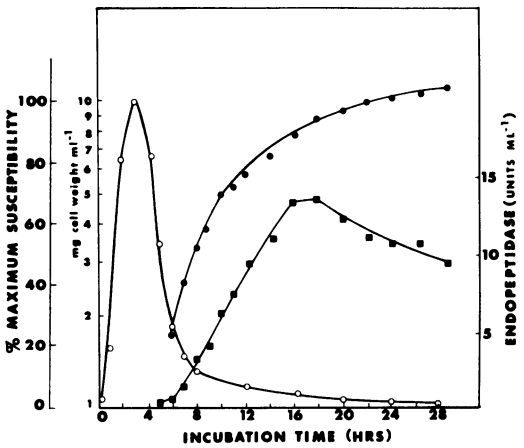

FIG. 2. *Relationship between growth, endopeptidase production, and susceptibility to lysostaphin for wild-type S. staphylolyticus in lysostaphin production medium. Cells were harvested at various stages of growth of the culture, washed, and resuspended to an optical density of 1.0 (620 nm). Lysostaphin (40 μg/ml) was added, and the decrease in optical density was recorded after 30 min. Susceptibility (○) is expressed as percent maximum susceptibility, all values being compared to an optical density change in 30 min of 0.583 U at the time of maximum susceptibility (3 h). Symbols: ■, endopeptidase concentration (units per milliliter); ●, dry cell weight (milligrams per milliliter).*

Chemical analysis of cell wall peptidoglycan of *S. staphylolyticus*. Table 1 shows the molar ratios of the amino acids and amino sugars per mole of lysine in the peptidoglycan of End⁻ mutants and wild-type *S. staphylolyticus* grown in lysostaphin production medium and the wild-type organism grown in CAA medium in which the endopeptidase is not produced. The chemical composition of the cell wall peptidoglycan in the End⁻ mutants was found to vary from the wild-type organism primarily in the relative amounts of serine and glycine found in the cross bridges. The average number of residues per cross bridge, relative amounts of serine and glycine, and a comparison of the sensitivity to lysostaphin for these organisms are presented in Table 2. These results suggest that the amount of serine in the cross bridges of the peptidoglycan may play a role in the resistance of *S. staphylolyticus* to lysis by the endopeptidase. Strains which were more susceptible to lysis by lysostaphin were found to have somewhat higher glycine-to-serine ratios in their cell wall peptidoglycan. These differences were small but reproducible. The most resistant End⁻ strain (EndNP-10), when grown in lysostaphin production medium containing a high concentration of glycine, had a concomitant increase in sensitivity and glycine-to-serine ratio in the peptidoglycan. It was found that the cells grown in this high-glycine medium also had a lower alanine content in their cell walls. A decreased alanine concentration in the cell wall of staphylococci grown in high-glycine concentrations also was shown by Hammes et al. (6). They reported that high-glycine concentrations apparently interfere with alanine incorporation in the peptidoglycan and lead to a decrease in the cross-linking of the tetrapeptide strands.

When the peptides glycylserine and serylglycine were incubated with lysostaphin and tested for hydrolysis products by thin-layer chromatography, no detectable hydrolysis products were found, indicating the endopeptidase was incapable of hydrolyzing these peptide bonds. Sloan

TABLE 1. *Molar ratios of amino acids and amino sugars in purified cell wall peptidoglycan of S. staphylolyticus*[a]

Strain	Molar ratio of:						
	Lysine	Glutamic acid	Glycine	Serine	Alanine	Glucosamine	Muramic acid
Wild type	1.00	1.02 (0.98–1.06)	2.34 (2.26–2.42)	1.31 (1.23–1.38)	1.83 (1.79–1.87)	0.79 (0.74–0.82)	1.07 (1.00–1.12)
NP-10	1.00	0.97 (0.95–0.98)	3.03 (3.00–3.05)	1.61 (1.56–1.65)	1.78 (1.75–1.79)	0.87 (0.85–0.89)	1.02 (1.00–1.04)
NP-A	1.00	0.92 (0.88–0.94)	2.86 (2.84–2.89)	1.36 (1.35–1.37)	1.82 (1.81–1.82)	0.80 (0.78–0.83)	1.07 (1.05–1.08)
NP-9	1.00	0.94 (0.94–0.95)	2.71 (2.69–2.75)	1.28 (1.26–1.30)	1.80 (1.80–1.81)	0.67 (0.67–0.68)	1.71 (1.14–1.19)
NP-10 + 1.5 M glycine	1.00	0.93 (0.87–1.00)	2.87 (2.83–2.89)	1.34 (1.30–1.40)	1.49 (1.42–1.58)	0.83 (0.79–0.90)	1.12 (1.08–1.18)
NP-H	1.00	0.90 (0.89–0.91)	2.79 (2.69–2.85)	1.29 (1.26–1.32)	1.82 (1.75–1.86)	0.77 (0.74–0.80)	1.09 (1.07–1.11)
Wild type in CAA medium	1.00	1.07 (1.05–1.09)	3.46 (3.38–3.50)	1.55 (1.54–1.55)	2.18 (2.16–2.19)	0.68 (0.62–0.71)	0.87 (0.84–0.88)

[a] Duplicate samples for each strain and condition were run. Each sample was analyzed twice on the analyzer. Results given represent the mean for these four values for each amino acid and amino sugar. Numbers in parentheses represent the range of values for the four determinations. The organisms were grown in lysostaphin production medium except as indicated.

TABLE 2. *Relative sensitivity to lysostaphin, serine and glycine content, and length of the cross bridges in peptidoglycan for S. staphylolyticus*[a]

Strain	Relative sensitivity to lysostaphin[b]	% Serine in cross bridges	Glycine/serine ratio	Avg. no. of residues in cross bridges[c]
Wild type	1.0	35.9 (35.2–36.3)	1.79 (1.75–1.84)	3.65 (3.49–3.80)
NP-10	2.5	34.7 (34.2–35.1)	1.88 (1.85–1.92)	4.64 (4.56–4.70)
NP-A	7.5	32.1 (32.1–32.2)	2.10 (2.10–2.11)	4.24 (4.19–4.26)
NP-9	9.0	32.1 (31.8–32.2)	2.12 (2.10–2.14)	3.99 (3.95–4.05)
NP-10 + 1.5 M glycine	10.6	31.8 (31.6–32.6)	2.14 (2.06–2.18)	4.21 (4.13–4.29)
NP-H	13.5	31.6 (31.4–31.9)	2.16 (2.13–2.19)	4.08 (3.95–4.17)
Wild type in CAA medium	19.0	30.9 (30.7–31.3)	2.23 (2.19–2.26)	5.01 (4.92–5.05)

[a] Data based on results given in Table 1 and Fig. 1. Numbers in parentheses represent the range of values for the four determinations.

[b] Based on lysis (change in optical density) after a 20-min reaction with 40 μg of lysostaphin per ml; all values compared to an arbitrary value of 1 for the wild-type organism.

[c] Sum of glycine and serine values from Table 1.

et al. (18) have previously shown that this enzyme will hydrolyze polyglycine substrates including diglycine. This lends support to the idea that resistance to lysis by lysostaphin in *S. staphylolyticus* cells may be due to the high serine content in their cell walls. This hypothesis also is supported by the work of Donegan and Riggs (5), Korman (9), and Zygmunt et al. (25) who showed that staphylococcal cells are more resistant to lysis by lysostaphin when they incorporate more serine and less glycine into their cross bridges.

The relative amounts of serine in the cross bridges may not be the only explanation as to why *S. staphylolyticus* is resistant to its own endopeptidase. *S. staphylolyticus* had shorter cross bridges than strains of *S. aureus*, which have been reported to have five to six glycine residues in their cross bridges (16). *S. staphylolyticus*, in conditions in which the endopeptidase was produced, had an average of approximately 3.65 residues in its cross bridges, whereas the End$^-$ strains of the organism, which were more susceptible to lysis by the endopeptidase, had more residues per cross bridge (Table 2). Longer cross bridges might reduce steric hindrance effects that limit the ability of the endopeptidase to approach susceptible bonds. It should be pointed out that there did not seem to be a direct correlation between the average number of residues in the cross bridges and the susceptibility of the End$^-$ cells to the endopeptidase. For example, strain EndNP-10 had longer cross bridges than did strain EndNP-9, but the latter organism was more sensitive to the endopeptidase.

Another possible reason for the resistance of *S. staphylolyticus* to the endopeptidase may be the presence of non-peptidoglycan components such as proteins, polysaccharides, lipids, or teichoic acids, which may protect the cross bridges from attack by the endopeptidase. We have found (G. L. Sloan, unpublished data) that treat-

ment of *S. staphylolyticus* cells with several different proteolytic enzymes (including trypsin, chymotrypsin, bromelain, subtilisin, and pronase) prior to reaction with lysostaphin does not cause an increase in the amount of cell lysis observed. The involvement of other non-peptidoglycan components in resistance to the endopeptidase cannot be ruled out by this study and indeed may be a factor in this resistance.

Tightness of the peptidoglycan net, i.e. the number of tetrapeptide chains linked by cross bridges, may also be involved in the resistance of *S. staphylolyticus* cells to the endopeptidase. The tighter the net, the greater the number of linkages that would have to be broken before the cell was lysed. Strain EndNP-10, when grown in the presence of a high concentration of glycine, may have had a looser network due to decreased cross-linking (6), perhaps partially explaining its increased susceptibility to the endopeptidase. Thickness of the peptidoglycan itself may be a factor in resistance to bacteriolytic enzymes. Suganuma (21) has shown that the thickness of the cell wall of staphylococci varies according to the strain and age of the culture. The walls of young cells are thin (about 15 nm), whereas those of older cultures are thick (about 80 nm). The smaller amount of peptidolgycan that must be removed to cause cell lysis may explain why the wild-type *S. staphylolyticus* cells were very susceptible to the endopeptidase during the early exponential phase of growth, even more so than cells grown to stationary phase in conditions in which the enzyme is not produced.

The results presented in this paper indicate that a phenotypic adaptation takes place in terms of the cell wall composition and resistance of the cell population to lysostaphin endopeptidase in cultures of *S. staphylolyticus* growing under conditions in which the enzyme is produced. The major quantitative differences in the peptidoglycan of *S. staphylolyticus* seem to involve the length of the interpeptide cross bridges and the relative amounts of serine and glycine in these bridges. Whether these differences can totally account for the resistance of the organism to the enzyme is uncertain.

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