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Adaptive Changes in Fatty Acids of *E. coli* Strains Exposed to a Quaternary Ammonium Salt and an Amine Oxide

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ABSTRACT. Resistant strains of *Escherichia coli* were obtained by stepwise cultivation in media with increasing concentration of antimicrobially active 1-(methyldodecyl)dimethylamine oxide and 1-(methyldodecyl)trimethylammonium bromide. Adaptive changes were determined in the fatty-acid (FA) composition in an isolated lipopolysaccharide sample from the outer membrane of these strains. The composition of this FA mixture from adapted strains was compared with that of FA from a sensitive strain. The differences were found in level of palmitic, heptadecanoic, heptadecenoic, heptadecadienoic and nonadecenoic acids. In addition, the adapted strains differed from each other in the content of myristic, pentadecanoic, stearic and linoleic acids.

Abbreviations

AO	amine oxide(s)	LPS	lipopolysaccharide(s)
FA	fatty acid(s)	OM	outer membrane
FAME	fatty acid methyl ester(s)	QAS	quaternary ammonium salt(s)
MTB	1-(methyldodecyl)trimethylammonium bromide	MDO	1-(methyldodecyl)dimethylamine oxide

QAS and structurally similar AO are membrane active antimicrobial agents. In subinhibitory concentration they are able to modulate various biological activities of bacteria (Čupková *et al*. 1988; Hoštacká *et al*. 1994, 1995; Majtánová *et al*. 1996). Moreover, it was found that QAS and AO themselves affect the metabolic activities of human leukocytes (Ferenčík *et al*. 1990).

OM of *E. coli* consists of LPS, which is a major component of the outer leaflet of the OM; the inner OM surface is formed by phospholipids. The OM composition is variable and depends on growth conditions (Carty *et al*. 1999; Guérin-Méchin *et al*. 1999; Kawahara *et al*. 2002). LPS consists of three domains: lipid A, the core sugar region and the O-antigen polymer.

We investigated the adaptive changes of FA composition in isolated LPS sample from OM of *E. coli* exposed to MTB and MDO. To follow the changes in OM FA due to adaptation, we modified sensitive strains of *E. coli* by cultivation in media containing subinhibitory concentration of QAS and AO.

MATERIAL AND METHODS

Microorganisms and culture media. The strain of *Escherichia coli* ATCC 11229 was sensitive to the AO MDO and to the QAS MTB. By cultivating this strain in synthetic medium C (McQuillen and Roberts 1954) with increasing concentrations of amine oxide and ammonium salt, two resistant strains were obtained (Dubničková *et al.* 2003). The original sensitive as well as the resistant strains were incubated in medium C at 37 °C till the end of the exponential phase, harvested by centrifugation, thrice washed in 0.85 % NaCl and lyophilized. The resistant strains were cultivated under the same conditions in the presence of 0.82 mmol/L MDO and 0.62 mmol/L MTB.

Extraction methods. LPS was obtained from 4 mg of lyophilized bacteria by phenol–water extraction method (Westphal and Jann 1965). It was then purified by ultracentrifugation (100 000 ***g***, 2 h; *Beckman*, USA), treated with RNAase and DNAase and with proteinase K (Rodriguez-Carvajal *et al.* 2001); the enzymes were purchased from *Sigma*.

GC-MS analysis of FA. *Hewlett Packard* 6890 (series II) gas chromatograph and HP-GC mass selective detector (5973 MSD) were used. FAME were prepared according to Metcalfe and Wang (1981) and analyzed by GC on an RTX 1 column (30 m, ID 0.32 mm, film thickness 0.25 mm; *Restek*, USA). The GC oven

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was programmed for 2 min at 40 °C, than an increase of 2 K/min to 300 °C and, finally, 20 min at 300 °C. The injector temperature was kept at 180 °C. The flow rate of the carrier gas (helium) was 25 mL/s. The MS detector operated at 200 °C, ionization energy was 11 aJ (70 eV). The scan range was from 30 to 700 *m*/*z* at 0.9 scan per s. Solvent delay was 9 min. FAME were identified by using mass-spectral libraries (*Wiley 7th* and *NIST-98*).

Significance of differences between the samples. A difference between the total FA composition of the two strains was considered to be significant if relative difference $d \geq 50$ %.

RESULTS AND DISCUSSION

The proportion of FA in cells and changes in the FA composition of the OM of *E. coli* cultivated in the presence of MDO and MTB were used as indicators of strain adaptation to antimicrobials; significant differences were found in LPS FA composition between the strains (Table I).

Table I. Fatty acid composition of *E. coli* outer membrane-LPS from the sensitive strain (control) and from strains adapted to MDO and MTB

Fatty acid	Control	MDO[a]		MTB[a]	
$C_{14:0}$	4.0	6.0	150*	5.1	127
$C_{15:0}$	3.6	1.4	39*	3.0	83
$C_{16:0}$	16.6	25.3	152*	34.0	205***
$C_{16:1}$	11.7	6.63	56	6.8	58
$C_{17:0}$	13.5	3.6	27*	6.2	46*
$C_{17:1}$	3.1	23.3	752***	8.6	277***
$C_{17:2}$	4.9	0.0	0	0.0	0
$C_{18:0}$	6.3	2.5	40*	5.9	94
$C_{18:1}$	30.0	21.5	72	19.4	65
$C_{18:2}$	5.4	1.6	30*	4.0	74
$C_{19:1}$	0.9	8.2	911***	7.0	778***

[a]*First columns* – % of total fatty acids compared with control (100 %); *second columns* – relative content (%) of individual FA in adapted strains compared to corresponding FA control value (the significance of relative difference (d) in FA composition between the control (100 %) and MDO or MTB samples:
* d = 50–75 %, ** d = 75–100 %, *** d > 100 %.

LPS of the MDO-exposed cells exhibited changes in FA with the exception of palmitic and oleic acids; in the presence of MTB, the adaptive changes included increase in palmitic, heptadecenoic and nonadecanoic acids and decrease in heptadecanoic acid.

The differences in FA content between the control and MTB-exposed cells were lower than between the control and the strain adapted to MDO. The FA profiles in cultures adapted to the two disinfectants differed significantly in only four acids: pentadecanoic, heptadecenoic, stearic and linoleic acids (Table II). Changes in the content of other acids in the two samples relative to the control were similar.

Table II. Comparison of relative change in FA composition (%)[a] of *E. coli* outer membrane between cells adapted to MDO and to MTB (100 %)

Fatty acid	MDO	Fatty acid	MDO
$C_{14:0}$	117	$C_{17:2}$	0
$C_{15:0}$	47*	$C_{18:0}$	42*
$C_{16:0}$	74	$C_{18:1}$	111
$C_{16:1}$	97	$C_{18:2}$	40*
$C_{17:0}$	58	$C_{19:1}$	117
$C_{17:1}$	271***		

[a]For further details *see* Table I.

Interestingly, heptadecadienoic acid disappeared in both adapted strains (Table I) but, simoultaneously, this loss was compensated in both strains by a large increase in nonadecenoic acid and by an increase of palmitic acid in MDO- and heptadecenoic acid in MTB-exposed cultures. The change in the relative content of shorter-to-longer acyls and increasing unsaturation may reflect the importance of maintaining the optimum OM fluidity and stability under adverse conditions.

Natural lipids A from bacteria and the modifications of their natural structure were studied both *in vivo* and *in vitro* as modulators of immune activity (Alexander and Rietschel 2001). Lipid A that was modified after the adaptation of the bacteria to the presence of amphiphilic compounds exhibited altered immunomodulatory activity and reduced the cardiac ischemia–reperfusion injury more than the natural lipid A from untreated cells (Dubničková *et al*. 2003; Kuželová *et al*. 2005).

Somerville *et al*. (1997) and Mueller *et al*. (2004) showed that myristic acid is a crucial component necessary for the ability of lipid A (FA carrier in LPS) to activate immune cells. We found that this acid increased in MDO-exposed cells (Table I). This observation correlates with our previous finding that the microbicidal activity of human leukocytes was significantly increased by lipid A from a strain adapted to AO (Dubničková *et al*. 2003). On the other hand, we found that OM extracts from the AO-adapted cells stimulated the microbicidal activity of human cells against *C. albicans* less than extracts from a sensitive strain of *E. coli* (Bukovský *et al*. 1998).

Amphiphiles use both electrostatic and hydrophobic interactions to bind the bacterial target sites and alterations of cell wall contribute to protection against them (Kopecká-Leitmanová *et al*. 1989). AO are likely to bind *via* their polar *N*-oxide group to the negatively charged diphosphate bridges joining LPS molecules in complexes. In such case the hydrophobic aliphatic chains of AO can bind to FA by hydrophobic interactions. On the other hand, the ammonium salt in solution dissociates and interacts with polar cell components, changes of membrane fluidity and permeability being the final consequences of this interaction. The resistance to amphiphiles is accompanied by changes in the composition of the cellular outer layers. The common mechanism in amphiphile resistance was shown to be the reduction of the negative surface charge by modification of the bacterial cell envelope (LPS and phospholipids) which leads to the repulsion of these compounds. The differences between the activity of AO and QAS depend on the structure of each amphiphile.

In Gram-negative bacteria, AO and QAS disturb the structure and integrity of OM (Kopecká-Leitmanová *et al.* 1989). A stepwise cultivation in the media with increasing subinhibitory concentrations of these agents increased the resistance of *E. coli*; the observed minimum inhibitory concentration of MDO was 0.28 mmol/L, that of MTB 0.18 mmol/L.

Adaptation of microorganisms to various environmental factors (*e.g.*, temperature, antimicrobial compounds) often leads to large chemical changes in their cell-surface structures (Guérin-Méchin *et al*. 1999; Dubois-Brissonnet *et al.* 2001; Chládková *et al*. 2004). *E. coli* strain adapted to antimicrobial AO significantly differed from the natural sensitive one in the FA and hydroxy FA profiles of lipid A, which can cause a decrease in the OM permeability due to reduction of its fluidity (*cf.* Bukovský *et al.* 1991).

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REFERENCES

ALEXANDER C., RIETSCHEL E.T.: Bacterial lipopolysaccharides and innate immunity. *J.Endotox.Res.* **7**, 167–202 (2001).

BUKOVSKÝ M., MLYNARČÍK D., NAGY A., BELLA J.: Outer membrane alterations in *Escherichia coli* cells adapted to amine oxides. *Acta Fac.Pharm.* **46**, 153–167 (1991).

BUKOVSKÝ M., MLYNARČÍK D., KOŠČOVÁ H.: Change of immunomodulating properties of *Escherichia coli* caused by artificial resistance to amine oxides. (In Slovak) *Farm.Obzor* **57**, 59–68 (1998).

CARTY S.M., SREEKUMAR K.R., RAETZ C.R.: Effect of cold shock on lipid A biosynthesis in *Escherichia coli*. Induction at 12 degrees C of an acyltransferase specific for palmitoleyl-acyl carrier protein. *J.Biol.Chem.* **274**, 9677–9685 (1999).

CHLÁDKOVÁ K., HENDRYCH T., GÁŠKOVÁ D., GORONCY-BERMES P., SIGLER K.: Effect of biocides on *S. cerevisiae*: relationship between short-term membrane affliction and long-term cell killing. *Folia Microbiol.* **49**, 718–724 (2004).

ČUPKOVÁ V., VINTER V., DEVÍNSKY F., LACKO I., MLYNARČÍK D.: Inhibitory effect of 1-methyldodecyldimethylamine oxide and *N*,*N*-bis(dodecyldimethyl)-1,2-ethanediammonium dibromide on the spores of *Bacillus cereus*. *Folia Microbiol.* **33**, 433–439 (1988).

DUBNIČKOVÁ M., BUKOVSKÝ M., MLYNARČÍK D.: Activation of human leukocytes by lipid A from *E. coli* strains adapted to quaternary ammonium salt and amine oxide. *Folia Microbiol.* **48**, 543–547 (2003).

DUBOIS-BRISSONNET F., MALGRANGE S., GUÉRIN-MÉCHIN L., HEYD B., LEVEAU J.Y.: Changes in fatty acid composition of *Pseudomonas aeruginosa* ATCC 15442 induced by growth conditions: consequences of resistance to quaternary ammonium compounds. *Microbios* **106**, 97–110 (2001).

FERENČÍK M., LACKO I., DEVÍNSKY F.: Immunomodulatory activity of some amphiphilic compounds. *Pharmazie* **45**, 695–696 (1990).

GUÉRIN-MÉCHIN L., DUBOIS-BRISSONNET F., HEYD B., LEVEAU J.Y: Specific variations of fatty acid composition of *Pseudomonas aeruginosa* ATCC 15442 induced by quaternary ammonium compounds and relation with resistance to bactericidal activity. *J.Appl.Microbiol.* **87**, 735–742 (1999).

HOŠTACKÁ A., MAJTÁN V.: Enzymic and permeability activity of *Pseudomonas aeruginosa* after treatment with sub-MICs of organic ammonium salts. *Folia Microbiol.* **39**, 197–202 (1994).

HOŠTACKÁ A., MAJTÁN V., HYBENOVÁ D.: Antimicrobial efficacy of quaternary bisammonium salts and the effect of their sub-MICs on *Pseudomonas aeruginosa* virulence factors. *Folia Microbiol.* **40**, 283–287 (1995).

KAWAHARA K., TSUKANO H., WATANABE H., LINDNER B., MATSUURA M.: Modification of the structure and activity of lipid A in *Yersinia pestis* lipopolysaccharide by growth temperature. *Infect.Immun.* **70**, 4092–4098 (2002).

KOPECKÁ-LEITMANOVÁ A., DEVÍNSKY F., LACKO I., MLYNARČÍK D.: Interaction of amine oxides and quaternary ammonium salts with membrane and membrane associated processes in *E. coli* cells. Mode of action. *Drug Metab.Drug Interact.* **7**, 29–51 (1989).

KUŽELOVÁ M., MLADONICKÁ M., ŠPERGLOVÁ L., STANKOVIČ M., JUSKO M., DUBNIČKOVÁ M., BUKOVSKÝ M., ADAMEOVÁ A., STANKOVIČOVÁ T., SEGINKO J.: Modified lipid A influences myocardial ischemia reperfusion injury at both *in vitro* and *in vivo* conditions. (In Slovak) *Farm.Obzor* **74**, 251–256 (2005).

MAJTÁNOVÁ Ľ., MAJTÁN V., HOŠTACKÁ A.: Effects of amphiphilic compounds on metabolic and biological activities of *Salmonella typhimurium*. *Arzneim.Forsch.Drug Res.* **46**, 64–67 (1996).

MCQUILLEN K., ROBERTS R.B.: The utilization of acetate for synthesis in *Escherichia coli*. *J.Biol.Chem.* **207**, 81–95 (1954).

METCALFE L.D., WANG C.N.: Rapid preparation of fatty-acid methyl-esters using organic base-catalyzed trans-esterification. *J.Chromatogr.Sci.* **19**, 530–535 (1981).

MUELLER M., LINDNER B., KUSUMOTO S., FUKASE K., SCHROMM A.B., SEYDEL U.: Aggregates are the biologically active units of endotoxin. *J.Biol.Chem.* **279**, 26307–26313 (2004).

RODRIGUEZ-CARVAJAL M.A., TEJERO-MATEO P., ESPARTERO J.L., RUIZ-SAINZ J.E., BUENDÍA-CLAVERÍA A.M., OLLERO F.J., YANG S.S., GIL-SERRANO A.M.: Determination of the chemical structure of the capsular polysaccharide of strain B33, a fast growing soy bean-modulating bacterium isolated from an acid region of China. *Biochem.J.* **357**, 505–511 (2001).

SOMERVILLE J.E. Jr., CASSIANO L., BAINBRIDGE B., CUNNINGHAM M.D.P., DARVEAU R.P.: A novel *E. coli* lipid a mutant that produces an antiinflammatory lipopolysaccharide. *J.Clin.Invest.* **97**, 359–365 (1997).

WESTPHAL O., JANN K.: Bacterial lipopolysaccharides. Extraction with phenol–water and further applications of the procedure, pp. 83–91 in R.L. Whistler, J.N. BeMiller, M. Wolfrom (Eds): *Methods in Carbohydrate Chemistry, Vol. 5*. Academic Press, New York–London 1965.