Edman Degradation on In Vitro Biosynthesized Peptidoglycans from *Staphylococcus epidermidis*

R. H. HILDERMAN and H. G. RIGGS, Jr.

Department of Microbiology, University of Missouri School of Medicine, Columbia, Missouri 65201

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Edman degradations were performed on the pentapeptide bridges of peptidoglycans which were biosynthesized in vitro by using six different species of seryl-transfer ribonucleic acid (tRNA). The ratio of glycine to serine in each of the five bridge positions varied with the concentration of crude glycyl-tRNA contained in the reaction mixtures, and serine appeared to be incorporated in a random manner. No single species of seryl-tRNA could be identified as the one active in in vivo pentapeptide bridge synthesis; however, it can be speculated that seryl-tRNA(s) from category I could be involved in the in vivo nonrandom incorporation of serine in the bridge.

The biosynthesis and structure of the staphylococcal cell wall has been extensively covered in two review articles (2, 6). Since then, it has been reported (3) that seven species of seryl-transfer ribonucleic acid (tRNA) can be isolated from *Staphylococcus epidermidis* strain Texas 26. Six of the seven species incorporated serine into in vitro biosynthesized peptidoglycans. It was also demonstrated that, by increasing the concentration of glycyl-tRNA in the in vitro reaction mixture, the amount of serine inserted into the pentapeptide bridge of the peptidoglycan was increased. This indicates that the amount of serine incorporated into the pentapeptide bridge appears to be dependent upon the concentration of glycyl-tRNA. The six species of seryl-tRNA participating in in vitro peptidoglycan biosynthesis could be grouped into two categories based on the glycine-to-serine ratio contained in the pentapeptide bridges. Category I tRNAs (seryl-tRNA from peaks I, II, and V) produced a high glycine-to-serine ratio in the pentapeptide bridges, while category II (seryl-tRNA from peaks III, VI, and VII) had a low glycine-to-serine ratio.

Tipper and Berman (9) performed Edman degradation on the pentapeptide bridges of peptidoglycans isolated from *S. epidermidis* strain Texas 26 and *S. aureus* Copenhagen. These investigators have shown that there are only four types of pentapeptide bridges in *S. epidermidis* strain Texas 26. The amino acid sequences in these bridges are as follows from the N-terminal end:

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>glycine</td>
<td>glycine</td>
<td>glycine</td>
<td>glycine</td>
<td>glycine</td>
</tr>
<tr>
<td>(2)</td>
<td>glycine</td>
<td>glycine</td>
<td>glycine</td>
<td>glycine</td>
<td>serine</td>
</tr>
<tr>
<td>(3)</td>
<td>glycine</td>
<td>glycine</td>
<td>glycine</td>
<td>serine</td>
<td>glycine</td>
</tr>
<tr>
<td>(4)</td>
<td>glycine</td>
<td>serine</td>
<td>glycine</td>
<td>glycine</td>
<td>glycine</td>
</tr>
</tbody>
</table>

Increasing the concentration of glycyl-tRNA in the in vitro reaction mixture, the amount of serine incorporated into the pentapeptide bridge of the peptidoglycan was increased. This suggests that serine is incorporated into the pentapeptide bridge in a nonrandom manner.

Since a species of glycyl-tRNA has been isolated that appears to be involved in the biosynthesis of the peptidoglycan of *S. aureus* (8), we were interested in determining whether a specific species of seryl-tRNA incorporated serine into the nonrandom bridge sequences described by Tipper and Berman (9).

**MATERIALS AND METHODS**

**Bacterial strain.** The organism used in this study was *S. epidermidis* strain Texas 26 provided by H. F. Browder, Mead-Johnson Laboratories.

**Chemicals.** Protamine sulfate, adenosine triphosphate, and uridine diphosphate-N-acetylmuramic acid.
were purchased from Sigma Chemical Co. Uniformly labeled \(^4\)C-serine (specific activity, 115 mCi/mmol), \(^4\)C-glycine (specific activity, 70 mCi/mmol), and \(^3\)H-glycine (specific activity, 5.87 Ci/mmol) were purchased from International Chemical and Nuclear Corp. Phenylisothiocyanate and trifluoroacetic acid (TFA) were purchased from Eastman Kodak Co.

**Media and cultivation.** The medium used was composed of (per liter of water): yeast extract, 10 g; peptone, 10 g; K\(_2\)HPO\(_4\), 5 g; and glucose, 2 g. Organisms were grown for isolation of tRNA and enzymes by the following procedure. *S. epidermidis* strain Texas 26 was grown overnight at 37 °C on a Trypticase soy agar slant. The slant was used to inoculate 1 liter of the growth medium. After 8 h of growth at 37 °C, the culture was added to a fermenter containing 15 liters of the above medium. The bacteria were harvested when the cell concentration was \(4 \times 10^8\) cells/ml.

**Isolation of tRNAs and enzymes.** Isolation of seven species of seryl-tRNA, crude seryl-tRNA, crude glycyl-tRNA, as well as the aminoacyl synthetases and the particular enzymes from *S. epidermidis* strain Texas 26, have been previously described (3).

**In vitro peptidoglycan biosynthesis.** The assay mixtures and conditions for in vitro peptidoglycan biosynthesis have been described (7), except \(^3\)H-glycine replaced unlabeled glycine and 3 \(\mu\)l of penicillin G (0.25 mg/ml) were added to prevent bridge closure. Descending paper chromatography was performed to separate the peptidoglycan from the reaction product. After incubation, the entire reaction mixture was spotted on Whatman 3 MM filter paper, and the chromatogram was allowed to develop for 12 h at room temperature in a solvent containing isobutyric acid and 1 N ammonium hydroxide (5:3). The peptidoglycan remained at the origin while the lipid intermediates and amino acids migrated away from the origin. The origin was cut out, dried, and used in the determinations of the amount of serine and glycine incorporated.

**Edman degradation of the pentapeptide bridge.** The procedure used for the degradations was a modification of procedures previously described (5, 9). Duplicate incubation mixtures for each peptidoglycan were prepared. Each mixture was spotted on filter paper, and the chromatograms were developed as described above. The origin of one chromatogram was cut out and counted in a liquid scintillation spectrometer. The counts per minute for \(^4\)C-serine and \(^3\)H-glycine were determined by the method of Chase and Rabinowitz (1). The picomoles of serine and glycine were calculated, and these data were recorded as the ratio of picomoles of glycine to picomoles of serine incorporated before Edman degradation. The peptidoglycan was eluted from the origin of the second chromatogram with water, recovering 86% of the total label, and it was then subjected to Edman degradation. The peptidoglycan was dried by lyophilization and dissolved in 100 \(\mu\)l of freshly prepared buffer, composed of \(N\)-ethyl-morpholine (6 ml), of 0.2 N acetic acid (15 \(\mu\)l), and 95% ethanol (5 ml) per 10 ml of water. The mixture was incubated at 37 °C for 3 h after 10 \(\mu\)l of phenylisothiocyanate were added. After incubation, the solution was lyophilized. The residue was extracted twice with 100 \(\mu\)l of benzene to remove uncoupled phenylisothiocyanate. The benzene was removed by aspiration, and the residue was dried under an infrared lamp.

Cyclization was performed by the addition of 100 \(\mu\)l of TFA to the dried residue. This mixture was incubated at room temperature for 60 min. Then the TFA was removed by lyophilization. The dried material was dissolved in 100 \(\mu\)l of 0.2 N acetic acid, spotted on Whatman 3 MM filter paper strips, and allowed to dry under an infrared lamp. Chromatography was performed by using benzene as the solvent. The nonpolar phenylthiocarbamyl amino acids had an \(R_f\) value of about 0.5 while the peptidoglycan remained at the origin. After chromatographic separation, the filter paper was cut into 2-mm strips and counted in a liquid scintillation spectrometer. The peptidoglycan was eluted from the origin with water, and the above cycle was repeated five times.

**RESULTS**

To determine whether serine was incorporated into the in vitro biosynthesized pentapeptide bridge in a nonrandom manner, experiments were performed with the six species of seryl-tRNA active in in vitro peptidoglycan biosynthesis (3). Figure 1 shows the results of Edman degradation of the pentapeptide bridges synthesized in which the concentration of glycyl-tRNA was 0.161 U at 260 nm, and the concentration of the six seryl-tRNA species isolated were as follows: peak I, 0.561 U; peak II, 0.500 U; peak III, 0.221 U; peak V, 0.245 U; peak VI, 0.387 U; and peak VII, 1.20 U. Serine was incorporated into all bridge positions except three: position 1 from the peak I seryl-tRNA bridge and positions 1 and 2 from the peak V seryl-tRNA pentapeptide bridge. This indicates that serine is incorporated in a random manner except in the three positions mentioned. Two bridges have a very high glycine-to-serine ratio in one of their positions: position 2 in the peak II bridge and position 3 in the peak V bridge had ratios of 28:1 and 25:1, respectively.

Experiments were performed using seryl-tRNA peaks from category I and increasing the concentration of crude glycyl-tRNA from 0.161 U to 1.60 U at 260 nm while the seryl-tRNA concentrations were the same as in Fig. 1. Only category I seryl-tRNAs were used, because at this high concentration of crude glycyl-tRNA the ratio of glycine to serine in the pentapeptide bridge decreased (3). This would determine whether increasing the concentration of glycyl-tRNA would (i) result in the insertion of serine into position 1 of pentapeptide bridges biosynthesized by seryl-tRNA from peak I as well as into positions 1 and 2 of pentapeptide bridges biosynthesized by peak V, and (ii) affect the high glycine-to-serine ratio at position 2 in the bridge biosynthesized by using peak II seryl-tRNA and at position 3 in the bridge biosynthe-
of serine
ated and pentaglycine bridge which might
ly, an enzyme involved in the formation of the pentaglycine bridge of S. aureus has been iso-
ated and purified 100-fold (4). No multicom-
ponent activity has been found by these investiga-
sized by peak V seryl-tRNA. The results (Fig. 2) show that serine is inserted into every bridge position. It is also apparent that the high glycine-to-serine ratios at position 2 of bridges biosynthesized using peak II and at position 3 of bridges biosynthesized during peak V are greatly reduced (28:1 to 3.2:1 and 25:1 to 3:1, respectively).

Discussion

The results of this study indicated that, in an in vitro system, serine was incorporated into the pentapeptide bridges in a random manner. Tipper and Berman (9) found that the pentapeptide bridge sequence was nonrandom by performing Edman degradation on cell walls isolated from viable staphylococcal cells. The reason(s) for the different results in these two studies is not apparent at the present time. However, it is possible that when the particu-
late enzymes used in this study were isolated, a molecular organization of this membrane component was either altered or destroyed, a situation which might cause a random incorporation of serine into the pentapeptide bridge. Recent-
ly, an enzyme involved in the formation of the pentaglycine bridge of S. aureus has been iso-
lated and purified 100-fold (4). No multicom-
ponent activity has been found by these investiga-
tors at this stage of purification; therefore, it is not known how many enzymes are involved in the formation of the pentapeptide bridge.

Before the peptidoglycan was subjected to Edman degradation, it was eluted off the chromatogram with water and 85% of the total label was recovered. This indicates that the elution of the peptidoglycan from the chromatogram was

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FIG. 1. Edman degradation of pentapeptides of in vitro biosynthesized peptidoglycans using six species of seryl-tRNA. (a) Ratio of picomoles of \(^{3}H\)-glycine to picomoles of \(^{14}C\)-serine in the pentapeptide bridge before degradation. This ratio was calculated from the chromatogram that was counted before degradation. (b) Ratio of picomoles of \(^{3}H\)-glycine to \(^{14}C\)-serine in the pentapeptide bridge after degradation. This ratio was calculated by determining the picomoles for both glycine and serine at each bridge position. Then the total amount of each amino acid in the bridge was determined. The total picomoles of glycine were divided by the total picomoles of serine to obtain the glycine-to-serine ratio after degradation.

FIG. 2. Edman degradation of pentapeptides of in vitro biosynthesized peptidoglycans using three species of seryl-tRNA. Ratios (a) and (b) are expressed as in Fig. 1.
efficient. Six cycles of Edman degradation were performed on each pentapeptide bridge to determine if more than five amino acids were incorporated into the pentapeptide bridge. There was no activity found in the sixth cycle of the degradation (not shown), which indicated that only five amino acids were incorporated into the pentapeptide bridge in an in vitro reaction. This is consistent with the work of Kamiryo and Matsuhashi (4). The glycine-to-serine ratios before and after degradation are approximately equal, with the exception of the bridge biosynthesized by using peak II seryl-tRNA (Fig. 1 and 2). This suggests that the glycine-to-serine ratio at each position is a true representation of the relative amounts of each of these amino acids inserted at a given position. The large differences in the glycine-to-serine ratio before and after degradation found in peak II (Fig. 1) was seen in repeated experiments, and the reason for this difference is not apparent.

Our data indicate that seryl-tRNA from peaks I and V could be involved in the biosynthesis of the nonrandom pentapeptide bridge (Fig. 1). Tipper and Berman (9) showed that serine was not inserted into positions 1 or 2 in any of the pentapeptide bridges. Only bridges from peak V seryl-tRNA do not have serine in positions 1 or 2, whereas bridges from peak I seryl-tRNA do not have serine in position 1 (Fig. 1). However, when the concentration of glycyl-tRNA used in the in vitro reaction was increased (Fig. 2), serine was inserted into all five positions with seryl-tRNA from peaks I and V. Therefore, the nonrandom sequence only occurs when the concentration of glycyl-tRNA used in the reaction mixture is low.

It is interesting that the two positions in all of the bridges having the highest glycine-to-serine ratio, position 2 in bridges biosynthesized by peak II seryl-tRNA and position 3 in bridges biosynthesized by peak V seryl-tRNA (Fig. 1), occur immediately before positions 3 and 4, which have been reported (9) to have the highest serine concentration in the cell walls of S. epidermidis strain Texas 26. It could be hypothesized that these high glycine concentrations at position 2 (peak II) and position 3 (peak V) could be a control which initiates the insertion of serine into any of the remaining bridge positions. This type of a control mechanism could account for the nonrandom incorporation of serine into the pentapeptide bridge sequences reported by Tipper and Berman (9) except for position 5. Again, when the concentration of glycyl-tRNA used in the reaction mixture increases with respect to the seryl-tRNA concentration used, these high glycine-to-serine ratios at position 2 (peak II) and positions 3 (peak V) decrease (Fig. 2).

ACKNOWLEDGMENTS

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LITERATURE CITED