EFFECT OF POLYMYXIN B ON THE FATTY ACID COMPOSITIONS OF OUTER MEMBRANES FROM *SERRATIA MARCESCENS*

Sir:

The binding ability of cationic polymyxin B (PB) with anionic components such as lipopolysaccharide (LPS) and phospholipids (PL) in outer membranes of gram-negative bacteria has been studied,[1] and the complexes formed have been characterized.[2,3] In addition, the degradative effect of PB on LPS[2,4] and PL[5] have also been demonstrated. In the case of phospholipid degradation, KUSANO *et al.*[6] attributed the degradative effect of PB to its activation of a phospholipase system. Since a phospholipase A_1 has been shown to exist in the outer membranes of gram-negative bacteria,[7] it seems feasible to study the damage effect of PB on outer membranes of gram-negative bacteria by analysing the fatty acid compositions of the outer membrane lipids isolated from cells after PB treatment. In this communication, we report the results of the fatty acid analysis of the extractable and bound lipids in outer membranes of *Serratia marcescens* before and after PB treatment.

Materials and Methods

The strains of *S. marcescens* used in this study have been previously described.[2] Cells were grown in an enriched medium with aeration at room temperature and harvested at an optical density between 0.50 and 0.55 units.

Outer membranes were isolated according to a slightly modified method of OSBORN and MUNSON.[7] In general the method involves the formation of spheroplast of cells washed with Tris buffer by treatment with lysozyme-EDTA in a sucrose Tris buffer solution, and sucrose density gradient centrifugation of the sonified spheroplasts. The outer membrane fractions were recovered from the high density region.

The *in vivo* treatment of the outer membrane is the same as that used for the *in vivo* treatment of whole cells.[8] Cells from 1 liter of culture medium were treated with 20 mg of PB (Burroughs Wellcome) in 75 ml of 0.9% NaCl solution, pH 7.3 for 1 minute at 37°C. Outer membranes were isolated from the treated cells.

Total extractable lipids (TEL) were extracted with chloroform - methanol (2: 1, v/v) as previously described.[9] The yields of TEL were determined gravimetrically. Both TEL and the bound lipids (in the residue of the extracted outer membranes) were hydrolysed in 6 N HCl for 24 hours, and the released fatty acids converted into methyl esters for gas chromatographic analysis.[9]

Results and Discussion

Table 1 shows the yields and the fatty acid compositions of the TEL from outer membranes of various strains of *S. marcescens* before and after PB treatment. The degradative effect of PB on outer membranes of *S. marcescens* is clearly shown by the significant decrease (17~42%) in the yields of the TEL. The fatty acid compositions of the TEL of the outer membranes isolated from cells before and after PB treatment differed more quantitatively than qualitatively. In both instances, the outer membranes of *S. marcescens* were enriched in saturated (C_{16}) and monounsaturated ($C_{18:1}$) fatty acids, with more saturated in the resistant strains (08 and 6292) and more monounsaturated in the sensitive strains (Bizio and 13378). An explanation for the high saturated fatty acid contents of TEL of outer membranes could be due to the relatively high amount of phosphatidylethanolamine in these membranes.[10] This phospholipid contains more saturated fatty acids than phosphatidylglycerol and diphosphatidylglycerol which are predominant in the cytoplasmic membranes. The significant decrease of both the yields of the TEL and the saturated fatty acids after PB treatment not only supports the finding of KUSANO *et al.*[5,6] on the *in vivo* activation of a phospholipase system by PB but also agrees with other reports[7] that the phospholipase in the outer membrane of *S. marcescens* and other gram-negative bacteria is the phospholipase A_1 which generally cleaves the saturated fatty acids located in the alpha position. The lower saturated/unsaturated fatty acid ratio in the TEL of the outer membranes of the sensitive cells before and after PB treatment might indicate that a decrease of hydrophobicity of the membrane lipids may cause an increase in the permeability of a cationic antibiotic such as PB. However, penetration of PB across the outer membranes of gram-negative bacteria is complicated by the presence of the LPS molecules which not only form complexes with PB[2] but are also degraded by

Table 1. Yield and fatty acid composition of total extractable lipids in outer membranes of *Serratia marcescens*, before and after polymyxin B treatment

	Strain	Yield of TEL (%)	C_{10}	C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{17}	U[e]	C_{18}	$C_{18:1}$	C_{19}	[d]Sat/unsat
Resistant	08[a]	40.0	T	2.5	4.4	69.4	0.5	T	2.9	7.3	13.0	Neg[c]	6.2
	08-PB[b]	23.0	T	1.9	2.6	48.7	13.1	Neg	4.8	5.9	14.7	8.2	2.1
	6292	35.0	T	1.9	6.3	62.8	5.6	3.2	7.5	2.8	9.9	Neg	5.0
	6292-PB	23.4	T	1.6	3.9	44.0	14.3	T	3.4	7.1	18.9	6.8	1.7
Sensitive	Bizio	51.0	6.5	2.8	9.5	45.1	T	7.1	2.3	4.2	22.8	Neg	3.3
	Bizio-PB	36.6	T	2.6	3.7	46.4	T	T	2.0	3.0	36.3	4.2	1.5
	13378	44.3	T	3.5	7.3	47.0	2.7	Neg	5.3	5.9	28.4	Neg	2.0
	13378-PB	36.5	T	1.9	3.5	34.1	8.6	Neg	2.3	2.8	4.15	5.4	0.8

[a] Outer membranes isolated from untreated whole cells.
[b] Outer membranes isolated from PB treated whole cells.
[c] Neg=negative.
[d] A ratio of saturated fatty acids *versus* unsaturated fatty acids. Values do not include the unknown and C_{19}.
[e] U=unidentified component.

Table 2. Fatty acid composition of bound lipids in outer membranes of *S. marcescens*, before and after polymyxin B treatment

	Strain	C_{12}	C_{14}	U_1[d]	C_{16}	$C_{16:1}$	C_{17}	U_2[d]	C_{18}	$C_{18:1}$	C_{14-OH}
Resistant	08[a]	7.7	24.3	11.0	35.1	T	Neg[c]	5.2	1.7	1.9	13.0
	08-PB[b]	11.3	19.8	3.4	38.2	1.6	Neg	3.3	5.6	Neg	16.9
	6292	15.2	23.4	5.2	26.8	1.7	Neg	6.8	3.7	Neg	17.3
	6292-PB	10.0	24.1	5.0	25.7	3.4	1.6	5.5	T	T	17.4
Sensitive	Bizio	11.3	16.4	5.0	36.5	T	Neg	2.3	10.6	Neg	17.8
	Bizio-PB	14.0	17.8	4.4	34.1	3.5	Neg	7.7	2.3	Neg	16.2
	13378	11.7	17.0	2.7	28.7	5.7	3.1	9.0	2.7	T	19.4
	13378-PB	18.6	24.7	4.6	25.5	1.1	2.8	6.2	4.1	2.3	10.2

[a] Outer membranes isolated from untreated whole cells.
[b] Outer membranes isolated from PB treated whole cells.
[c] Neg=negative.
[d] U=unidentified component; U_1=unidentified component 1; U_2=unidentified component 2.

PB.[4] Hydrophobic interaction of the acyl group of PB with the lipid A moiety of the LPS can cause inactivation of endotoxin (LPS-protein complex)[11] as well as facilitating the penetration of the antibiotic through the hydrophobic interior of the outer membrane.

In order to study the effect of PB on lipid A of the LPS of *S. marcescens*, the fatty acid composition of the bound lipids of the outer membranes before and after PB treatment were analysed (Table 2). The fatty acid compositions of the bound lipids resemble those of the LPS[9] and the lipid A.[12] The major fatty acids identified were C_{12}, C_{14}, C_{16}, and beta-hydroxy C_{14}. After PB treatment there was little change in the overall composition. It can be concluded that TEL is the site of the damage caused by PB treatment which most probably activates the phospholipase A_1 system in the outer membranes of *S. marcescens* and other gram-negative bacteria.

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