

FATTY ACID COMPOSITION OF LIPOPOLYSACCHARIDES FROM  
SERRATIA MARCESCENS, SENSITIVE AND RESISTANT TO POLYMYXIN B

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The fatty acid composition of lipopolysaccharides (LPS) from six strains of Serratia marcescens, resistant and sensitive to polymyxin B, were analyzed. In all strains the fatty acids detected were laurate, myristate, palmitate, two unknowns and B-hydroxy myristate. The major component identified was B-hydroxy myristate (34.9-46%). No quantitative difference was observed in the fatty acids of the LPS from strains with different antibiotic sensitivities. However, the amount of LPS and its glucosamine content was shown to be higher in the resistant strains.

Lipopolysaccharides (LPS) are characteristic components of the outer membrane of the cell envelope of gram-negative bacteria. LPS is a phosphorous containing heteropolymer consisting of a Lipid A moiety covalently linked to a core polysaccharide which is attached to the O-specific side chain. The Lipid A moiety is an acylated poly-D-glucosamine phosphate. Although recent work has shown that Lipid A can cause many biological effects, such as tumor inhibition, pyrogenicity and toxicity (Galanos, 1972), no correlation has been shown between Lipid A and antibiotic sensitivity.

The polymyxins are cationic polypeptide antibiotics with both lipophilic and lipophobic groups. It has been shown that polymyxin treatment of isolated LPS resulted in general in the breakdown of its structure (Lopes, 1969). This degradative action was explained by an electrostatic interaction between the antibiotic and the LPS molecule (Bader, 1973). In addition, it is possible that a hydrophobic interaction between the 6-methyl-octanoic acid of the polymyxin B and the fatty acids in Lipid A of the LPS could be involved.

Although the fatty acid composition of Lipid A from Serratia marcescens has been reported (Alaupovic, 1966; Adams, 1969), its role in antibiotic resistance against polymyxins remains unknown. In this study we report the isolation of LPS from strains of Serratia marcescens sensitive and resistant to polymyxin B and their fatty acid composition.

MATERIALS AND METHODS

Clinical and nonclinical isolates of Serratia marcescens, sensitive and resistant to polymyxin B, were grown on an enriched medium and harvested

at the late log phase (Alaupovic, 1966). The clinical isolates were obtained from the microbiology laboratory of the Peoria School of Medicine, Peoria, Illinois.

LPS was extracted by the hot aqueous phenol method of Westphal, et. al. (1952) with a slight modification. After removal of phenol by dialysis, the extract was submitted to RNase treatment and repeated ultracentrifugation at 105,000 xg until freed of nucleic acids. The bottom gel layer was lyophilized to give the LPS fraction. The LPS was analyzed for carbohydrate, KDO, phosphorous, glucosamine and lipid content. Total carbohydrate was determined by the total anthrone-positive material (Koebler, 1952); 2-keto-3-deoxy octonic acid (KDO) by the method of Weissbach and Hurwitz (1959), phosphorous determination by the Bartlett method (1958), and glucosamine determination by the method of Randle and Morgan (1955). Methylated fatty acids (Metcalfe, 1961) from hydrolyzed LPS were analyzed on a gas chromatograph (Varian Aerograph, model 1400), equipped with a six foot glass column packed with di-ethylene glycol succinate polyester (DEGS) on chromosorb W (60-80 mesh). The column temperature was 150°C, while the injector and detector temperatures were 210 and 250 C respectively. The carrier gas (argon) was adjusted to 25 psi and a flow rate of 54.5 ml/min, while hydrogen and oxygen were adjusted to 20 and 25 psi respectively with flow rates of 30 and 200 ml/min respectively.

#### RESULTS AND DISCUSSION

Differences in total extractable lipid (unbound lipids) or fatty acid composition, or both, have been associated with increased antibiotic resistance in various bacteria and it has been suggested that the lipid composition may be important in preventing the entrance or binding of the antibiotic to the cell (Anderes, 1971; Chang, 1972). In recent studies, Bishop and Birmingham (1973), as well as Winshell and Neu (1974), found no difference in the lipid content of several bacterial strains resistant and sensitive to antibiotics. However, Norrington and James (1970) and others (Hugo, 1967; Mackenzie, 1970) found that the total extractable lipids in antibiotic resistant strains were 2-5 times higher than the antibiotic sensitive strains, and Chang, et. al., (1972) observed the total extractable lipids and phospholipids in a nonpigmented antibiotic sensitive strain of *Serratia marcescens* to be 3 times higher than a pigmented antibiotic resistant strain. Therefore, it appears that total extractable lipids may or may not play a significant role in the antibiotic sensitivity.

The bound lipid (Lipid A) in LPS has been shown to be the biologically active component of endotoxin (LPS-protein complex) (Galanos, 1972; Luderitz, 1973), which can be inactivated by polymyxin B treatment (Cooperstock, 1974; Craig, 1974). It was suggested that the site of inactivation may be the Lipid A moiety of the LPS. In addition to the electrostatic interaction between polymyxin and LPS (Bader, 1973), it is possible that a hydrophobic interaction between the bound lipids and the fatty acid moiety of polymyxin B might also be involved. The overall effect would be the prevention of the antibiotic from reaching the cytoplasmic membrane where a lethal action may occur with the phospholipids (HsuChen, 1973).

Table 1 summarizes the yields and chemical compositions of the isolated LPS. KDO, the characteristic component of LPS, was positive for all LPS

TABLE 1. Partial Chemical Composition of Lipopolysaccharides Preparations

Strains	Yields	KDO	Anthrone(+) carbohydrate %	Phosphorous %	Glucosamine %
<u>Polymyxin B Resistant</u>					
08	<sup>a</sup> 17.1	+	15.0	2.4	8.3
6292	20.7	+	17.0	1.4	9.8
2736	3.6	+	15.6	1.6	2.5
<u>Polymyxin B Sensitive</u>					
Bizio	8.0	+	22.7	1.8	4.3
3910	8.1	+	15.1	1.3	3.4
13378	11.8	+	22.7	3.0	2.7

<sup>a</sup>Yields of LPS is expressed in mg/g whole cells.

preparations. The total anthrone-positive material (15-22.7%) revealed no differences among the strains. The yields of LPS are, in general, higher in the resistant strains (3.6-20.7 mg/g whole cells) as are the glucosamine contents (2.5-8.3%). Since the side chain of *Serratia marcescens* 08 also contains glucosamine (Turesky, 1973), an increase in glucosamine content in this strain may reflect a higher content in the side chain and/or the Lipid A moiety. A quantitative analysis of the content of Lipid A and side chain polysaccharide from LPS is needed to verify this suggestion.

The fatty acid compositions of the LPS from three sensitive and three resistant strains of *Serratia marcescens* are summarized in Table 2. The major and characteristic fatty acid was 3-hydroxy myristate (34.9-46%). Other fatty acids detected were laurate (8.5-15.7%), myristate (10.6-20%), palmitate (10.5-20.8%) and two unknowns. No relative difference between sensitive and resistant strains was observed among the fatty acids. Our results of fatty acid composition in LPS are consistent with those of the isolated lipid A reported by Alaupovic, et. al. (1956) and Adams and Singh (1969). Alaupovic, et. al. (1956) analyzed the lipid A of strains 08 and Bizio and found four major fatty acids: laurate, myristate, palmitate and 3-hydroxy myristate. The glucosamine content in the isolated lipid A of 08 was reported to be higher than that of Bizio. Similar results of

TABLE 2. Fatty Acid Composition of Lipopolysaccharide Preparations from Serratia marcescens

Strains	C <sub>12</sub> Laurate %	C <sub>14</sub> Myristate %	U <sub>1</sub> %	C <sub>16</sub> Palmitate %	U <sub>2</sub> %	B-OH-C <sub>14</sub> B-Hydroxy Myristic Acid %
<u>Polymyxin B Resistant</u>						
08	8.5	20.0	6.0	10.5	4.0	46.0
6292	14.5	13.1	5.6	12.6	7.8	40.8
2736	13.0	18.5	4.8	19.4	2.8	38.3
<u>Polymyxin B Sensitive</u>						
Bizio	12.3	10.9	4.9	13.2	6.7	41.0
3910	15.7	17.0	6.7	20.8	2.1	34.9
13378	12.6	15.0	6.0	15.4	5.7	40.5

fatty acid compositions were also reported in the Temple strain (Adams, 1969).

Results of this study showed that it was possible to isolate LPS from Serratia marcescens in spite of their difference in antibiotic sensitivities. However, the yields of LPS from the resistant strains are slightly higher than those from the sensitive strains. Whether this result is significant or not in the mechanism of polymyxin resistance remains to be demonstrated. When endotoxins are inactivated, the nature of the antibiotic sensitivity of cells, from which endotoxins are isolated, seems to be unrelated and unimportant (Cooperstock, 1974). The lack of any observable difference in the fatty acid composition from sensitive and resistant strains might indicate that Lipid A is involved in the inactivation process, rather than in the mechanism of polymyxin resistance.

#### ACKNOWLEDGEMENT

The authors express deep appreciation for the financial support for this investigation from the Brown-Hazen Grant of Research Corporation, and Dr. M. A. Miller of Peoria School of Medicine, Peoria, Illinois for the supply of the clinical isolates.

## LITERATURE CITED

- ADAMS, C. A., and P. O. Singh. 1970. The chemical constitution of Lipid A from Serratia marcescens. Can. J. Biochem. 48:55-62.
- ALAUPOVIC, P., A. C. Olson, and J. C. Tsang. 1966. Studies on the characterization of lipopolysaccharides from two strains of Serratia marcescens. Ann. N. Y. Acad. Sci. 133:546-565.
- ANDERES, E. A., W. E. Sandline, and P. R. Elliker. 1971. Lipids of antibiotic-sensitive and -resistant strains of Pseudomonas aeruginosa. Can. J. Microbiol. 12:1357-1365.
- BADER, J., and M. Teuber. 1973. Action of polymyxin B on bacterial membranes. I. Binding to the O-antigenic lipopolysaccharides of Salmonella typhimurium. Z. Naturforsch. 28c:442-430.
- BARTLETT, G. R. 1959. Colorimetric assay methods for free and phosphorylated glyceric acids. J. Biol. Chem. 234:469-471.
- BISHOP, E. A., and M. A. C. Birmingham. 1973. Lipid composition of gram-negative bacteria, sensitive and resistant to streptomycin. Antimicrob. Ag. Chemother. 4:373-379.
- CHANG, C. Y., R. E. Molar, and J. C. Tsang. 1972. Lipid content of antibiotic-resistant and -sensitive strains of Serratia marcescens. Appl. Microbiol. 24:972-976.
- COOPERSTOCK, M.S. 1974. Inactivation of endotoxin by polymyxin B. Antimicrob. Ag. Chemother. 6:422-425.
- CRAIG, W. A., J. H. Turner, and C. M. Kunin. 1974. Prevention of the generalized Schwartzman reaction and endotoxin lethality by polymyxin B localized in tissues. Infect. Immunity. 10:287-292.
- GALANOS, C., E. T. Rietschel, O. Luderitz, and O. Westphal. 1972. Biological effects of Lipid A complexed with bovine-serum albumin. Eur. J. Biochem. 31:230-233.
- HSUCHEN, C. C., and D. S. Feingold. 1973. Selective membrane toxicity of the polyene antibiotics: studies on lecithin membrane models (liposomes). Antimicrob. Ag. Chemother. 4:1008-1012.
- HUGO, W. B. 1967. The mode of action of antibacterial agents. J. Appl. Bacteriol. 30:17-50.
- KOHLER, L. G. 1952. Differentiation of carbohydrates by anthrone reaction of rate and color intensity. Anal. Chem. 24:1576-1579.
- LOPES, J., and W. D. Inniss. 1969. Electron microscopy of effect of polymyxin B on Escherichia coli lipopolysaccharides. J. Bacteriol. 100:1128-1130.

- LUDERITZ, O., C. Galanos, V. Lehmann, M. Nurninen, E. T. Reitschel, G. Rosenfelder, M. Simon, and O. Westphal. 1973. Lipid A: chemical structure and biological activity. J. Infect. Dis. 128(Suppl.): 17-29.
- MACKENZIE, C. R., and D. C. Jordan. 1970. Cell wall phospholipid and viomycin resistance in Rhizobium meliloti. Biochem. Biophys. Res. Commun. 40:1008-1012.
- METCALFE, L. D., and A. A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatograph analysis. Anal. Chem. 33:363-364.
- NORRINGTON, F. E., and A. M. James. 1970. The cell wall lipids of cells of tetracycline-sensitive and -resistant strains of Streptococcus pyogenes. Biochem. Biophysics Acta. 218:269-277.
- RONDIE, G. J. M. and W. T. J. Morgan. 1955. The determination of glucosamine and galactosamine. Biochem. J. 61:586-589.
- TARCSAY, L., C. S. Wang, S. C. Li, and P. Alaupovic. 1973. Composition and structure of the O-specific side chain of endotoxin from Serratia marcescens 08. Biochem. 12:1946-1954.
- WEISSBACH, A., and J. Hurwitz. 1959. The formation of 2-ketodeoxyheptonic acid in extracts of Escherichia coli B. J. Biol. Chem. 234:705-709.
- WESTPHAL, O., O. Luderitz, and F. Bister. 1952. Über die extraktion von Phenol/wasser. Z. Naturforsch. 7:148.
- WINSHELL, E. B., and H. C. Neu. 1974. Relation of cell wall lipid content of Serratia marcescens to resistance to antimicrobial agents. Antimicrob. Ag. Chemother. 6:73-75.