THE EFFECT OF POLYMYXIN B ON OUTER MEMBRANE OF *SERRATIA MARCESCENS*: ACTIVATION AND DISSOCIATION OF OUTER MEMBRANE ASSOCIATED ALKALINE PHOSPHATASE

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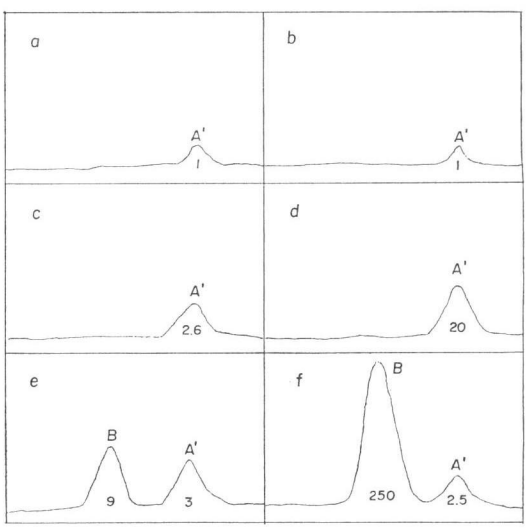
Various reports have indicated that the cationic cyclic-peptide antibiotic, polymyxin B (PB), interacts with a number of outer membrane components of gram-negative bacteria[1~5]. PB degrades lipopolysaccharide (LPS)[1,2] and phospholipids[3] and forms complexes with these molecules[4,5]. Such effects appear to be due to a multiplicity of actions of this antibiotic.

More recently, the PB degradation of phospholipids in *Pseudomonas aeruginosa* and *Escherichia coli* was suggested to be caused by an *in vivo* activation of the phospholipase.[6] Since phospholipases are outer membrane associated enzymes in gram-negative bacteria[7], it follows that one means of studying the effect of PB on the outer membrane is to examine its effect on the activity of enzymes associated with the outer membrane. In this communication, we wish to report the PB induced activation and dissociation of the alkaline phosphatase associated with the outer membrane of *Serratia marcescens*.

Two strains of *S. marcescens* were used: strain 08 (PB resistant) and strain Bizio (PB sensitive). Cells were grown in an enriched medium with aeration at room temperature and harvested at an optical density between 0.50 and 0.55[2,4]. Outer membranes were isolated by sucrose density gradient centrifugation of sonified lysozyme-EDTA treated spheroplasts as previously described[8]. Two types of PB treatment of outer membranes were performed: an *in vitro* treatment in which isolated outer membranes (1 mg) were treated with 0.05 mg of PB in 1.5 M sodium chloride, and an *in vivo* treatment in which outer membranes were isolated from cells that had been previously treated with PB[2] (20 mg of PB in 1.5 M sodium chloride for the amount of cells harvested from 1 liter of growth medium for 1 minute at 37°C). Individual active alkaline phosphatase components in the untreated outer membranes, and in those after either *in vitro* or *in vivo* PB treatment, were separated by SDS-polyacrylamide gel electrophoresis and their activities quantitatively assayed by incubating the electrophoresed gels in 0.001 M *p*-nitrophenyl phosphate in 1 M Tris buffer, pH 8[9]. Gels were scanned at 410 nm after 6 hours, and the areas under the scans measured.

Fig. 1 shows the scans of the separated alkaline phosphatase activity associated with the outer membranes before and after PB treatment. The untreated outer membranes from both strain 08 and strain Bizio contained an active component of 190,000 daltons (A′) (Fig. 1–a and 1–b). The addition of PB to the isolated outer membranes from the untreated cells resulted in an enhance-

Fig. 1. Polymyxin B induced activation and dissociation of alkaline phosphatase associated with outer membranes of two strains of *S. marcescens*. Untreated outer membranes from resistant strain 08 (a) and sensitive stain Bizio (b); outer membranes after *in vitro* polymyxin B treatment from strain 08 (c) and strain Bizio (d); outer membranes after *in vivo* polymyxin B treatment from strain 08 (e) and strain Bizio (f).
The molecular weights of the active alkaline phosphatase components (A′ and B) were estimated as follows: A′ 190,000 daltons and B 110,000 daltons.
Numbers within the scans represent relative activity of the components using the activity of the untreated outer membranes of the corresponding strains as 1.



ment of the activity of component A′. In strain 08 the activity of A′ was almost triple (Fig. 1-c), while that of strain Bizio increased about 20 fold (Fig. 1-d). *In vivo* PB treatment of the outer membranes resulted in the appearance of another component of 110,000 daltons (B) as well as the activation of A′ (Fig. 1-e and 1-f). Both the activation and the dissociation effects on the outer membranes from the PB sensitive strain Bizio were much more distinct than those of the PB resistant strain 08. Such a discriminating effect of PB, both *in vitro* and *in vivo*, on the sensitive strain of *S. marcescens* has been reported on other occasions[2,4].

Since it has been suggested that alkaline phosphatase of gram-negative bacteria exists as and forms complexes with LPS[10,11] and phospholipids[11], it seems likely that the activation and dissociation induced by PB on outer membrane associated alkaline phosphatase of *S. marcescens* is caused by either the degradative effect or binding effect of PB or both with these molecules in the outer membranes. Because of the lack of activation of phospholipase under *in vitro* conditions[6], the activation effect of PB on alkaline phosphatase is probably the result of its interaction with LPS and not its degradation effect on phospholipids. On the other hand, the dissociation effect of PB in the *in vivo* systems (Fig. 1-e and 1-f) may be explained by its degradative actions on both LPS and phospholipid molecules which may serve as binding sites for the subunits (B) to form the polymeric structure (A′).

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References

1) Lopes, J. & W. E. Inniss: Electron microscopy of effects of polymyxin B on *Escherichia coli* lipopolysaccharide. J. Bact. 100: 1128～1130, 1969
2) Tsang, J. C.; D. A. Brown & D. A. Weber: Effects of polymyxin B on cell morphology and lipopolysaccharide composition of *Serratia marcescens*. Microbios 14: 43～54, 1975
3) Kusano, T.; K. Izaki & H. Takahashi: Degradation of phospholipids in *Pseudomonas aeruginosa* induced by polymyxin B. J. Antibiotics 28: 689～695, 1975
4) Tsang, J. C.; D. A. Weber & D. A. Brown: Evidences for complex formation between polymyxin B and lipopolysaccharides from *Serratia marcescens*. J. Antibiotics 29: 735～742, 1976
5) Teuber, M. & J. Bader: Action of polymyxin B on bacterial membranes. Binding capacities for polymyxin B of inner and outer membranes isolated from *Salmonella typhimurium* G 30. Arch. Microbiol. 109: 51～58, 1976
6) Kusano, T.; K. Izaki & H. Takahashi: *In vivo* activation by polymyxin B of phospholipase from *Pseudomonas aeruginosa* and *Escherichia coli*. J. Antibiotics 29: 674～675, 1976
7) Osborn, M. & R. Munson: Separation of inner (cytoplasmic) and outer membranes of gram-negative bacteria. Methods in Enzymology 3: 642～653, 1974
8) Tsang, J. C.; D. A. Brown & D. M. Kranz: Detection of lipopolysaccharide components in outer membranes isolated from *Serratia marcescens*. Microbios Lett. 1: 209～217, 1976
9) Kranz, D. M.; L. V. Page & J. C. Tsang: Alkaline phosphatase from clinical and non-clinical strains of *Serratia marcescens* grown in two types of media. Microbios 14: 183～193, 1975
10) Lindsay, D. S.; B. Wheeler, K. E. Sanderson, J. W. Costerton & K. J. Cheng: The release of alkaline phosphatase and of lipopolysaccharide during the growth of rough and smooth strains of *Salmonella typhimurium*. Canad. J. Microbiol. 19: 335～343, 1973
11) Day, D. F. & J. M. Ingram: *In vitro* studies of an alkaline phosphatase-cell wall complex from *Pseudomonas aeruginosa*. Canad. J. Microbiol. 21: 9～16, 1975