Morphological alterations of *Pseudomonas aeruginosa* and *Escherichia coli* by nocardicin A

(Scanning electron microscopy; transmission electron microscopy; bulges; spheroplast cell; lysis)

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1. SUMMARY

*Pseudomonas aeruginosa* and *Escherichia coli* were exposed to nocardicin A, and were subsequently observed with transmission and scanning electron microscopes. Although the nocardicin A-induced morphological alterations such as bulges and spheroplast formations were observed both in *P. aeruginosa* and *E. coli*, their positions on the cell surface were different in the two species.

2. INTRODUCTION

Nocardicin A [1,2], one of the monocyclic β-lactam antibiotics, was reported to have a significant bactericidal effect on Gram-negative bacteria [3,4]. Other reports [5-7] of its effects on *P. aeruginosa* and *E. coli* suggested that the bactericidal effect was due to its inhibitory action on the synthesis of cross-linked peptidoglycan, and also to its interaction with penicillin-binding proteins (PBPs). These were biochemical studies of the action of nocardicin A on the bacteria, but there have been no studies aimed at the morphological alterations caused by the antibiotic. We report in this paper the nocardicin A-induced morphological alterations in *P. aeruginosa* and *E. coli*.

3. MATERIALS AND METHODS

Clinically isolated *P. aeruginosa* and a standard strain, NIHJC-2 of *E. coli*, were used throughout. Minimal inhibitory concentrations (MICs) of nocardicin A to the two species were determined by the agar dilution method [8].

The bacteria were incubated in Pennassay broth (Difco) at 37°C under aerobic conditions and, after 3 h of incubation when the bacteria were in logarithmic growth, nocardicin A was added. At 1 and 2 h after the antibiotic addition, samples were removed for transmission and scanning electron microscopy. Electron microscopic studies were carried out as previously reported by Nishino and Nakazawa [9]. In scanning electron microscopy, the alterations of 100 cells each of *P. aeruginosa* and *E. coli* were observed.

4. RESULTS AND DISCUSSION

The successive stages of the morphological alteration of *P. aeruginosa* from the rodlike shape through the round spheroplast to the ghost are documented in Fig. 1. Fig. 1A shows *P. aeruginosa* E2 in the process of division. Exposure to 50 μg/ml (MIC) of nocardicin A produced single
and multiple bulges on the cell surface which admitted the cytoplasm flow (Fig. 1B and 2A). The structure of bulges consisted of both outer and inner (cytoplasmic) membrane, and were similar to those produced in *E. coli* by colicin M [10] and a new cephamycin, MT-141 [11]. Bulges developed most frequently, but not exclusively, at the growing zone of the cells. The expansion of the bulge formed a spheroplast cell (Fig. 1, C and D), which eventually became a ghost (Fig. 1E). In *E. coli*, nocardicin A at 100 μg/ml (MIC) induced bulges exclusively near the constricted site (Fig. 2B), and afterwards spheroplasts, and finally ghosts. Unlike most other β-lactams, particularly antipseudomonal agents [12,13], nocardicin A at concentrations as low as its MIC induced bulges and spheroplasts both in *P. aeruginosa* and *E. coli* which resulted in cell lysis. This gave a morphological demonstration of the fact that the antibiotic possesses bactericidal and bacteriolytic activities and that it inhibits the functions of PBP 1a and 1b in *E. coli* [6] and PBP 1b in *P. aeruginosa* [7]. The PBPs of *P. aeruginosa* are similar to those of *E. coli*, except that PBP 1a and 1b of *P. aeruginosa* correspond to PBP 1b and 1a of *E. coli*, respectively. These PBPs are concerned with cell elongation, and are peptidoglycan transpeptidases [14,15]. Carbenicillin interacts with PBP 1a and b, 2, 3, and 4 in *P. aeruginosa* [7], and nocardicin A with those PBPs except PBP 3 in *E. coli* [6]. Both antibiotics induced bulges near the constricted area of each respective species-cells (Fig. 2B). These results suggested that there should be no difference between the action of nocardicin A on *E. coli* and that of carbenicillin on *P. aeruginosa* in respect to morphological alterations as well as to antibiotic-sensitive sites. The sites of nocardicin A-induced bulges were, however, different in *P. aeruginosa* and *E. coli* (Fig. 2). The cell wall of *P. aeruginosa* has a thinner peptidoglycan layer than that of *E. coli*, as is observed in ultrathin-section electron microscopy in *P. aeruginosa* [16].
might account for the greater visibility of the damage on the lateral wall caused by nocardicin A in *P. aeruginosa* than in *E. coli*. This account, however, contradicts the fact that bulges are induced near the constricted area by the action of carbenicillin on *P. aeruginosa* [10,11]. It has been reported that the positions of bulges are β-lactam antibiotics-sensitive sites [17–19]. Thus, we conclude that there should be a difference in the location of the nocardicin A-sensitive site in *P. aeruginosa* and *E. coli*.

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**REFERENCES**