INHIBITORY EFFECT OF POLYMYXIN B ON PRODIGIOSIN BIOSYNTHESIS IN *SERRATIA MARCESCENS*

Sir:

Prodigiosin or prodigiosin-like antibiotic pigments are tripyrrole compounds synthesized by *Serratia marcescens*, *Vibrio psychroerythrus*, *Pseudomonas magnesorubra* and *Alteromonas rubra* as well as several genera of actinomycetes.[1,2] Although the structures of several of the pigments are known, the site of localization and biosynthesis is less than clear.[3,4] Studies of inhibition of prodigiosin formation in nonproliferating cells of *S. marcescens* by certain antibiotics and antimetabolites indicated that macromolecular synthesis might be involved in the biosynthesis.[5,6] These macromolecules might serve as binding sites for the prodigiosin molecules or their intermediates.[6,7] The fact that pigment formation was more sensitive to inhibition than growth of cells led to the suggestion that prodigiosin biosynthesis might involve pathways other than those primarily required for cellular growth.[8,9] In the course of our studies on the effect of polymyxin B on outer membranes of *S. marcescens*,[10,11,12] we observed that polymyxin B exerted an inhibitory effect on prodigiosin biosynthesis without disturbing cellular growth.

S. marcescens 08, a chromogenic polymyxin B resistant strain (MIC>1,000 μg/ml) was used in our studies. Inhibition of pigment formation was monitored by both the agar-diffusion technique and spectrophotometric analysis. *S. marcescens* 08 was cultured in 10 ml of Nutrient Broth (Difco) at pH 7 and incubated at room temperature for 8 hours. An aliquot of 0.1 ml of the bacterial suspension was spread on each of the Trypticase Soy Agar (TSA) plate at pH 7. To the sterilized disc blands (6.5 mm in diameter), concentrations of 5,000, 500, 400, 300, 250, 150, 100, 50, 25, 12.5 and 0 μg/ml of polymyxin B sulfate (Aerosporin, Burrough Wellcome) were added. The antibiotic discs were placed on the surface of the seeded agar plates. Each concentration of polymyxin B was carried out in quadruplicates. The plates were then incubated at room temperature for 48 hours. Diameters of pigment inhibition zones were measured and expressed to the nearest mm.

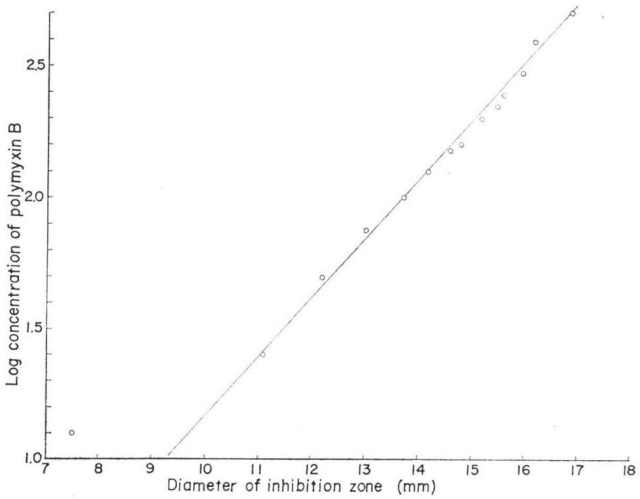
In order to quantify the actual reduction of prodigiosin biosynthesis *S. marcescens* 08 was grown in the presence of 12.5 μg/ml of polymyxin B (the MIC at which pigment was inhibited). Four hundred milliliters of a 48-hour culture (in light) were harvested and extracted with acetone, followed by partition with petroleum ether according to the method of WILLIAMS, *et al.*[13] The water-free petroleum ether extract was evaporated to dryness and reconstituted in 5 ml of dichloroethane for spectrophotometric analysis in a Beckman ACTA VI Spectrophotometer at 537 nm.

Table 1. Zone inhibition of pigment formation by polymyxin B at various concentrations.

Concentration of polymyxin B (μg/ml)	Diameter of inhibited zone* (mm)
12.5	7.5
25	11.1
50	12.2
75	13.0
100	13.7
125	14.2
150	14.6
175	14.8
200	15.2
225	15.5
250	15.6
300	16.0
400	16.2
500	16.9

* Average values of four experiments.

Fig. 1. The relationship of polymyxin B concentration on inhibition zone.



The results of zone inhibition of prodigiosin formation by polymyxin B at various concentrations are summarized in Table 1. From these results, the minimal concentration inhibiting pigment formation was estimated to be 12.5 μg/ml. At a concentration of 5,000 μg/ml of polymyxin B, neither pigment formation nor cellular growth was observed. Otherwise, an opaque zone of pigmentless cells was observed. A plot of the logarithm of the polymyxin B concentration against the diameters of the pigment inhibition zone shows a linear relationship similar to that typical of antibiotic inhibition of bacterial growth (Fig. 1). Since *S. marcescens* 08 used in this study is resistant to more than 1,000 μg/ml of polymyxin B, it can be concluded that the pigment inhibition effect of polymyxin B is independent of its antibiotic activity. The inhibitory effect of plymyxin B on pigment synthesis in *S. marcescens* 08 was demonstrated by the quantitative determination of the actual amount of pigment reduced in cells grown in Tryptic Soy Broth (TSB) (Difco) in the presence of polymyxin B at a concentration of 12.5 μg/ml. Table 2 shows that polymyxin B at this concentration caused a reduction of over 80% of the pigment. It is not known at this time if the reduction was the result of a decrease of synthesis or an increase of degradation of the pigment(s).

When the pigmentless cells from the inhibition zones were recultured to fresh TSA plates without polymyxin B, pigment synthesis resumed after about 48 hours. Cells exposed to polymyxin B, regardless of polymyxin B concentrations, required extended time period for pigment synthesis to start. Normally, pigment formation occurs usually after 24 hours of incubation at room temperature (23~24°C). Our experiment showed that the effect of polymyxin B on pigment synthesis in *S. marcescens* 08 was transient in nature without genetic damage to the pigment synthesis system. The additional time required for pigment synthesis to occur might be necessary because the membrane systems were partially damaged by polymyxin B. As a membrane disrupting antibiotic, polymyxin B is capable of binding to several outer membrane components,[14,15] causing morphological changes in the membranes of the cell envelope of Gram-negative bacteria.[16] The competitive binding of polymyxin B to these membrane components[17] may displace some of the macromolecular site(s) required for the pigment synthesis. The ability of polymyxin B to activate some degradative enzymes in the outer membrane[18,19,20] in the damaged membrane would possibly contribute to the instability and degradation of the pigment(s), once it is synthesized.

Table 2. Per cent inhibition of pigment formation by polymyxin B in broth cultures.

Condition of growth	Amount of pigment formed* (Absorbance units at 537 nm)	Inhibition (%)
Control flask	4.66	0
Flask containing polymyxin B (12.5 μg/ml)	0.95	80.5

* Average values of two experiments. Values represent the amount of pigment formed in 0.4 liters of cultures, as extracted by the method of WILLIAMS, *et al.* and dissolved in 5 ml of dichloroethane for spectrophotometric analysis.

Finally, it should be mentioned that the inhibitory action of pigment synthesis by polymyxin B in *S. marcescens* might be useful in the developing of a simple and quantitative assay of polymyxin B in clinical and biological fluids.

JOSEPH C. TSANG*
XANTHE SHEUNG

Department of Chemistry
Illinois State University
Normal-Bloomington, IL 61761
U.S.A.

(Received December 10, 1979)

References

1) GERBER, N. N.: Prodigiosin-like pigments. Crit. Rev. Microbiol. 3: 469~485, 1975
2) GERBER, N. N. & M. J. GAUTHIER: New prodigiosin-like pigment from *Alteromonas rubra*. Appl. Environ. Microbiol. 37: 1176~1179, 1979
3) MORRISON, D. A.: Prodigiosin synthesis in mutants of *Serratia marcescens*. J. Bacteriol. 91: 1599~1604, 1966
4) TSANG, J. C. & D. M. KALLVY: Association of prodigiosin with outer cell wall components. Trans. Ill. State Acad. Sci. 64: 22~26, 1971
5) QUADRI, S. M. H. & R. P. WILLIAMS: Biosynthesis of tripyrrole bacterial pigment prodigiosin, by nonproliferating cells of *Serratia marcescens*. Texas Reports Biol. Med. 30: 73~83, 1972

* To whom all correspondence should be addressed.

6) WINKLER, U.; K. B. HELLER & R. FOLLE: Pleiotropic consequences of mutations towards antibiotic-hypersensitivity in *Serratia marcescens*. Arch. Microbiol. 116: 259~268, 1978
7) YOSHIDA, S.: A study of a water-soluble complex of prodigiosin produced by a strain of *Serratia marcescens*. Canad. J. Biochem. Physiol. 40: 1019~1024, 1962
8) WILLIAMS, R. P.: Biosynthesis of prodigiosin—a secondary metabolite of *Serratia marcescens*. Appl. Microbiol. 25: 356~402, 1973
9) WITNEY, F. R.; M. L. FAILLA & E. D. WEINBERG: Phosphate inhibition of secondary metabolism in *Serratia marcescens*. Appl. Environ. Microbiol. 33: 1042~1046, 1977
10) BROWN, D. A. & J. C. TSANG: Effect of polymyxin B on the fatty acid compositions of outer membranes from *Serratia marcescens*. J. Antibiotics 30: 194~196, 1976
11) BROWN, D. A. & J. C. TSANG: Chemical and electrophoretic changes induced by polymyxin B on outer membrane components from *Serratia marcescens*. J. Antibiotics 31: 603~609, 1978
12) WEBER, D. A.; M. J. NADAKAVUKAREN & J. C. TSANG: Electron microscopic observations of polysaccharide components in polymyxin B treated outer membranes from *Serratia marcescens*. J. Antibiotics 32: 66~72, 1979
13) WILLIAMS, R. P.; J. A. GREEN & D. A. RAPPOPORT: Studies of pigmentation of *Serratia marcescens*. I. Spectral and paper chromatographic properties of prodigiosin. J. Bacteriol. 71: 115~120, 1956
14) BADER, J. & M. TEUBER: Action of polymyxin B on bacterial membranes. I. Binding to the O-antigenic lipopolysaccharide on *Salmonella typhimurium*. Z. Naturforsch. 28c: 422~430, 1973
15) TEUBER, M. & J. BADER: Interaction of polymyxin with outer membrane of *Salmonella typhimurium* binding to isolated lipopolysaccharides and lipid A. Abstr. Com. Meeting Fed. Europ. Biochem. Soc. 8: 26, 1972
16) SCHINDLER, P. R. G. & M. TEUBER: Action of polymyxin B on bacterial membranes: Morphological changes in the cytoplasm and in the outer membrance of *Salmonella typhimurium* and *Escherichia coli*. Antimicr. Agents & Chemoth. 8: 95~104, 1975
17) SCHINDLER, M. & M. J. OSBORN: Interaction of divalent cations and polymyxin B with lipopolysaccharide. Biochemistry 18: 4425~4430, 1979
18) KUSANO, T.; K. IZAKI & H. TAKAHASHI: Degradation of phospholipid in *Pseudomonas aeruginosa* induced by polymyxin B. J. Antibiotics 28: 689~695, 1975
19) 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
20) TSANG, J.C.; C.M. KRANZ & D.A. BROWN: The effect of polymyxin B on outer membrane of *Serratia marcescens*: Activation and dissociation of outer membrane associated alkaline phosphatase. J. Antibiotics 30: 270~271, 1977