EFFECT OF GLYCL-tRNA CONCENTRATION ON IN VITRO SERINE INCORPORATION INTO THE PEPTIDOGLYCAN OF S. EPIDERMIDIS*

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SUMMARY

Seven species of seryl-tRNA have been isolated from S. epidermidis strain Texas 26 by the use of Plaskon CTFE powder as the support for Adogen 464 in reversed-phase chromatography. All but one of these seven seryl-tRNA incorporated ^{14}C-serine into in vitro biosynthesized peptidoglycan. This suggested that at least one of the functional roles of six of the seven seryl-tRNA peaks isolated may be involved in in vivo cell wall synthesis. It has also been shown that the ratio of glycine to serine in the pentapeptide bridge depended upon the concentration of glycyl-tRNA in the assay mixture. By increasing the concentration of glycyl-tRNA the amount of serine inserted into the bridge is increased. Thus the amount of serine incorporated into the pentapeptide bridge appears to be dependent upon the concentration of glycyl-tRNA.

The structure of the staphylococcal peptidoglycan has been reviewed by Ghuysen (1) and the biosynthesis of the peptidoglycan, for the most part has been determined (2-8). Tipper and Berman (9) have reported that there are only four types of pentapeptide bridges in Staphylococcus epidermidis strain Texas 26 peptidoglycans: pentaglycine (20%) and pentapeptides with glycine replaced by serine in the third position (55%), the first and third positions (15%) and the second position (10%) from their N-termini. This suggests that serine is incorporated into the pentapeptide bridge in a nonrandom manner. In the present communication, we report that by increasing the concentration of glycyl-tRNA used in in vitro peptidoglycan biosynthesis the amount of serine incorporated into the pentapeptide bridge increases.

METHODS

Organism. Stock cultures of S. epidermidis strain Texas 26 were maintained on tryp ticase soy agar with monthly transfers. A semi-defined medium composed of 0.5%

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K_2HPO_4, 1.0% yeast extract, 1.0% peptone and 0.2% glucose was used to grow the cells for the isolation of tRNA.

RNA isolation. The procedure for breaking the staphylococcal cells was that of Sharon and Jeanloz (10) except bentonite (10 mg/ml) and 1 mM magnesium acetate-0.1 M Tris-HCl (pH 7.4) were added to the cell suspension before disruption. After disruption the nucleic acids were precipitated from the mixture by the procedure of Zubay (11).

Isolation of Enzymes. All enzymes used in this study were isolated from S. epidermidis strain Texas 26 by procedures previously described by Petit et al. (8). The supernatant enzymes, containing the amino acid synthetases, were isolated by protamine sulfate precipitation and were used in both ^{14}C-acceptance assays and peptidoglycan biosynthesis. The particulate enzymes were isolated by differential centrifugation and were only involved in peptidoglycan biosynthesis.

tRNA Assay. The procedure used was that of Kelmer et al.(12) except 2 µl of ^{14}C-serine (specific activity of 115 mCi/pmole) were used.

Peptidoglycan Biosynthesis Assay. The assay mixtures and conditions have been described (8) except, where indicated, ^{3}H-glycine (specific activity 5.87 Ci/pmole) replaced unlabeled glycine. To isolate the peptidoglycan, 0.048 ml of each assay mixture was subjected to descending paper chromatography on Whatman 3MM filter paper with an isobutyric acid-IN ammonium hydroxide (5:3) eluting solution. Chromatography was performed for 12 hours at room temperature. 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 counted in a liquid scintillation spectrometer.

RESULTS

Isolation of Seryl-tRNA. Figure 1 presents a representative 260 nm elution profile of the nucleic acids isolated from S. epidermidis strain Texas 26 after DEAE-cellulose chromatography. The 280/260 ratio was approximately 0.5 which indicated that the eluted material was free of protein (13).

The entire 260 nm elution profile from DEAE-cellulose chromatography was

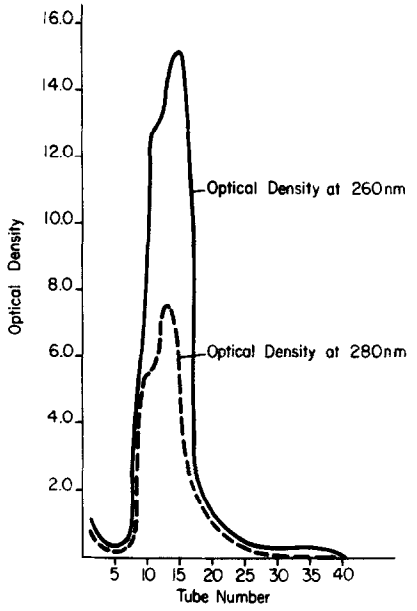

Figure 1. Optical density profile after DEAE-cellulose chromatography of nucleic acids isolated from S. epidermidis strain Texas 26. The column used was 2.5 by 45 cm. A discontinuous gradient was used to elute the tRNA. The column was first washed with one liter of a buffer containing 1 mM $MgCl_2$, 1 mM EDTA and 0.2M Tris-HCl (pH 7.0) plus 0.3M NaCl to remove soluble nucleotides from the column. Then one liter of the above buffer plus 0.7M NaCl was applied to elute off the tRNA. The flow rate was 1 ml/min and the effluent was collected in 10 ml fractions. OD at 260 nm _____. OD at 280 nm --------.

pooled and the tRNA was precipitated and discharged by the procedure of Kelmers et al. (12). Then 1700 optical density units of this sample were applied to a reversed-phase column in which Plaskon CTFE powder was used as a support for Adogen 464.

The elution profile after reversed-phase chromatography is shown in Fig. 2a. Figure 2b presents results of ^{14}C-serine acceptance assays for each tube of the elution profile from reversed-phase chromatography. The acceptance profile shows that there are seven well defined peaks of seryl-tRNA. The isolation of seven peaks of seryl-tRNA is of interest since by the most recent genetic code there are only six codons which bind seryl-tRNA.

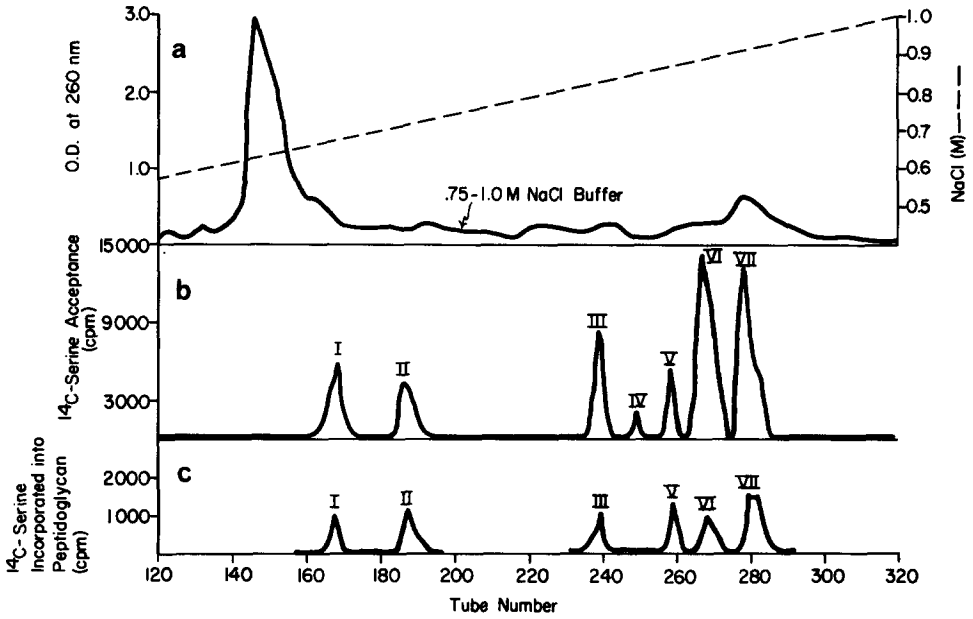

Figure 2. Reversed-phase chromatography of the nucleic acids isolated from DEAE-cellulose chromatography. (a) 260 nm elution profile after reversed-phase chromatography. The procedure used for preparation and packing of the column has been described previously (14) except a 2.5 by 45 cm column was used. The tRNA was eluted from the column by a linear gradient generated from one liter of 0.35M NaCl in Buffer A (0.01M $MgCl_2$, 1 mM 2-mercaptoethanol and 0.01M Tris-HCl (pH 7.0) and one liter of 0.75M NaCl in Buffer A. When this gradient was complete, a second gradient was formed from one liter of 0.75M NaCl in Buffer A and from one liter of 1M NaCl in Buffer A. NaCl -------. (b) ^{14}C-serine acceptance activity measured in cpm/0.1 ml. (c) ^{14}C-serine incorporated into the peptidoglycan measured in cpm/0.048 ml.

The data for the *in vitro* incorporation of ^{14}C-serine into the peptidoglycan using the seven seryl-tRNA peaks are presented in Fig. 2c. All of the peaks except No. IV stimulated *in vitro* peptidoglycan biosynthesis. These *in vitro* data suggest that at least one of the functional roles *in vivo* of six of the seven seryl-tRNA may be participation in cell wall synthesis.

In Vitro Peptidoglycan Biosynthesis. The ratio of pmoles of glycine incorporated

TABLE I

Comparison of pMoles of Amino Acids Incorporated into Pentapeptide Using Crude Glycyl-tRNA and Seryl-tRNA from Peaks I, II, III, V, VI, and VII.

Seryl-tRNA Peak	Total pMoles Inc.	pMoles Ser Inc.	pMoles Gly Inc.	Ratio[a]
I	113	14 12.4%[b]	99 87.6%	7.1:1
II	91	12 13.2%	79 86.8%	6.6:1
III	210	55 26.2%	155 73.8%	2.8:1
V	240	30 12.5%	210 87.5%	7.0:1
VI	58	13 22.4%	45 77.6%	3.5:1
VII	47	9 19.1%	38 80.9%	4.2:1

The reaction assay is the same as described in the Methods Section except ^{3}H-glycine (specific activity 5.87 Ci/mmole) replaced unlabeled glycine. Each of the six seryl-tRNA peaks were pooled and precipitated by the procedure of Kelmers et al.(12). The concentration of each peak was measured in optical density units per ml at 260 nm. The concentrations were as follows: Peak I-.561, Peak II-.560, Peak III-.221, Peak V-.245, Peak VI-.387, and Peak VII-1.20. These are the concentrations of seryl-tRNA used in this experiment. The concentration of the crude glycyl-tRNA was .161. The crude glycyl-tRNA was isolated by DEAE-cellulose chromatography. The glycyl-tRNA peak was identified by ^{14}C-glycine isoacceptance activity. A ^{14}C-serine isoacceptance assay was also performed to insure the glycyl-tRNA peak was free of seryl-tRNA. This peak of glycyl-tRNA was pooled and precipitated by the method of Kelmers et al. (12).

(a) Ratio was determined by dividing the total pmoles of ^{3}H-glycine by the total pmoles of ^{14}C-serine.

(b) Percent was calculated by dividing the pmoles of serine or glycine by the pmoles incorporated.

into the pentapeptide bridge to the pmoles of serine incorporated was determined for each species of seryl-tRNA by varying the concentrations of glycyl and seryl-tRNAs used in the assay mixture. Table I shows the results of in vitro peptidoglycan biosynthesis experiments using the six species of seryl-tRNA active in in

vitro peptidoglycan biosynthesis and crude glycyl-tRNA. From the ratio of pmoles glycine:pmoles serine it is possible to divide the six species of seryl-tRNA into two catagories. Category I contains seryl-tRNA from peaks I, II and V which biosynthesized pentapeptide bridges with glycine:serine ratios, ranging from 6.6:1 to 7.1:1. Whereas, Category II seryl-tRNAs (from peaks III, VI and VII) are involved in the formation of pentapeptide bridges with glycine:serine ratios, ranging from 2.8:1 to 4.2:1. These results suggest that the ability of seryl-tRNA to incorporate serine into the pentapeptide bridge is dependent upon the concentrations of both glycyl and seryl-tRNA in the cell.

Peptidoglycans were biosynthesized using crude glycyl-tRNA and crude seryl-tRNA at various concentrations to determine if the concentrations of either one or the other tRNAs exerted a control on the amount of serine residue in the pentapeptide bridge. The results of these experiments are shown in Table II. By increasing the concentration of crude glycyl-tRNA, the ratio of pmoles of glycine: pmoles of serine in the pentapeptide bridge decreases. When the concentration of seryl-tRNA is double the concentration of glycyl-tRNA in the assay mixture, there is a higher incorporation of both amino acids into the pentapeptide bridge, with a glycine:serine ratio of 6.6:1. This ratio of incorporation resulting from crude seryl-tRNA corresponds to that resulting from the isolated peaks of seryl-tRNA falling in Catagory I. However, when the concentration of crude seryl and glycyl-tRNA is equal or when the concentration of glycyl-tRNA is double the concentration of seryl-tRNA, there is a slight drop in the total amino acid incorporation into the pentapeptide bridges but there is significant change in the glycine:serine ratio, 2.9:1 and 2.1:1, respectively. These ratios fall in the range obtained when the isolated peaks of seryl-tRNA in Category II were used.

Experiments were then performed using each tRNA from Category I to determine if increasing the glycyl-tRNA concentration would lower the glycine:serine ratio of the biosynthesized bridge. The results of these experiments are shown in Table III. These data show that the ratios of glycine:serine in the bridge do decrease when the higher glycyl-tRNA concentration is used in their biosynthesis.

TABLE II

Comparison of pMoles of Amino Acids Incorporated into the Pentapeptide Using Crude Glycyl and Seryl-tRNAs

Ratio of Glycyl-tRNA: Seryl-tRNA	Total pMoles Inc.	pMoles Ser Inc.	pMoles Gly Inc.	Ratio[a]
1:2	137	18 13.1%[b]	119 86.9%	6.6:1
1:I	126	32 25.4%	94 74.6%	2.9:1
2:I	117	38 32.5%	79 67.5%	2.1:1

In the assay mixture ^{3}H-glycine (specific activity 5.87 Ci/mmole) was used in place of unlabeled glycine. (Crude glycyl and crude seryl-tRNAs were isolated by DEAE-cellulose chromatography and each species of tRNA was identified respectively by ^{14}C-glycine isoacceptance activity and ^{14}C-serine isoacceptance activity.) The tubes of each species of tRNA that did not overlap were pooled and precipitated by the procedure of Kelmers et al.(12). The concentrations of tRNA used in these experiments were expressed as optical density units per ml at 260 nm and are as follows: (1) 1:2 is .800 units of crude glycyl-tRNA and 1.6 units of crude seryl-tRNA; (2) 2:1 is 1.6 units of glycyl tRNA and 1.6 units of seryl-tRNA; (3) 2:1 is 1.6 units of glycyl-tRNA and .800 units of seryl-tRNA.

(a) Ratio was determined by dividing the total pmoles of ^{3}H-glycine by the total pmoles of ^{14}C-serine.

(b) Percent was calculated by dividing the pmoles of serine or glycine by the total pmoles incorporated.

At the high glycyl-tRNA concentration the ratio of glycine:serine in the bridges biosynthesized by using Peaks I, II and V are 4.4:1, 4.2:1 and 3.8:1, respectively These are now similar to the ratio of glycine:serine found when there are equal concentrations of crude glycyl and seryl-tRNAs. Tables I and III show that the percentage of pmoles of glycine incorporated decreased slightly at the higher

TABLE III

Comparison of pMoles of Amino Acids Incorporated into the Pentapeptide Using Crude Glycyl-tRNA and Seryl-tRNA from Peaks I, II, and V.

Seryl-tRNA Peak	Total pMoles Inc.	pMoles Ser Inc.	pMoles Gly Inc.	Ratio[a]
I	125	23 18.4%[b]	102 81.6%	4.4:1
II	115	22 19.1%	93 80.9%	4.2:1
V	188	39 20.7%	149 79.3%	3.8:1

Same as Table I except 1.61 O.D. units of crude glycyl-tRNA was used.

(a) Ratio was determined by dividing the pmoles of ^{3}H-glycine by the total pmoles of ^{14}C-serine.

(b) Percent was calculated by dividing the pmoles of serine or glycine by the total pmoles incorporated.

concentration of crude glycyl-tRNA. However, the percentage of pmoles of serine incorporated into the bridges for each peak increased significantly at the higher concentration of crude glycyl-tRNA. These data are consistent with the data using crude glycyl and seryl-tRNAs (Table II) in that pentapeptide bridges biosynthesized by each peak of seryl-tRNA have an increase of serine and a decrease of glycine at concentrations of glycyl-tRNA which are either equal or greater than the concentrations of seryl-tRNAs. Thus it appears that the concentration of glycyl-tRNA acts as a control for serine incorporation into the pentapeptide bridge of the peptidoglycan.

DISCUSSION

The results of this study show that there are seven well defined peaks of seryl-tRNA in S. epidermidis strain Texas 26. Other investigations have reported only four species of seryl-tRNA from bacterial systems (8). However, Kelmers and

Heatherly (15) have reported four to eight poorly defined peaks of seryl-tRNA from calf liver, while Hatfield and Portugal (16) have isolated six species of seryl-tRNA from beef liver. Since there are only six codons which bind seryl-tRNA , and since Peak IV (Fig.2) does not participate in in vitro peptidoglycan biosynthesis, it would be of interest to determine if Peak IV can participate in in vitro protein synthesis in order to determine its functional role(s) in the cell.

This study also indicates that the amount of serine incorporated into the pentapeptide bridges depends, in part, upon the concentration of glycyl-tRNA. The optimum effect of glycyl-tRNA on serine incorporation appeared to be reached when the concentration of glycyl-tRNA:seryl-tRNA was 1:1 (Table II). This indicates that the amount of serine incorporated into the pentapeptide is dependent upon the concentration of glycyl-tRNA. This hypothesis is supported by the results of Petit et al. (8) who showed that glycine incorporation into the pentapeptide bridge of S. epidermidis strain Texas 26 depended upon the presence of glycyl-synthetase while the incorporation of serine depended upon the presence of both seryl- and glycyl-synthetases.

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