Note

Binding of D-Methionine to Peptidoglycan in the Presence of Inhibitors of Cell Wall Synthesis in *Escherichia coli*

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Certain D-amino acids such as D-methionine are directly incorporated into peptidoglycan of *Escherichia coli*, where they inhibit the binding of lipoprotein to the peptidoglycan.¹⁾ This second binding reaction may be important in the normal proliferation of *E. coli*; its inhibition by D-amino acids may enhance the bacteriolytic activities of most β -lactam compounds (penicillins, cephalosporins, and others; ref. 2, and T. Tsuruoka *et al.*, in preparation), a synergistic phenomenon first observed by Lark and Lark.³⁾ Lark, Bradley, and Lark⁴⁾ observed the incorporation of D-methionine into the cell wall of *Alkaligenes*, a Gram-negative bacterium, and found that the incorporated amino acid had both the amino and carboxyl groups substituted. The mode of its binding to peptidoglycan was not known.

Izaki, Matsushashi, and Strominger, based on their enzymatic study of the biosynthesis of peptidoglycan,⁵⁾ proposed that D-amino acids are incorporated into peptidoglycan through a reverse reaction of a DD-transpeptidase (a peptidoglycan-crosslinking enzyme) sensitive to penicillin because of penicillin-binding protein-1Bs.⁶⁾ By this enzyme activity, D-amino acids could bind to peptidoglycan by exchange between the amino group of the D-amino acid and the D-center amino group of *meso*-diaminopimelic acid (A_2pm) that has been involved in crosslinking of peptidoglycan. This reaction would form a peptide linkage between the amino group of the D-amino acid and the carboxyl group of the terminal D-alanine moiety of the tetrapeptide (L-alanyl-D-glutamyl-*meso*-diaminopimelyl-D-alanine) side chain of the peptidoglycan. Consequently, the carboxyl group of the D-amino acid could remain unsubstituted, which is not in agreement with the observation of Lark *et al.*⁴⁾

The incorporation of D-methionine into peptidoglycan

in *E. coli* is unaffected by the presence of 500 μ g/ml β -lactam compounds such as benzylpenicillin and ampicillin.¹⁾ Therefore, this incorporation does not involve the mechanism proposed by Izaki *et al.*⁵⁾ The D-amino acid incorporated into peptidoglycan had an amino group substituted,¹⁾ but whether the carboxyl group of D-amino acid was also substituted, as suggested by Lark *et al.*⁴⁾ was

FIG. 1. Hydrazinolysis of D-[¹⁴C]Methionyl Muropeptides.

An autoradiogram of a paper electropherogram is shown. A is D-[¹⁴C]methionyl muropeptides; B, hydrazinolysate of A; C, D-[¹⁴C]methionine.

Preparation of D-[¹⁴C]methionyl muropeptides by incorporating D-[¹⁴C]methionine into peptidoglycan in *E. coli* and isolating the peptidoglycan followed by digestion with lysozyme was as described elsewhere (1). Hydrazinolysis was as follows. D-[¹⁴C]methionyl muropeptide (1050 dpm, 4 μ l) in a small glass tube (6 mm in diameter) was mixed with unlabeled DL-methionyl-DL-methionine (2 μ mol, 20 μ l) carrier and freeze-dried. Anhydrous hydrazine (Aldrich, 200 μ l) was added and the tube sealed under evacuation and heated at 100°C for 8 hr. Then the tube was opened and the mixture dried *in vacuo*. The residue was dissolved in 20 μ l of water, 10 μ l of which was subjected to paper electrophoresis using Toyo-Roshi No. 51B filter paper with pyridine-acetic acid-water (10:0.4:90 by volume). The electrophoresis was for 20 min at 22°C with the electric voltage difference of 55 V per cm. Formation of both methionine and methionine hydrazide from the carrier dipeptide was checked by ninhydrin reaction.

not known.

Here, the presence of the free carboxyl group of D-methionine in the D-methionine-peptidoglycan complex is studied. Hydrazinolysis of D-[14 C]methionyl muropeptides obtained by lysozyme treatment of a complex of peptidoglycan and D-[14 C]methionine gave a radiolabeled product that had the same paper electrophoretic mobility as [14 C]methionine (Fig. 1). No radioactivity was found in the position of the methionine hydrazide. The radioactive product was also identical with methionine in two-dimensional thin-layer chromatography on Silica Gel G (E. Merck; solvent, 1st run, *n*-butanol-acetic acid-water, 4:1:2, v/v/v, 2nd run, isobutyric acid-1 M ammonia, 1:0.6, v/v; data not shown).

These results may mean that the D-methionine was bound to peptidoglycan by its amino group, and that the carboxyl group did not participate in the binding, at least in the final form of the complex. Probably other D-amino acids bind to peptidoglycan in a similar way.

We could not understand one observation. [14 C]Methionyl muropeptide obtained by lysozyme digestion of the reaction product gives two radioactive spots upon paper chromatography (solvent, isobutyric acid-1 M ammonia, 1:0.6, v/v), which we referred to elsewhere as products A (with the higher *R_f*) and B (with the lower *R_f*).¹⁾ The *R_f* of product A was higher than that of *N*-acetylglucosaminyl-*N*-acetylmuramyl-tetrapeptide (L-Ala-D-Glu-*meso*-A₂pm-D-Ala referred to as C6) and the *R_f* of product B was slightly lower. Upon elution of products A and B from the paper with water and chromatography, both fractions had chromatographic mobility similar to that of the original product B. Treatment of the eluted fractions of products A and B with penicillin-insensitive DD-endopeptidase,⁷⁾ which hydrolyses the crosslinked muropeptide dimer molecule bis(*N*-acetylglucosaminyl-*N*-acetylmuramyl-tetrapeptide) to give two molecules of C6, did not change the chromatographic mobilities of the compounds in the eluted fractions (data not shown). Probably product A is labile and easily hydrolyzed by drying, to form a compound with an *R_f* similar to that of product B. This degradation product could be product B, but we are not sure. In the hydrazinolysis experiment here, we used the same lysozyme-treated reaction mixture that gave two spots on the

paper chromatogram, and it seems unlikely that product A decomposed before its contact with hydrazine to form a product that released free methionine by hydrazinolysis.

We have not yet found which group in the peptidoglycan binds to the amino group of the D-amino acid. There are several possibilities: peptidoglycan has several carboxyl groups in its repeating-unit muropeptides. The most likely is the carboxyl group at the L-center of *meso*-diaminopimelic acid, because this group is the place where lipoprotein binds, linking the peptidoglycan to the outer membrane.⁸⁾ Addition of certain D-amino acids decreased the ratio of the peptidoglycan-bound form of lipoprotein over the free form.¹⁾ If we assume that the linkage between the D-amino acid and peptidoglycan was at the L-center carboxyl group of *meso*-diaminopimelic acid, the binding of D-amino acids to peptidoglycan may be done by an LD-transpeptidase. A similar mechanism probably fractions in binding the ϵ -amino group of the C-terminal lysine moiety of the lipoprotein to the L-center carboxyl group of *meso*-diaminopimelic acid of the peptidoglycan.

REFERENCES

- 1) T. Tsuruoka, A. Tamura, A. Miyata, T. Takei, K. Iwamatsu, S. Inouye and M. Matsuhashi, *J. Bacteriol.*, **160**, 889 (1984).
- 2) M. Matsuhashi, T. Tsuruoka, Y. Yamada, H. Goi, A. Miyata, A. Tamura, T. Takei, S. Amano and S. Inouye, Proc. 13th Int. Congress of Chemotherapy, Vol. Tom 4, 1983, pp. 58/1 ~ 58/5.
- 3) C. Lark and K. G. Lark, *Can. J. Microbiol.*, **5**, 369 (1959).
- 4) C. Lark, D. Bradley and K. G. Lark, *Biochim. Biophys. Acta.*, **78**, 278 (1963).
- 5) K. Izaki, M. Matsuhashi and J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.*, **55**, 656 (1966).
- 6) J. Nakagawa, S. Tamaki, S. Tomioka and M. Matsuhashi, *J. Biol. Chem.*, **259**, 13937 (1984).
- 7) S. Tomioka and M. Matsuhashi, *Biochem. Biophys. Res. Commun.*, **84**, 978 (1978).
- 8) V. Braun, *Biochim. Biophys. Acta*, **45**, 335 (1975).