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Cadaverine Is Covalently Linked to Peptidoglycan in *Selenomonas ruminantium*

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Cadaverine was found to exist as a component of cell wall peptidoglycan of *Selenomonas ruminantium*, a strictly anaerobic bacterium. [^{14}C]cadaverine added to the growth medium was incorporated into the cells, and about 70% of the total radioactivity incorporated was found in the peptidoglycan fraction. When the [^{14}C]cadaverine-labeled peptidoglycan preparation was acid hydrolyzed, all of the ^{14}C counts were recovered as cadaverine. The [^{14}C]cadaverine-labeled peptidoglycan preparation was digested with lysozyme into three small fragments which were radioactive and were positive in ninhydrin reaction. One major spot, a compound of the fragments, was composed of alanine, glutamic acid, diaminopimelic acid, cadaverine, muramic acid, and glucosamine. One of the two amino groups of cadaverine was covalently linked to the peptidoglycan, and the other was free. The chemical composition of the peptidoglycan preparation of this strain was determined to be as follows: L-alanine–D-alanine–D-glutamic acid–*meso*-diaminopimelic acid–cadaverine–muramic acid–glucosamine (1.0:1.0:1.0:1.0:1.1:0.9:1.0).

The cell wall of a gram-negative bacterium such as *Escherichia coli* and *Salmonella typhimurium* consists of two layers: the outer membrane layer and the peptidoglycan layer. The outer membrane contains lipopolysaccharide, protein, phospholipid, and a free form of lipoprotein described by Inouye et al. (8). The peptidoglycan layer contains lipoprotein described by Braun and Rehn that is covalently linked to the peptidoglycan (bound form of lipoprotein) (1). The bound form of lipoprotein at least plays a role in the maintenance of the structural integrity of the outer membrane of the cell envelope (21, 17). The *lpo* or *mlp* mutant of *E. coli* shows the physiological and morphological alterations of the cell envelope, such as cell lysis by EDTA and the formation of membrane blebs by Mg^{2+} starvation (21, 17). Kamio and Takahashi reported previously (11) that the wild type of *Selenomonas ruminantium* subsp. *lactilytica*, a strictly anaerobic, gram-negative strain, contains no detectable protein component in the cell wall fraction corresponding to the lipoprotein in *E. coli*. However, no significant cell lysis of *S. ruminantium* occurs with a high level of EDTA (11). These results led us to examine the composition of the peptidoglycan of *S. ruminantium* chemically. In this paper, we report the presence of cadaverine, which is a component of the peptidoglycan of *S. ruminantium*, and describe the chemical composition of the peptidoglycan of this strain. (A preliminary account of this paper has appeared [9a].)

MATERIALS AND METHODS

Bacterial strain. *Selenomonas ruminantium* subsp. *lactilytica*, described in a previous paper (11), was used.

Medium and cultural conditions. *S. ruminantium* was grown in a yeast extract-glucose medium (11) supplemented with 0.01% sodium *n*-valerate at 37°C under anaerobic conditions (12).

Preparation of ^{14}C-amino acids- or [^{14}C]cadaverine-labeled cells. The yeast extract-glucose medium (20 ml) containing *n*-valerate and either 50 μCi of an L-*U*-^{14}C-amino acid mixture or 10 μCi of [1,5-^{14}C]cadaverine (4.5 μM) was inoculated with 0.1 ml of an overnight culture of *S. ruminantium* and incubated at 37°C for 4 h. The cells were collected and used for preparation of the peptidoglycan.

Preparation of peptidoglycan. Preparation of the peptidoglycan was done essentially by the procedure of Yanai et al. (20) with the following exceptions. (i) During the preparation, α-amylase was used to degrade the high-molecular-weight glycogen (19) which contaminated the peptidoglycan fraction. (ii) Pronase or trypsin, used for release of the bound form of lipoprotein from the peptidoglycan of *E. coli*, was not used at any stage during the isolation procedure because of the absence of the bound or free form of lipoprotein in this strain. (iii) Finally, the peptidoglycan fraction was treated with 3% cold perchloric acid. The typical protocol is shown in Fig. 1.

For preparation of peptidoglycan from ^{14}C-amino acids- or [^{14}C]cadaverine-labeled cells, a similar but scaled-down procedure was employed.

Solvent systems. The following solvent systems were used for cellulose thin-layer chromatography, silica gel thin-layer chromatography, and paper chromatography: (i) ethanol-acetic acid-water (2:1:2 [vol/

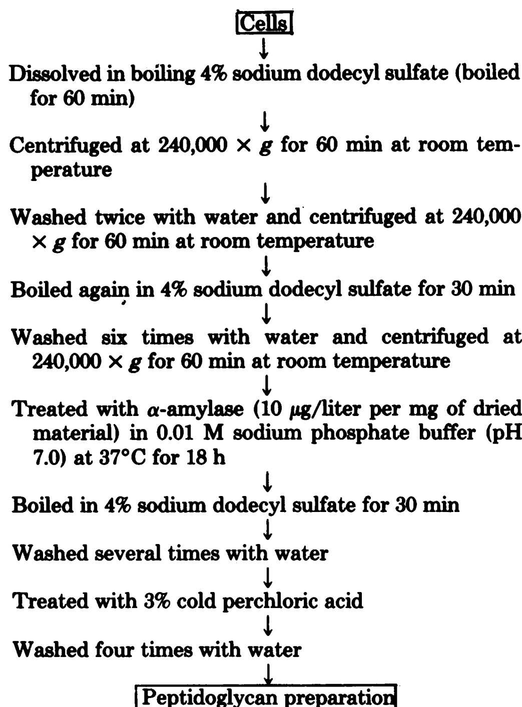

FIG. 1. *Flow sheet of peptidoglycan preparation.*

vol/vol]); (ii) *n*-butanol-acetic acid-water-pyridine (15:3:12:10 [vol/vol/vol/vol]); (iii) *n*-propanol-11.5 N HCl-water (8:3:2 [vol/vol/vol]); (iv) phenol-water (250:75 [wt/wt]) in NH_3 atmosphere; (v) methyl cellosolve-propionic acid-water (14:3:3 [vol/vol/vol]), saturated with NaCl; (vi) benzyl alcohol-chloroform-methanol-water-15 N NH_4OH (30:30:30:6:2 [vol/vol/vol/vol]); (vii) methanol-15 N NH_4OH (7:3 [vol/vol]); (viii) the upper phase of *n*-butanol-acetic acid-water (4:1:5 [vol/vol/vol]); and (ix) methanol-pyridine-10 N HCl-water (80:10:2.5:17.5 [vol/vol/vol/vol]).

Isolation of glutamic acid, alanine, and diaminopimelic acid from the HCl hydrolysate of the peptidoglycan preparation. The purified peptidoglycan preparation was hydrolyzed in 6 N HCl at 100°C for 14 h, and HCl was removed by evaporation. The hydrolysate was applied to a Dowex 50-X8 (type H) column. The amino acids and amino sugars were eluted with 2 N NH_4OH. Then the eluate was evaporated to dryness, and the residue was applied to paper chromatography by using solvent system (ix). Glutamic acid, alanine, and diaminopimelic acid were eluted from the paper with distilled water. The purity of each amino acid was checked by cellulose thin-layer chromatography by using solvent systems (i), (ii), and (iv). These purified amino acids were used for determination of their optical configurations.

Digestion of the peptidoglycan preparation with lysozyme. Lysozyme digestion was carried out by the method of Primosigh et al. (15).

Analytical procedure. UV absorption of the peptidoglycan suspension was measured with a Shimazu (Kyoto, Japan) UV200 recording spectrophotometer.

The amount of amino acid and amino sugar in the acid hydrolysate of the peptidoglycan preparation was determined quantitatively by a Hitachi (Tokyo, Japan) 835 automatic amino acid analyzer with alanine, glutamic acid, diaminopimelic acid, glucosamine, and muramic acid as standards. For quantitative determination of cadaverine, a portion of the acid hydrolysate of the peptidoglycan preparation was applied to paper for electrophoresis with cadaverine as the standard by the method of Inoue and Mizutani (7).

Optical configuration of glutamic acid was determined by the method of Kotani et al. (14). Glutamic acid from the peptidoglycan fraction and standard L- and D-glutamic acids were treated with L-glutamic acid decarboxylase, and the residual amount of glutamic acid was determined by ninhydrin reaction after a portion of the reaction mixture was paper chromatographed by using solvent system (ix). The amount of D-alanine was determined by a modification (16) of the method described by Johnson et al. (9). *meso*-Diaminopimelic acid was determined by the method of Bricas et al. (3).

2-Keto-3-deoxyoctulosonic acid and phospholipids were determined by methods described previously (10).

Radioactivity was quantitated with a Packard 3255 liquid scintillation spectrometer with the scintillation fluid of Bray (2).

Materials. Sodium dodecyl sulfate was obtained from BDH Chemical, Ltd., Poole, England. α-Amylase (4×, crystallized from *Bacillus subtilis*), egg white lysozyme, diaminopimelic acid, muramic acid, and D-amino acid oxidase were obtained from Sigma Chemical Co., St. Louis, Mo. Glucosamine hydrochloride, cadaverine dihydrochloride, and L-glutamic acid decarboxylase were from Wako Pure Chemical Industries Ltd., Tokyo, Japan. Cadaverine dihydrochloride was recrystallized in ethanol-water at 4°C. L-^{14}-amino acid mixture and [1,5-^{14}C]cadaverine dihydrochloride were purchased from New England Nuclear Corp., Boston, Mass. The other chemicals used were of the best grade commercially available.

Cellulose thin-layer plates and Silica Gel 60 plates were purchased from E. Merck AG, Darmstadt, Germany. For paper chromatography, Whatman 3MM filter paper was obtained from W. & R. Balston Ltd., England.

RESULTS

Purity of the peptidoglycan preparation. The peptidoglycan suspension in water did not show any absorption peak between 250 and 350 nm. Neither protein, lipopolysaccharide, or phospholipid was detected in the peptidoglycan preparation. From these results, it was determined that the peptidoglycan preparation was free from nucleic acids, aromatic compounds, and membrane components.

Amino acid and amino sugar composition

of *S. ruminantium* peptidoglycan. The elution profile of the acid hydrolysate of the peptidoglycan preparation is shown in Fig. 2. Glutamic acid, alanine, diaminopimelic acid, muramic acid, and glucosamine were found, but no other amino acid, especially lysine and arginine, which are representative amino acids of bacterial lipoprotein, was found. These results do not conflict with previous results (11) which revealed the absence of the protein corresponding to the bound form of lipoprotein of *E. coli* in this strain.

Cellulose thin-layer chromatography of the acid hydrolysate of the peptidoglycan preparation. A thin-layer chromatogram of the acid hydrolysate of the ^{14}C-amino acids-labeled peptidoglycan preparation is shown in Fig. 3. Four radioactive spots were detected. Spots a, b, and c had R_f values identical to those of authentic DL-glutamic acid, diaminopimelic acid, and DL-alanine, respectively. In addition, we found a unique radioactive spot (designated as spot d) which was positive in ninhydrin reaction.

Isolation and identification of the spot d compound. The purified peptidoglycan preparation (15 g) from the cells of a 2,000-liter culture was hydrolyzed. The hydrolysate was applied to a Dowex 50-X8 (type H) column (3 by 35 cm). After amino acids and amino sugars were eluted with 2 liters of 2 N NH_4OH, the column was washed with water and 1 N HCl until no ammonium ion was detected. Then, the spot d compound was eluted with 1 liter of 6 N HCl. Crystals were formed during the evaporation to remove HCl from the eluate. Recrystallization was performed in ethanol-water at 4°C. The crystals (650 mg) of the compound thus obtained were analyzed and determined to be cadaverine dihydrochloride from the following results. (i) The crystals had R_f values identical to those of authentic cadaverine dihydrochloride as determined on cellulose thin-layer plates by use of the solvent systems (i) to (v) described above. (ii) The crystals were subjected to elemental analysis and were found to contain the following: C, 34.3%; H, 9.22%; N, 15.9%; and Cl, 40.6%. Calculations for cadaverine dihydrochloride revealed the following: C, 34.3%; H, 9.21%; N, 16.0%; and Cl, 40.5%. (iii) The crystals were subjected to infrared spectrum and nuclear magnetic resonance analyses. The infrared spectrum of the crystals (Fig. 4A) was identical to that of authentic cadaverine dihydrochloride (Fig. 4B). The characteristically large absorption of $-NH_3^+$ derived from the hydrochloride salt of the primary amine, which appeared between 2,900 and 3,200 cm^{-1}, was detected in both samples. The nuclear magnetic resonance spectrum of the crystals was also identical to that of authentic cadaverine dihydrochloride (Fig. 5A and B). The nuclear magnetic resonance spectrum of the crystals indicated the following: (i) the methyl group was absent, and all signals were from methylene group; and (ii) the carbon chain was straight and symmetrical. These results indicated that the crystals of the spot d compound were cadaverine dihydrochloride.

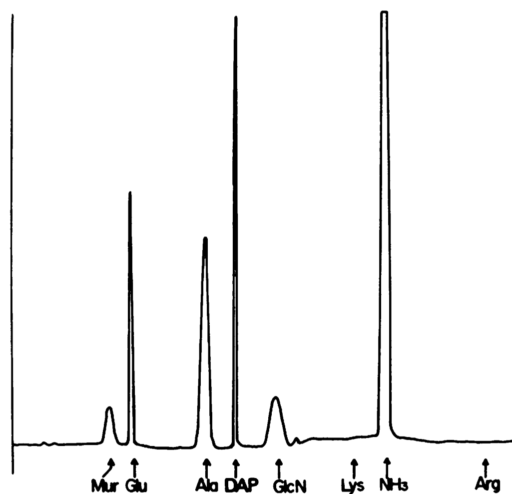

FIG. 2. *Elution profile of the hydrolysate of S. ruminantium cell wall peptidoglycan on an automatic amino acid analyzer. (Mur) Muramic acid; (Glu) glutamic acid; (Ala) alanine; (DAP) diaminopimelic acid; (GlcN) glucosamine; (Lys) lysine; (Arg) arginine.*

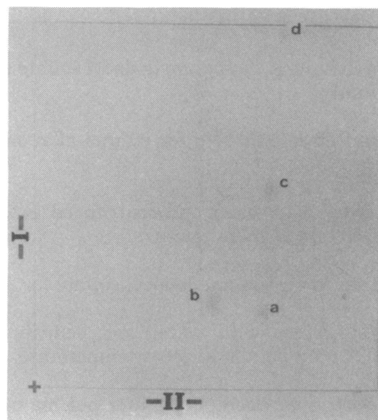

FIG. 3. *Radioautogram of the acid hydrolysate from the ^{14}C-amino acids-labeled peptidoglycan preparation. Solvent systems (iv) and (i) (see the text) were used for the first (I) and second (II) dimensions, respectively.*

Determination of optical configurations of glutamic acid, alanine, and diaminopimelic acid. The quantity of glutamic acid was unchanged on treatment with L-glutamic acid

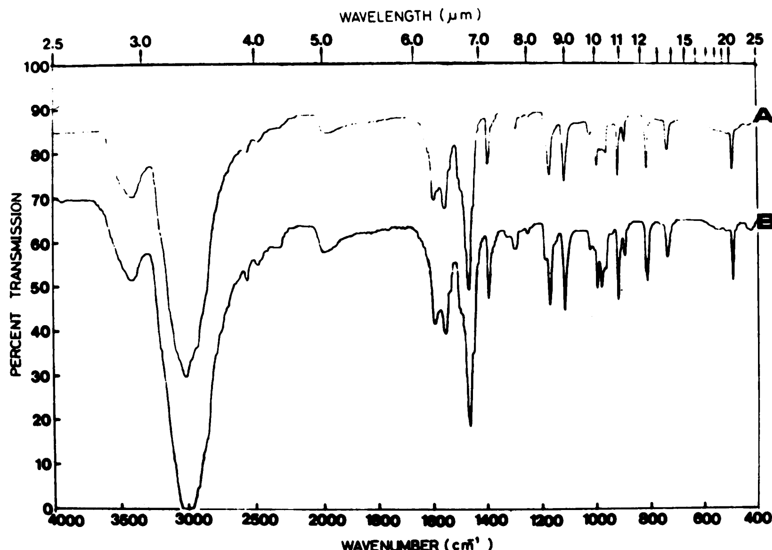

FIG. 4. *Infrared absorption spectra of the crystals (A) and authentic cadaverine dihydrochloride (B) (KBr). The absorption spectra were recorded with a Hitachi 285 infrared spectrophotometer.*

decarboxylase, and about 50% of the alanine disappeared after treatment with D-amino acid oxidase. These enzymatic analyses indicated that the glutamic acid residue had a D configuration and that the ratio of D-alanine to L-alanine residues was about one. To elucidate the optical configuration of diaminopimelic acid, the sample and authentic diaminopimelic acid (a mixture of D,D, L,L, and *meso* types) were treated with dinitrophenol and were silica gel thin-layer chromatographed by using solvent system (vi). Under these conditions, dinitrophenyl-D,D-diaminopimelic acid and dinitrophenyl-L,L-diaminopimelic acid have identical R_f values, and dinitrophenyl-*meso*-diaminopimelic acid gives an R_f value smaller than either of the former (3). As shown in Fig. 6, the dinitrophenylated sample had an R_f value identical to that of dinitrophenyl-*meso*-diaminopimelic acid, and no spot corresponding to the spot of dinitrophenyl-D,D-diaminopimelic acid and dinitrophenyl-L,L-diaminopimelic acid was detected. These results clearly indicate that the diaminopimelic acid residue of the peptidoglycan of this strain is a *meso* type.

Incorporation of labeled cadaverine into the peptidoglycan preparation. To determine the location of cadaverine in *S. ruminantium*, [1,5-^{14}C]cadaverine was added to the growth medium of the cells. About 70% of the total radioactivity incorporated into the cells was recovered in the peptidoglycan fraction. The [^{14}C]cadaverine-labeled peptidoglycan preparation was divided into two portions, one of which was hydrolyzed and cellulose thin-layer chromatographed. The other was digested with lysozyme and was paper chromatographed. When the [^{14}C]cadaverine-labeled peptidoglycan fraction was acid hydrolyzed, all of the ^{14}C counts were found to be recovered as cadaverine (Fig. 7). Three radoactive and ninhydrin-positive spots, including one major spot (designated "A") appeared in the paper chromatogram of the lysozyme digest (Fig. 8, sample 1), whereas no radioactive spot except at the origin was detected in the sample without lysozyme treatment (Fig. 8, sample 2). When the spot "A" compound was eluted with water from the paper, hydrolyzed in 6 N HCl, and applied to cellulose thin-layer chromatography with solvent systems (i) and (ii), alanine, diaminopimelic acid, glutamic acid, cadaverine, muramic acid, and glucosamine were detected. These results showed that cadaverine was one of the components of the peptidoglycan of this strain. The size of the spot "A" compound has not been determined yet.

Linkage of cadaverine to the peptidoglycan. [^{14}C]cadaverine-labeled peptidoglycan preparation was dinitrophenylated and acid hydrolyzed. The hydrolysate was silica gel thin-layer chromatographed. As shown in Fig. 9, dinitrophenyl [^{14}C]cadaverine migrated to the front, and [^{14}C]cadaverine stayed at the origin on the silica gel plate. The hydrolysate of the dinitrophenylated peptidoglycan migrated to the area corresponding to an R_f value of 0.4. These results led us to the conclusion that one of two amino groups were covalently linked to the peptidoglycan, and the other was free.

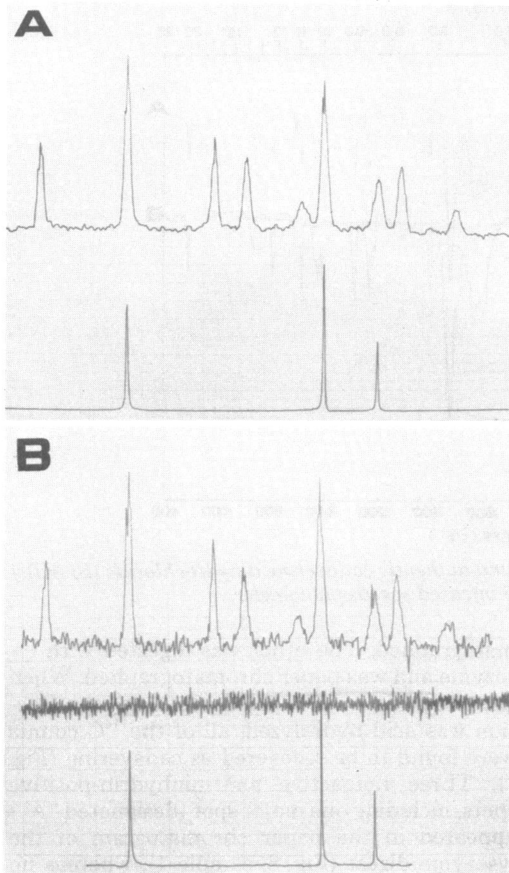

FIG. 5. *Nuclear magnetic resonance spectra of the crystals (A) and authentic cadaverine dihydrochloride (B). The ^{13}C-Four Transform nuclear magnetic resonance spectra were recorded in sample tubes of 10-mm diameter on a JEOL FX-100 nuclear magnetic resonance spectrometer at 25.5 MHz.*

It should be also noted that the radioactive cadaverine was not released from the peptidoglycan preparation despite the treatment with 10% boiled trichloroacetic acid for 30 min or chloroform-methanol (1:3 [vol/vol]) at 80°C for 60 min.

Quantitative analysis of amino acid, amino sugar, and cadaverine of the peptidoglycan preparation. The amounts of amino acid, amino sugar, and cadaverine of the acid hydrolysate were determined quantitatively. The following results were obtained: glutamic acid-alanine-diaminopimelic acid-cadaverine-muramic acid-glucosamine (689:1430:695:754:653:703 [nmol/mg of peptidoglycan preparation]). From this result, the molar ratios of L-alanine, D-alanine, *meso*-diaminopimelic acid, cadaverine, muramic acid, and glucosamine to D-glucose were calculated to be 1.0, 1.0, 1.0, 1.1, 0.9, and 1.0, respectively.

DISCUSSION

The occurrence, biosynthesis, and function of polyamines in a wide variety of organisms have been demonstrated (4, 18). However, no work

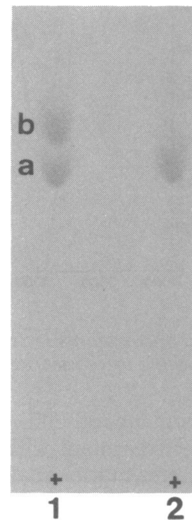

FIG. 6. *Thin-layer chromatogram of dinitrophenyl diaminopimelic acid from the peptidoglycan preparation. Dinitrophenylation was carried out by the method of Yanai et al. (20). (1) Standards. (a) Dinitrophenyl-meso-diaminopimelic acid; (b) dinitrophenyl-D,D-diaminopimelic acid plus dinitrophenyl-L,L-diaminopimelic acid. (2) Dinitrophenyl diaminopimelic acid from the hydrolysate of the peptidoglycan preparation.*

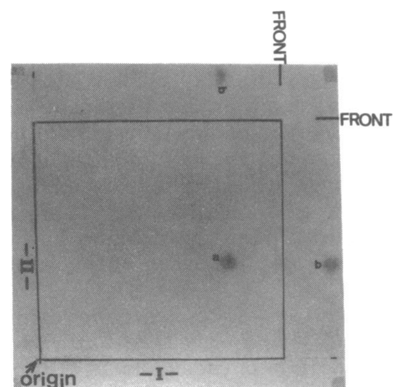

FIG. 7. *Radioautogram of the hydrolysate from the [^{14}C]cadaverine-labeled peptidoglycan preparation. Solvent systems (i) and (iii) (see the text) were used for the first (I) and second (II) dimensions, respectively. (a) Radioactive spot from the sample; (b) authentic [^{14}C]cadaverine.*

concerning the presence of polyamines in cell wall peptidoglycan has been reported. Our present studies clearly demonstrated the presence of cadaverine as one of the components of the peptidoglycan in *S. ruminantium* and also confirmed the absence of the bound form of lipoprotein in this strain. Gmeiner showed in *Proteus mirabilis* (6) that although the exponentially growing cells do not contain any covalently linked lipoprotein, the stationary cells possess it in an amount similar to those found in *E. coli* and *S. typhimurium* during all growth phases. In *S. ruminantium,* the free and bound forms of lipoprotein were not detected in either exponential or stationary phases. To our knowledge, this is the first report which pointed out the presence of covalently linked polyamine to the peptidoglycan in bacteria. With regard to the origin of cadaverine, our recent studies revealed that cadaverine in *S. ruminantium* is synthesized from L-lysine by lysine decarboxylase in vitro (unpublished data).

In *E. coli,* the bound form of lipoprotein has an important role in the outer membrane assembly on peptidoglycan in vitro (5). Although the biological function(s) of the peptidoglycan-bound cadaverine in *S. ruminantium* remains to be elucidated, we propose that the cadaverine in peptidoglycan might associate with the outer membrane components, such as phospholipids or acidic proteins, by ionic interaction and might play a similar role to that of the bound form of lipoprotein in *E. coli.*

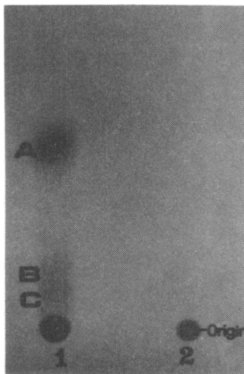

FIG. 8. *Radioautogram of the lysozyme digest of the [^{14}C]cadaverine-labeled peptidoglycan preparation. [^{14}C]cadaverine-labeled peptidoglycan was incubated at 37°C for 20 h with one mg of lysozyme in 0.5 ml of 0.1 N ammonium acetate containing 5 µl of toluene. The reaction mixture was examined by descending paper chromatography on Whatman 3MM paper at room temperature for 50 h with solvent system (viii) (see the text).*

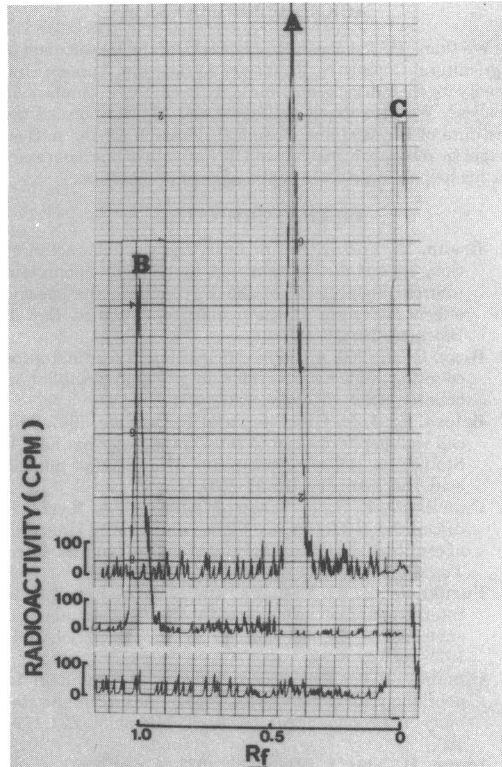

FIG. 9. *Radioscanner patterns of the hydrolysate from the dinitrophenylated peptidoglycan preparation. (A) Hydrolysate from the dinitrophenylated peptidoglycan. (B) Dinitrophenyl [^{14}C]cadaverine. (C) [^{14}C]cadaverine. Radioactivity was detected by an Irigaku (Tokyo, Japan) TRM 1-B radioscanner equipped with a windowless gas flow counter.*

Kato et al. reported recently (13) the presence of lanthionine, a sulfur-containing diamino acid, in the peptidoglycan of *Fusobacterium nucleatum,* which is an anaerobic bacterium. They also showed that the lanthionine is an essential dibasic amino acid involved in cross-linkages between stem peptide subunits in *F. nucleatum* (13). In *S. ruminantium,* cadaverine seems not to be involved in cross-linkage because one of the two amino groups of cadaverine is free. From our preliminary data of N-terminal and C-terminal amino acid analyses, *meso*-diaminopimelic and residue seemed to participate in cross-linkage. To confirm this possibility, the isolation and identification of some fragments of a cross-linked portion of the peptidoglycan obtained by use of a lytic enzyme or partial hydrolysis should be performed.

At present, our studies are focused on how and where cadaverine is covalently linked to the peptidoglycan and on its biological function.

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LITERATURE CITED

1. **Braun, V., and K. Rehn.** 1969. Chemical characterization, spatial distribution and function of lipoprotein (murein lipoprotein) of the *E. coli* wall: the specific effects of trypsin on the membrane structure. Eur. J. Biochem. **10:**426–438.
2. **Bray, G. A.** 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. **1:**279–285.
3. **Bricas, E., J.-M. Ghuysen, and P. Dezelee.** 1967. The cell wall peptidoglycan of *Bacillus megaterium* KM. I. Studies on the stereochemistry of α-diamino pimelic acid. Biochemistry **6:**2598–2607.
4. **Canellakis, E. S., D. Viceps-Madore, D. A. Kyriakidis, and J. S. Heller.** 1979. The regulation and function of ornithine decarboxylase and of the polyamines. Curr. Top. Cell. Regul. **15:**155–202.
5. **Furukawa, H., H. Yamada, and S. Mizushima.** 1979. Interaction of bacteriophage T4 with reconstituted cell envelopes of *Escherichia coli* K-12. J. Bacteriol. **140:** 1071–1080.
6. **Gmeiner, J.** 1979. Covalent linkage of lipoprotein to peptidoglycan is not essential for outer membrane stability in *Proteus mirabilis*. Arch. Microbiol. **121:**177–180.
7. **Inoue, H., and A. Mizutani.** 1973. A new method for isolation of polyamines from animal tissues. Anal. Biochem. **56:**408–416.
8. **Inouye, M., J. Shaw, and C. Chen.** 1972. The assembly of a structural lipoprotein in the envelope of *Escherichia coli*. J. Biol. Chem. **247:**8154–8159.
9. **Johnson, K., C. Duez, J. M. Frere, and J.-M. Ghuysen.** 1975. β-Lactamases (*Actimonycetes* species). Methods Enzymol. **43:**687–697.

9a. **Kamio, Y., Y. Itoh, Y. Terawaki, and T. Kusana.** 1980. A new form of structural peptidoglycan in *Selenomonas ruminantium*: existence of polyamine in peptidoglycan. Agric. Biol. Chem. **44:**2523–2526.

10. **Kamio, Y., and H. Takahashi.** 1980. Isolation and characterization of outer and inner membranes of *Selenomonas ruminantium:* lipid compositions. J. Bacteriol. **141:**888–898.
11. **Kamio, Y., and H. Takahashi.** 1980. Outer membrane proteins and cell surface structure of *Selenomonas ruminantium*. J. Bacteriol. **141:**899–907.
12. **Kanegasaki, S., and H. Takahashi.** 1967. Function of growth factors for rumen microorganisms. I. Nutritional characteristics of *Selenomonas ruminantium*. J. Bacteriol. **93:**456–463.
13. **Kato, K., T. Umemoto, H. Sagawa, and S. Kotani.** 1979. Lanthionine as an essential constituent of cell wall peptidoglycan of *Fusobacterium nucleatum*. Curr. Microbiol. **3:**147–151.
14. **Kotani, S., I. Yanagida, K. Kato, and T. Matsuda.** 1970. Studies on peptides, glycopeptides and antigenic polysaccharide-glycopeptide complexes isolated from an L-11 enzyme lysate of the cell wall of *Mycobacterium tuberculosis* strain H37Rv. Biken J. **13:**249–275.
15. **Primosigh, J., H. Pelzer, D. Maass, and W. Weidel.** 1961. Chemical characterization of mucopeptides released from the *E. coli* B cell wall by enzymatic action. Biochim. Biophys. Acta **46:**68–80.
16. **Shepherd, S. T., H. A. Chase, and P. E. Reynolds.** 1977. The separation and properties of two penicillin-binding proteins from *Salmonella typhimurium*. Eur. J. Biochem. **78:**521–532.
17. **Suzuki, H., U. Nishimura, S. Yasuda, A. Nishimura, M. Yamada, and Y. Hirota.** 1978. Murein-lipoprotein of *Escherichia coli*: a protein involved in the stabilization of bacterial cell envelope. Mol. Gen. Genet. **167:**1–9.
18. **Tabor, C. W., and H. Tabor.** 1976. 1,4-Diaminobutane (putrescine), spermidine, and spermine. Annu. Rev. Biochem. **45:**285–306.
19. **Wallace, R. J.** 1980. Cytoplasmic reserve polysaccharide of *Selenomonas ruminantium*. Appl. Environ. Microbiol. **39:**630–634.
20. **Yanai, A., K. Kato, T. Beppu, and K. Arima.** 1976. Peptidoglycan of *Pseudomonas aeruginosa*. Agric. Biol. Chem. **40:**1505–1508.
21. **Yem, D. W., and H. C. Wu.** 1978. Physiological characterization of an *Escherichia coli* mutant altered in the structure of murein lipoprotein. J. Bacteriol. **133:**1419–1426.