Electron Microscopic Studies on Mode of Action of Polymyxin

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The antibacterial activity of the polymyxin group against gram-negative bacteria has been explained as owing to increased permeability of the cell envelope consisting of cell wall and cytoplasmic membrane and the resultant leakage of the cell contents (7). Consequently, morphological studies should contribute much to the elucidation of the mechanism of action of these agents (2). We have made detailed observations of the changes in the cell envelope caused by polymyxin B and colistin, using Escherichia coli B and Pseudomonas aeruginosa P29 (furnished by Y. Homma, Institute of Medical Science, Tokyo University).

The cells of these gram-negative bacteria, grown in Nutrient Broth (Difco), were harvested in the logarithmic phase by centrifugation and washing with tris(hydroxymethyl)aminomethane buffer (pH 7.2), and they were finally suspended in the same buffer to give 5 x 10^6 viable cells per ml. The cell suspension was added to one-ninth volume of polymyxin B sulfate (PLB, Taizophizer Co., Tokyo, Japan), colistin sulfate, or colistin methanesulfonate (CL and CLM, Kaken Co., Tokyo, Japan) solutions of various concentrations. After incubation at 37 C for various time intervals, each mixture was examined for the viable cell count and prepared for electron microscopy. A cell wall fraction from E. coli B disrupted in a French pressure disintegrator cell was also treated with PLB as described above. Action of PLB on the spheroplasts of E. coli B obtained by Repaske's procedure (8) was measured by the decrease of optical density of the spheroplast suspension at 660 nm. For electron microscopy, the cells were doubly fixed with 1% glutaraldehyde solution and 1% OsO4 solution, dehydrated through the graded alcohol solution, and embedded in Epon 812. The specimens were cut with a Porter-Blum MT2 Ultramicrotome and examined in a JEM7 electron microscope. The cell wall fraction was negatively stained with phosphotungstic state (pH 7.0).

The viability of the cells of these bacteria was reduced by 10^-2 or less within 10 min contact with 25 µg of PLB per ml and CL and 250 µg of CLM per ml. Figure 1a shows a section of the untreated P. aeruginosa P29. Figure 1b is a section of the same organism treated with 25 µg of PLB per ml for 30 min. Figure 1c is the same bacterium treated with 250 µg of CLM per ml for 30 min. Numerous projections appear on the cell wall; the cytoplasmic membrane appears to be damaged, and part of the cytoplasmic material is released in fibrous forms through the cracks (Fig. 1b). Figure 1d shows a magnified part of a treated cell; the outer layer of the cell wall bears projections as wide as 12 to 14 nm.

Figure 2 illustrates the appearance of E. coli cells treated in the same way. In the case of E. coli B, in contrast to P. aeruginosa, the projections appear to be blebs derived from outer layers of the cell wall (Fig. 2c). Although such blebs are occasionally seen even in normal bacteria, their occurrence in such large numbers is apparently due to the action of PLB. The number of the projections decreased with reduction in concentration of the drugs, and this reduction was paralleled by the higher residual viable cell count. The antagonistic action of Mg^{2+} to PLB (5) was correlated with the electron microscopically observable reduction in the number of the projections.

These projections were also clearly visible on the purified cell wall fraction treated with PLB. Normal cell wall has an outer layer with a smooth surface (Fig. 3a, 3b), whereas those subjected to the action of PLB have numerous projections on their surface (Fig. 3c, 3d).

It has been shown that polymyxin acts not only on the cell wall but also on the cytoplasmic membrane (Fig. 1b). Observations of the effect on spheroplasts revealed that lysis occurred rapidly after addition of PLB (Fig. 4). Fig. 5a shows a picture immediately after addition of PLB; blebs have been formed apparently on the outer layer but no changes are observed in the cytoplasm. After exposure for 30 min, the protoplast lost its contents and the cytoplasmic membrane was disorganized (Fig. 5b).

The cell wall of gram-negative bacteria is composed of three layers (1, 3, 4). These experiments revealed that polymyxin-caused projections originated from the outermost layer of the three layers.

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FIG. 1. Thin sections of P. aeruginosa P29. (a) Untreated cell. × 74,000. (b) A cell treated with 25 µg of PLB per ml for 30 min. Numerous projections from the cell wall can be observed, and streaming of the cytoplasmic content through the cracks of the cell envelope is also visible (arrows). × 74,000. (c) A cell treated with 250 µg of CLM per ml for 30 min. Many projections are seen. × 74,000. (d) A higher magnification of a cell treated with 25 µg of PLB per ml for 30 min. A longitudinal section of a projection (arrow) shows clearly that the projection was derived from the outer layer of the cell wall. × 190,000. All magnification markers represent 0.1 µm.
FIG. 2. Thin sections of E. coli B. (a) Untreated cell. × 63,000. (b) A cell treated with 25 μg of PLB per ml for 30 min. Bleb-like projections on the cell wall are seen. × 95,000. (c) A magnified picture of a part of (a). × 190,000. These blebs are composed of the outer layer of the cell wall. Markers represent 0.1 μm.
Fig. 3. Cell wall fraction of *E. coli* B. (a) Negatively stained normal cell walls. X 72,000. (b) Thin section of the normal cell walls. X 81,000. (c) Negatively stained cell walls treated with PLB. X 72,000. (d) Thin section of the same as (c). X 81,000. Markers represent 0.1 μm.

Fig. 4. Lysis of the spheroplast of *E. coli* B after treatment with PLB (25 μg/ml). Symbols: O, no treatment; ●, treatment with 25 μg of PLB per ml.
and partially disorganized the cytoplasmic membrane. The chemical nature of the combining site of the cell wall with polymyxin is reported to be a phospholipid (6). Recently, we confirmed that the receptor sites for T3, T4, and T7 phages on E. coli B were specifically destroyed by treatment with polymyxin, whereas the receptor sites for T2 and T6 were not affected (Koike et al., unpublished data).

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LITERATURE CITED


