ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 9, pp. 1042-1049. © Pleiades Publishing, Inc., 2006.
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Role of Polyamines in Formation of Multiple Antibiotic Resistance of *Escherichia coli* under Stress Conditions

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Received April 13, 2006
Revision received May 22, 2006

Abstract—Under stress conditions, polyamines decreased the permeability of the outer membrane of *Escherichia coli*. This effect is caused by at least three mechanisms providing for an increase in the resistance to antibiotics transported through porin channels (fluoroquinolones, β-lactams): a positive modulation of the gene *micF* transcription (its product antisense RNA inhibits the synthesis of porin proteins on the translational level); a positive effect on the cell content of the multiple stress resistance factor σ^S (it is accompanied by a decrease in the porin transport because of suppression of *ompF* transcription and induction of cadaverine synthesis); a direct inhibition of the transport activity of porin channels. The production of cadaverine in *E. coli* cells significantly increased in response to various antibiotics, and this was likely to be a manifestation of oxidative stress.

DOI: 10.1134/S0006297906090148

Key words: polyamines, stress, antibiotic resistance, porin transport, σ^S, expression

Influences of environmental factors on microorganisms in their natural habitats contribute to development of universal defense mechanisms promoting their survival on exposure to various stresses, including the presence of antibiotics.

The long-term persistence of antibiotic resistant microorganisms under natural conditions is a fundamental aspect of the problem of multiple drug resistance. It can occur even in the case of limited use of antibiotics in order to weaken the selective pressure and is caused by the ability of structures, which are responsible for antibiotic resistance to concurrently provide for other vitally important functions of the cell, in particular, maintaining intracellular pH homeostasis [1, 2]. The resistance of microorganisms to various stresses is controlled by global regulators, which also manage expression of the adaptation genes. Based on similarity of the DNA signaling sequences complementary to the regulator under consideration, these genes are combined into groups (regulons) responsible for the tolerance to a specific kind of stress. The pronounced homology of amino acid sequences of such global regulators as SoxS, MarA, and Rob underlies the gain of the cross resistance to oxidative stress, antibiotics, and organic solvents under the influence of any of these factors [3]. A similar dependence has been also described for other stresses, such as decrease in pH and hyperosmotic and thermal shock [4]. Any of these exposures activates a common mechanism of multiple drug efflux structurally organized as channel-forming proteins of the AcrAB-TolC type in the envelope of gram-negative bacteria, the functions of which are directed to expel damaging compounds from the cell [5]. A similar system of cross resistance acts on the level of restriction of the transport by means of regulation of the number of porin channels in the outer membrane of gram-negative microorganisms [6, 7]. In this process the two-component system EnvZ/OmpR of signal transduction [8] and also the small regulatory RNA of *micF* [9] are involved.

Polyamines or other biogenous polycations, which have protective functions during various stresses, were shown to positively modulate gene *micF* expression under conditions of superoxide stress [10-12]. Moreover, polyamines were shown to immediately regulate the activity of the transport of compounds through porin channels, and this explains their positive role in the adaptation of microorganisms to acidic stress and increase in the resistance to some antibiotics [13].

Abbreviations: IPTG) isopropyl-β-thiogalactoside; MIC) minimal inhibitory concentration; σ^S) multiple stress resistance factor; ADB) absolutely dry biomass.

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Thus, it appears that one of the main functions of polyamines in the defense of microorganisms against various stresses can be associated with a limitation of the envelope permeability of gram-negative microorganisms on the level of regulation of the transport activity and quantity of channel-forming proteins. Therefore, it was suggested that polyamines should play a significant role in formation of the multiple antibiotic resistance under conditions of various stresses due to lowering the antibiotic entrance into the cell.

The purpose of the present work was to study mechanisms responsible for the influence of polyamines on formation of multiple antibiotic resistance of *E. coli*, in particular, under stress exposures.

MATERIALS AND METHODS

Objects of the study. Strains of *E. coli* used in the present work are listed in Table 1, with indication of their genotypes and source.

To determine the level of gene expression, gene *lacZ* fusions were used, these being obtained by transduction with bacteriophage λ as a vector [14, 15].

To deliberately switch on *rpoS* expression, the *E. coli* strain HS1600DE3Y (pRPOS) was used, with this gene induced by addition into the culture of a non-metabolized *lac*-inducer isopropyl-β-thiogalactoside (IPTG) at the concentration of 0.3 mM [16]. The presence in cells of this strain of the chromosomal fusion *osmY* (the target gene of the *rpoS* regulon) with the reporter gene *lacZ* allowed us to assess the efficiency of the *rpoS* switching on.

To determine the dependence of the *rpoS* expression on the polyamine content in the cell, we constructed the *E. coli* strain SHT03 based on the polyamine-deficient strain HT306. To evaluate the expression of the gene *lacZ*-fusions constructed on the basis of this strain, a chromosomal *lacZ*$^-$-mutant was selected unable to synthesize the functional β-galactosidase. The mutants were selected after the *E. coli* HT306 cells were cultured on LB-agar supplemented with *o*-nitrophenyl-β-galactopyranoside (40 mg/ml), toxic products of its degradation causing death of the cells possessing the β-galactosidase activity [17]. By transduction with the λRZ5 phage the gene fusion *rpoS742:lacZ*[hybr] was transferred into the isolated HT306 *lac*$^-$ mutant strain. The phage carrying this gene fusion was preliminarily prepared via its induction by UV-irradiation of the *E. coli* R091 cells (λRZ5:*rpoS742:lacZ*[hybr]) for 20 sec at the dose of about 300 erg/cm^2 [17].

Microorganism cultures. The *E. coli* strains maintained on LB-agar slants were plated onto LB-broth supplemented with streptomycin (25 μg/ml) or kanamycin

Table 1. Bacterial strains used in the present work

Strains of *E. coli*	Genotype	Source or reference
N9212 (pBR322)	8452, but *micF:lacZ*	Martin [3]
N8452	*rob*$^-$, *mar*$^-$, *sox*$^+$, Kan-R	
M2073 (pBR322)	GC4468 (Str-R) *mar*$^+$ *marR::lacZ*	
M2076 (pBR322)	N7840 (Δ*marRAB*) *marR:lacZ*	
N7840	*mar*$^-$, *rob*$^+$, *sox*$^+$, (Str-R)	
RO91	GC4468 (Str-R) *mar*$^-$, *rob*$^+$, *sox*$^+$, (Kan-R)	
MC4100	MC4100(λRZ5:*rpoS742:lacZ*[hybr])	Hengge-Aronis [15]
HT306	F$^-$ Δ(*arg-lac*)*U169 araD139 rpsL150 ptsF25 flbB5301 rbsR deoC relA1*	Tabor [18]
SHT03	*thr-1*, *araC14*, Δ*speD98*, Δ(*gpt-proA*)*62*, *lacY1*, *gln V44*(AS), *galK2*(Oc), λ$^-$, Δ*(SpeB-SpeA)97*, Δ(*SpeC-glcB*)*63*, *rpsL25*(strR), *xylA5*, *mtl-1*, *thi-1*, *ampCp-1*, *cadA2*	
HS1600DE3Y (pRPOS)	HT306, but *lacZ*$^-$ DE3 (λRZ5:*rpoS742:lacZ*[hybr])	present work
	MC4100DE3 *rpoS13::Tn10 osmY::lacZ*	Schellhorn [16]
Plasmids		
pBR322	4361-bp *rep* and *rop* from pMB1 *bla* from Tn3 *tet* from pSC101	Biological Institute, St. Petersburg State University
pRPOS	the 1298-bp fragment from pGC1, cloned into pET21, *T7lac*-promoter in the gene *rpoS* orientation	Schellhorn [16]

(50 μg/ml). When the strains transformed with the plasmid pBR322 were used, the medium was additionally supplemented with ampicillin (25 μg/ml) and tetracycline (20 μg/ml). After growing for 11 h in a thermostat at 37°C, the cells were transferred onto M-9 medium containing the antibiotic in the abovementioned concentration and cultured for 13 h in a thermostatted shaker (120 rpm) at the same temperature in a 500-ml flask with 300 ml of M-9 medium. The grown culture was used as an inoculum for seeding into 250-ml flasks containing 100 ml of M-9 medium and antibiotic, and grown under the same conditions. To grow *E. coli* HT306 and SHT03, M-9 medium was supplemented with thiamine (1 μg/ml), proline (100 μg/ml), and pantothenate (1 μg/ml) [18].

Cell biomass was evaluated after preliminarily diluting the culture in saline by absorption (OD_{600}) using an SF-46 spectrophotometer (LOMO, Russia).

Activity of β-galactosidase was determined in cells pretreated with a mixture of SDS (Sigma, USA) and chloroform by Miller's method [17].

Polyamine content was determined by two methods: thin layer chromatography [12] and HPLC using an LC-10Avp high-pressure chromatograph (Shimadzu, Japan). Pretreatment of samples for the analysis included extraction with a subsequent derivatization of polyamines by dansylation [12]. Polyamines were separated on a Luna C18(2) column (250 × 4.6 mm) filled with 5-μm particles (Phenomenex, USA) at 25°C. Water and acetonitrile were flowed onto the column at the rate of 1 ml/min with a linear gradient of acetonitrile concentration from 40 to 100% within 35 min, with a subsequent equilibrating for 10 min with 40% acetonitrile. The dansylated polyamine derivatives were detected using an RF-10AXL flow fluorimetric detector (Shimadzu) at the excitation and emission wavelengths of 400 and 516 nm, respectively. The polyamine concentrations were calculated with predetermined calibration coefficients and related to the values of the microorganisms' biomass used for the extraction of the samples.

Transport activity of porin channels of the *E. coli* outer membrane was determined according to a modification of the method of Zimmermann and Rosselet [19] based on photometric measurement of the cephalosporin concentration in the medium during their passing through the outer membrane porin channels of gram-negative microorganisms and cleavage by periplasmatic β-lactamase. This was performed using cefazolin with absorption maximum at 270 nm. For determinations, the *E. coli* strains used were transformed with the pBR322 plasmid carrying the β-lactamase gene. The permeability coefficient was calculated using the K_m value of β-lactamase equal to 750 μM.

Minimum inhibitory concentration of the antibiotic was determined by two methods: the gradient on plates [20] and the micro method of serial dilutions on immunological trays as recommended in [21]. *Prior to* the antibiotic dilution, the pH value in the LB-broth was adjusted to 7.4-5.8 (in the studies on acidic stress) with NaOH or HCl. In the studies on the σ^S effect on the antibiotic resistance, *rpoS* was induced by addition of 0.3 mM IPTG. After the microorganism culture under test had been plated, the plates were incubated overnight in a thermostat at 37°C. The results were recorded by automated photometry of the wells using a Uniplan reader (Russia) at 492 nm.

The results were processed statistically with Statistica for Windows 5.0 standard program package (StatSoft, Inc., 1995). The figures present the mean data for series of similar experiments (no less than three), and vertical segments show the standard errors. The significance of differences between the experimental and control data was evaluated using the unpaired Student's *t*-test, $p \le 0.05$.

RESULTS

Influence of polyamines on antibiotic resistance under conditions of oxidative stress. The antibiotic resistance of microorganisms significantly depends on the quantity and activity of transport proteins. Antibiotics are known to be transported across the outer membrane of gram-negative microorganisms mainly through OmpF porin

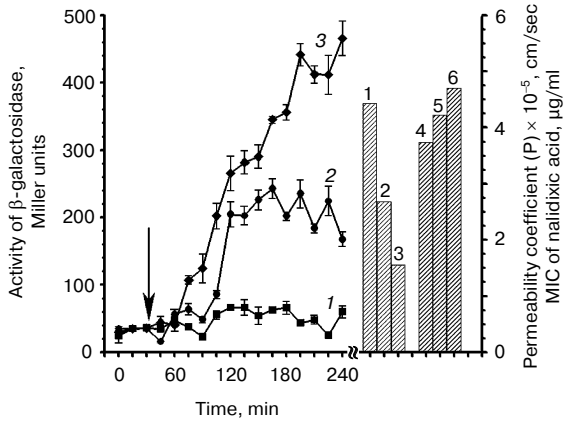

Fig. 1. Dependence of porin transport activity and antibiotic resistance of *E. coli* N9212 on the *micF* expression under conditions of its positive modulation with polyamines during oxidative stress. Curves: *1-3*) *micF* expression in culture without additions, on addition of 50 μM paraquat, and on addition of 50 μM paraquat and 5 mM putrescine, respectively. Columns *1-3* present the permeability coefficients in cultures 1-3, respectively, whereas columns *4-6* are the MIC values for nalidixic acid in cultures 1-3, respectively. The microorganisms were cultured on M-9 medium under standard conditions. The arrow indicates the injection of paraquat as an inducer of superoxide stress. In the microorganisms cultured for 240 min, the porin transport activity and MIC for nalidixic acid were determined by the gradient method on dishes with LB-agar supplemented with paraquat and putrescine in the same concentrations as in the liquid cultures.

channels, the content of which is regulated by the antisense RNA of *micF* [9].

During superoxide stress, putrescine stimulated the expression of gene *micF*, and this considerably limited the transport of substances across the outer membrane as a result of a decrease in the quantity of porin channels in the outer membrane (Fig. 1). This was associated with an increase in the minimal inhibitory concentration (MIC) for nalidixic acid transported into the cells through the porin channels, and this indicated a positive role of polyamines in the formation of antibiotic resistance via the positive modulation of *micF* (Fig. 1).

Polyamines as regulators of the transport activity of porin channels. The range of polyamine concentrations inhibiting the transport activity of porin channels markedly varies and depends on the number of amino groups in the polyamine molecule (Table 2). The greatest effect was displayed by spermidine, which contained three amino groups, whereas putrescine, cadaverine, as well as synthetic analogs of polyamines 1,4-diamino-2-butanone and 1,3-diaminopropane had the less pronounced effect. Low concentrations of polyamines slightly, but significantly stimulated the porin transport (Table 2). Although the nature of this effect is still unknown, it seems to be somewhat associated with the electrostatic interaction of polyamines with the surface charges of the outer membrane lipopolysaccharides that can influence the conformation of porins and change their activity [22].

To evaluate the efficiency of polyamines as modulators of porin transport during functioning of the efflux system, the operon *marRAB* was induced by cultivation of *E. coli* in medium supplemented with 5 mM sodium salicylate. Despite a considerable decrease in the transport activity of the induced cells, polyamines continued to inhibit the porin transport, in addition to salicylate.

Nature of synergism of putrescine and salicylate effects on antibiotic resistance. To determine the nature of synergism of polyamine and salicylate effects, we studied whether putrescine can be involved in the positive modulation of expression of the salicylate-induced operon *marRAB*. The *E. coli* strain with the chromosome fusion *marR:lacZ* was used (Fig. 2). Putrescine had virtually no effect on the expression of the *marRAB* operon. Thus, polyamine involvement in the positive regulation mechanism of multidrug efflux pumps is unlikely.

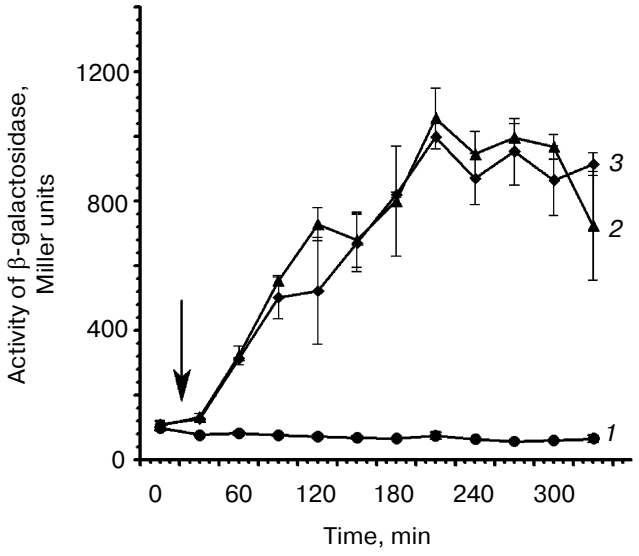

Fig. 2. Changes in the expression of the *E. coli* M2073 operon *marRAB* depending on additions of salicylate and putrescine. The arrow indicates the introduction of salicylate. Putrescine was added initially into the nutrient medium. *1-3*) The expression in cultures without additions, in the presence of 5 mM salicylate, and in the presence of 5 mM putrescine and 5 mM salicylate, respectively. The microorganisms were cultured on M-9 medium under standard conditions.

To test whether the polyamine and salicylate effects are synergic in the alternative mechanism of *micF* induction, the dependence of the minimal inhibitory concentration of levofloxacin transported through porins on the

Table 2. Changes in porin transport activity under the influence of biogenous polyamines and their synthetic analogs

Concentration of polyamines, mM	Porin transport activity depending on addition of polyamines and salicylate									
	putrescine		cadaverine		spermidine		1,4-diaminobutanone		1,3-diaminopropane	
	SL^-	SL^+	SL^-	SL^+	SL^-	SL^+	SL^-	SL^+	SL^-	SL^+
0	100	54.9 ± 4.0	100	54.9 ± 4.0	100	54.9 ± 4.0	100	54.9 ± 4.0	100	54.9 ± 4.0
10					51.5 ± 8.9	34.0 ± 6.4				
50	149.2 ± 5.0	110.3 ± 0.3	126.7 ± 1.9	53.7 ± 1.4	34.7 ± 1.9	28.2 ± 4.4	99.7 ± 3.8	68.6 ± 7.5	121.0 ± 6.8	90.6 ± 11.1
100	130.9 ± 4.2	92.6 ± 7.2	94.3 ± 9.4	47.9 ± 0.9	31.5 ± 1.4	15.2 ± 2.2	89.8 ± 1.1	62.4 ± 1.3	104.5 ± 5.1	73.7 ± 5.3
200	95.0 ± 1.1	85.5 ± 2.2	84.5 ±10.8	37.4 ± 2.8			71.7 ± 0.7	36.6 ± 4.8	55.1 ± 11.6	43.9 ± 2.1
300	73.2 ± 3.7	76.1 ± 4.4	53.1 ± 4.9	36.9 ± 6.9			36.6 ± 1.8	25.2 ± 2.3	50.9 ± 3.4	39.6 ± 3.1

Note: SL^-, in the absence of salicylate; SL^+, in the presence of 5 mM salicylate. Mean values of permeability coefficients ± *m* are presented in percent relatively to the control (transport in the absence of polyamines and salicylate).

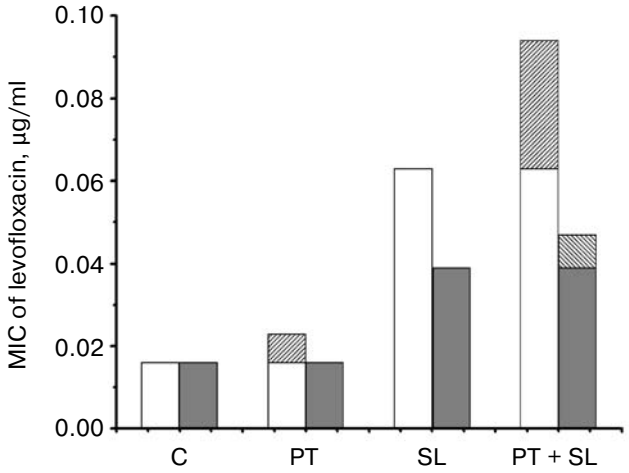

Fig. 3. Additive effects of putrescine and salicylate on the antibiotic resistance of *E. coli* M2073(*mar*$^+$) (light columns) and M2076(*mar*$^-$) (dark columns). Hatched parts of the columns indicate the effect of putrescine as compared to the corresponding control. The microorganisms were cultured under standard conditions in flasks on M-9 medium without additions (C), with addition of 5 mM putrescine (PT), 5 mM salicylate (SL), and of both 5 mM putrescine and 5 mM salicylate (PT + SL). The *mar* operon induction was monitored by an increase in the expression of the *marR:lacZ* fusion. On reaching the maximum expression (3-4 h of culture), the cells were pelleted from the medium and inoculated into wells of immunological plates with LB-broth supplemented with the same additions as were present in M-9 medium and also with antibiotics. The MIC values were determined after 18 h of culturing at 37°C.

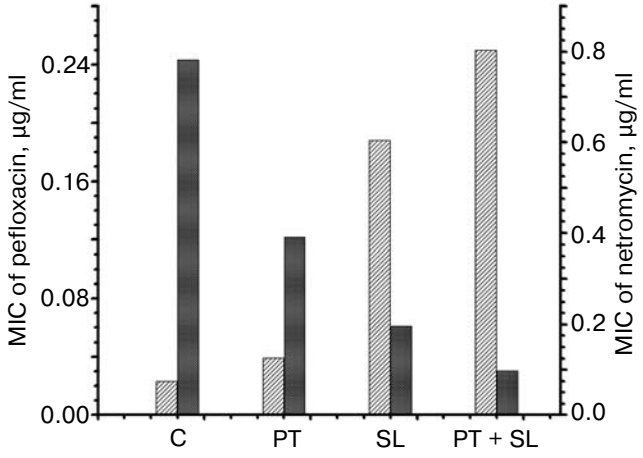

Fig. 4. Dependence of *E. coli* M2073 sensitivity to pefloxacin (hatched columns) and netromycin (dark columns) on putrescine and salicylate. The microorganisms were cultured under standard conditions in flasks on M-9 medium without additions (C), with addition of 5 mM putrescine (PT), 5 mM salicylate (SL), and of both putrescine and salicylate (PT + SL) at the same concentrations. After culturing for 3-4 h, the cells were pelleted from the medium and used for determination of the MIC under the conditions described in the caption for Fig. 3.

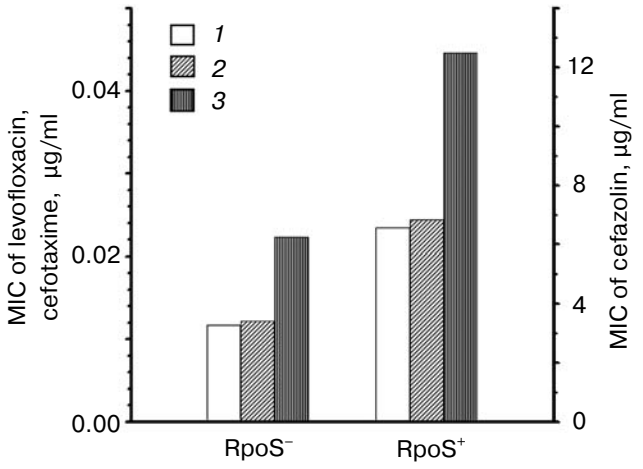

Fig. 5. Dependence of *E. coli* HS1600DE3Y (pRPOS) resistance to levofloxacin (*1*), cefotaxime (*2*), and cefazolin (*3*) on *rpoS* induction. The microorganisms were cultured under standard conditions in flasks on M-9 medium. The *rpoS* expression was induced by addition to one of the cultures of 0.3 mM IPTG (RpoS$^+$), while the other culture was intact (RpoS$^-$). The induction was monitored by the increase in the expression of the target, the gene fusion *osmY:lacZ*. On reaching the maximum induction, the cells of the two cultures were liberated from the medium and used for determination of the MIC by dilutions on immunological plates, adding IPTG to the induced cells.

presence of putrescine and salicylate was studied using two strains of *E. coli* (Fig. 3). One of these strains had the normal *marRAB* operon (*mar*$^+$), and the other had a deletion in the place of its location on the chromosome (*mar*$^-$).

Putrescine at the concentration of 5 mM noticeably increased the MIC in the wild strain (*mar*$^+$) culture, but its effect on the mutant (*mar*$^-$) culture, if any, was at the limits of the sensitivity of the method (the antibiotic titration step). But when added in the presence of salicylate, putrescine significantly increased the MIC in the cells of the two strains, more markedly in the *mar*$^+$ cells. Because the effect of salicylate on the MIC in the absence of the *mar* regulon was positive, it was suggested to be mainly due to limitation of porin transport by means of *micF* induction. This seems to be a factor determining the synergism of its effect with that of putrescine.

The MIC values of two classes of antibiotics transported into cells through porin channels (pefloxacin) and cell membranes (netromycin) were compared, and the mechanism of the transport occurred to be crucial for the effect of putrescine and salicylate on the sensitivity to the antibiotics (Fig. 4). The effects of these compounds on pefloxacin were very similar to their effects on another fluoroquinolone, levofloxacin, but in the case of netromycin, which is a representative of the second generation of aminoglycosides, the dependence was quite opposite. Thus, the synergism of putrescine and salicylate effects on fluoroquinolones and aminoglycosides (Fig. 4) seems to have a different nature.

Polyamines as modulators of antibiotic resistance during acidic stress. *Escherichia coli* reacts to acidic stress, in particular, by induction of expression of the gene *rpoS* encoding the alternative σ^S-subunit of RNA polymerase, which controls some systems of acidic resistance [23]. We have shown earlier that polyamines are positive modulators of the *rpoS* expression [12]. Based on some recent data [24], it was suggested that a mechanism functioning during acidic stress and providing for multiple drug resistance of *E. coli* could be caused by induction of σ^S synthesis.

This hypothesis was checked using the *rpoS* gene induction in cells of the *E. coli* strain HS1600DE3Y (pRPOS) (Fig. 5). The induced cells displayed a pronounced increase in the MIC for fluoroquinolones (levofloxacin) and β-lactam antibiotics (cefotaxime, cefazolin) that indicated an essential role of σ^S in formation of the multiple antibiotic resistance in *E. coli*. Hence, it was suggested that the role of polyamines in the development of multiple antibiotic resistance of *E. coli* could be determined by their positive influence on the content in the cell of the σ^S-subunit of RNA polymerase.

To follow the supposed cause–effect relations in the chain polyamines → σ^S → antibiotic resistance, a culture of strain SHTO3 constructed by us was used. Addition of putrescine to the cells of the polyamine-deficient strain markedly stimulated *rpoS* expression (Fig. 6). A similar although less pronounced influence was displayed by salicylate, which is a weak acid capable of causing acidic stress similarly to acetate [25]. Simultaneous addition of putrescine and salicylate manifested a summed effect. The MIC values for levofloxacin and pefloxacin suggested strong positive influences of putrescine and salicylate on the antibiotic resistance, which was similar to the effects of these compounds on *rpoS* expression (Fig. 6). This confirms the hypothesis that one of the mechanisms of multiple antibiotic resistance, in particular, under conditions of acidic stress, is realized via the influence of polyamines on the σ^S content in the cell.

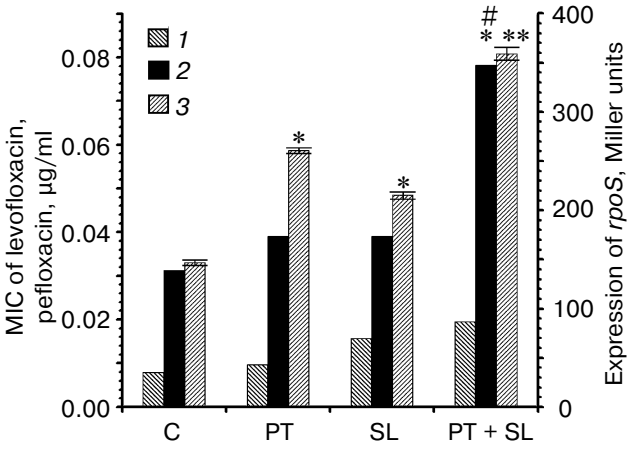

Fig. 6. Dependence of *E. coli* SHT03 resistance to levofloxacin (*1*) and pefloxacin (*2*) on *rpoS* expression (*3*) in the presence of putrescine and salicylate. The microorganisms were cultured under standard conditions in flasks on M-9 medium without additions (C), in the presence of 5 mM putrescine (PT), 5 mM salicylate (SL), and of both 5 mM putrescine and 5 mM salicylate (PT + SL). The *rpoS* expression was monitored by the activity of β-galactosidase using the gene fusion *rpoS:lacZ*. Upon reaching the maximum expression (~4 h of culturing), the cells were freed from the medium and used for determination of the MIC, as indicated in the captions to Fig. 3 and in "Materials and Methods". Significant differences at $p \le 0.05$ (considering Bonferroni's correction): *, from C; **, from PT; #, from SL.

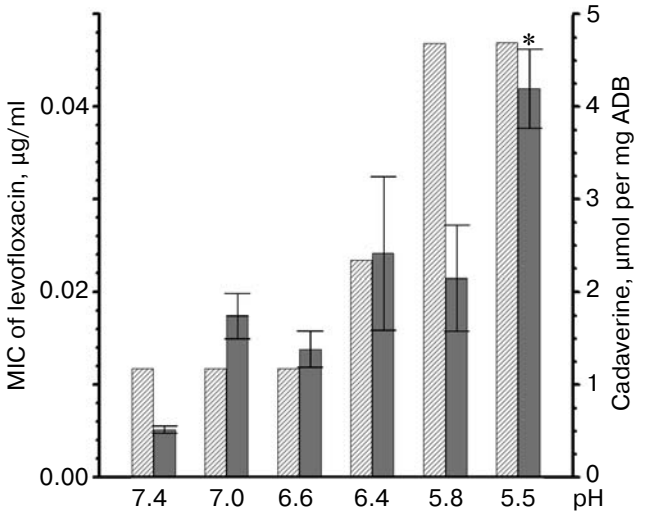

Fig. 7. Dependence of *E. coli* M2073 resistance to levofloxacin (hatched columns) on the cadaverine content in the cells (dark columns) under changes in pH of the medium in the presence of the antibiotic. The microorganism cultures for analysis were taken from the wells of immunological plates in the experiments for determination of the MIC. *, Significant difference from the control at $p \le 0.05$ with consideration of the Bonferroni correction.

The acidic stress caused by a decrease in pH of the medium was accompanied by an increase in the content of a polyamine cadaverine in the cells (Fig. 7), which significantly limited the transport activity of the porin channels (Table 2). This increased the antibiotic resistance (Fig. 7).

In addition to medium acidification, the production of cadaverine considerably increased in response to sublethal concentrations of the antibiotics, which were most effective at pH 5.5 (Fig. 8). The cadaverine content was significantly increased in the cells treated with fluoroquinolones transported through porin channels. This suggested that the restriction of the porin protein activity owing to induction of cadaverine can be a reaction of cell defense against antibiotics.

DISCUSSION

Hydrophilic compounds are transported into cells of gram-negative microorganisms through outer membrane

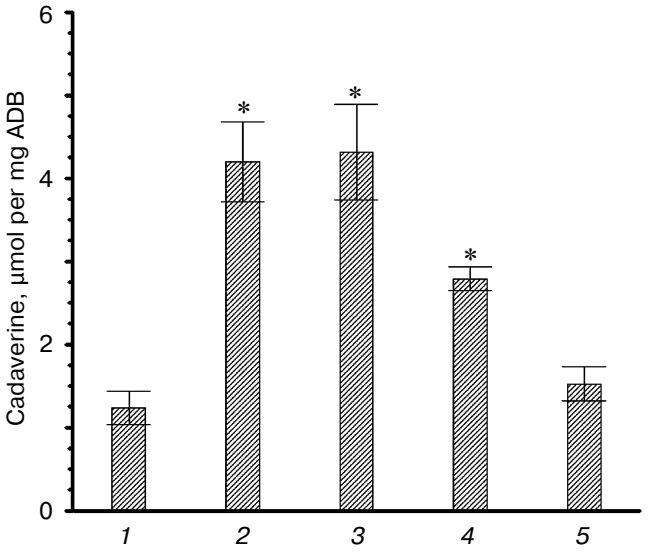

Fig. 8. Changes in the cadaverine content in *E. coli* M2073 cells in response to sublethal concentrations of antibiotics in the medium at pH 5.5. *1*) Culture without antibiotics; *2-5*) supplemented with 0.023 μg/ml levofloxacin, 0.047 μg/ml pefloxacin, 1.563 μg/ml nalidixic acid, and 1.563 μg/ml netromycin, respectively. *, Significant difference from the control at $p \leq 0.05$ with consideration of the Bonferroni correction. The microorganism cultures for analysis were taken from the wells of immunological plates in the experiments for determination of the MIC.

channels (porins), among which OmpF is preferable for transporting such toxic compounds as antibiotics and bile salts [26]. A mechanism negatively regulating the OmpF content on the level of protein synthesis is based on the interaction of *micF* antisense RNA with the 5′-fragment of the *ompF* mRNA which inhibits the translation [9]. The *micF* gene encoding the regulatory RNA is a constituent of the antioxidant defense regulon *soxRS*, induction of which is positively modulated by polyamines under conditions of oxidative stress [11]. The limitation of the transport activity of the cells caused by of the decrease in OmpF content due to this effect increases the antibiotic resistance (Fig. 1).

The rate of antibiotic entrance into the cells of gram-negative microorganisms is determined by the ratio between the oppositely directed flows: on one hand, the transport into the cell through porin channels, and on the other hand, the removal from the cell by the multidrug efflux pumps [27]. The synthesis of these pumps is controlled by the transcriptional regulator MarA induced by sodium salicylate [28], which can also induce the *micF* antisense regulatory RNA which suppresses OmpF synthesis on the translational level. The study on the transport activity of porin channels in the presence of salicylate has shown that polyamines inhibit this parameter even on the background of induction of the multidrug efflux system (Table 2). Moreover, the additivity of polyamine and salicylate effects is also due to their stimulation of *micF*.

Some antibiotics, in particular, aminoglycosides, cannot enter the cell through porin channels and across bilayer membranes at the cost of the cell membrane potential. The increase in the *E. coli* sensitivity to aminoglycosides observed in the presence of salicylate (Fig. 4) is explained by its effect as a weak acid elevating the membrane potential, which amplifies the electrophoretic transfer of positively charged aminoglycosides into the cell [29]. However, the polyamine-deficient mutants possess a decreased sensitivity to the first generation aminoglycoside streptomycin [30]. It seems that the ability of polyamines to bind with the 30S and 50S subunits of ribosomes can strengthen or weaken the effect of different antibiotics having influence on the level of ribosomes [31]. Thus, the synergism of the putrescine and salicylate effects in the case of fluoroquinolones (Fig. 3) is caused by the common action mechanisms (inhibition of transport through OmpF), but in the case of netromycin (Fig. 4) their synergism seems to be based on a different mechanism: the enhancement of the transport across the cytoplasmic membrane for salicylate and the influence on the target of the antibiotic (ribosomes) for polyamines.

The multiple antibiotic resistance under conditions of medium acidification can be realized via the system of multidrug efflux pumps [4] and also unidentified alternative mechanisms [25]. The pH dependence of the *E. coli* sensitivity to antibiotics suggests (Fig. 7) that one such mechanism can be associated with the accumulation of cadaverine in the cells on acidification of the medium as a result of induction of the *cadA* gene encoding inducible lysine decarboxylase [32].

As a mechanism increasing the production of cadaverine, oxidative stress seems to be a result of "suicidal response" of the microorganisms to a sublethal exposure to environmental factors [33]. This interpretation is confirmed by data on the induction of lysine decarboxylase by superoxide stress [11], the *cadA* belonging to the *soxRS* regulon of the antioxidant defense [34], and also the ability of some antibiotics to cause oxidative stress [35]. And σ^S seems to play a significant role in the elevation of the cadaverine production at the cost of inducible lysine decarboxylase LdcI [36].

Based on the known functions of σ^S in the defense of microorganisms against starvation, stationary phase, oxidative, acidic, osmotic, and other stresses, it is considered to be one of main regulators of their adaptive reactions [37]. This functional role of σ^S can be, in particular, associated with its strong negative influence on *ompF* expression on the translational level [7], which imparts to microorganisms additional resistance to deleterious environmental factors, including antibiotics. In this respect polyamines, which are also considered as factors of adaptation to various stresses [10, 12], are also synergic and enhance the resistance to antibiotics that are transported through the porin channels and lower the cell envelope permeability. This effect is provided for by at least three

different mechanisms: the restriction of the synthesis of porin channels on the translational level by the positive modulation of *micF* (Fig. 1), the direct negative effect on their transport activity (Table 2), and also the positive influence of polyamines on σ^S in the cell (Figs. 5 and 6) accompanied by a decrease in the porin transport due to suppression of the *ompF* transcription and the induction of cadaverine synthesis (Fig. 8).

The authors are sincerely grateful to R. G. Martin (National Institutes of Health, Bethesda, USA) for the presented *E. coli* strains N9212, N8452, M2073, M2076, and N7840; to R. Hengge (Berlin University, Berlin, Germany) for the *E. coli* strain R091; to H. Tabor (National Institutes of Health, Bethesda, USA) for the strain HT306; and also to H. Schellhorn (McMaster University, Hamilton, Canada) for the *E. coli* strain HS1600DE3Y (pRPOS).

This work was performed under the "Molecular and Cell Biology" Program of the Presidium of the Russian Academy of Sciences and was supported by the Russian Foundation for Basic Research (project Nos. 04-04-97501, 05-04-48091, 04-04-96062) and the Department of Science and Education of the Perm Region.

REFERENCES

1. Krulwich, T. A., Lewinson, O., Padan, E., and Bibi, E. (2005) *Nat. Rev. Microbiol.*, **3**, 566-572.
2. Lewinson, O., Padant, E., and Bibi, E. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 14073-14078.
3. Martin, R. G., and Rosner, J. L. (2002) *Mol. Microbiol.*, **44**, 1611-1624.
4. Ma, D., Alberti, M., Lynch, C., Nikaido, H., and Hearst, J. E. (1996) *Mol. Microbiol.*, **19**, 101-112.
5. Eswaran, J., Koronakis, E., Higgins, M. K., Hughes, C., and Koronakis, V. (2004) *Curr. Opin. Struct. Biol.*, **14**, 741-747.
6. Heyde, M., and Portalier, R. (1987) *Mol. Genet. Genom. (Hist. Arch.)*, **208**, 511-517.
7. Liu, X. Q., and Ferenci, T. (2002) *Microbiol. Sgm.*, **147**, 2981-2989.
8. Pratt, L. A., Hsing, W. H., Gibson, K. E., and Silhavy, T. (1996) *J. Mol. Microbiol.*, **20**, 911-917.
9. Storz, G., Opdyke, J. A., and Zhang, A. X. (2004) *Curr. Opin. Microbiol.*, **7**, 140-144.
10. Tkachenko, A., Nesterova, L., and Pshenichnov, M. (2001) *Arch. Microbiol.*, **176**, 155-157.
11. Tkachenko, A. G. (2004) *Biochemistry (Moscow)*, **69**, 188-194.
12. Tkachenko, A. G., Shumkov, M. S., and Akhova, A. V. (2006) *Biochemistry (Moscow)*, **71**, 185-193.
13. Samartzidou, H., Mehrazin, M., Xu, Z. H., Benedik, M. J., and Delcour, A. H. (2003) *J. Bacteriol.*, **185**, 13-19.
14. Martin, R. G., Gillette, W. K., and Rosner, J. L. (2000) *Mol. Microbiol.*, **35**, 623-634.
15. Muffler, A., Barth, M., Marschall, C., and Hengge-Aronis, R. (1997) *J. Bacteriol.*, **179**, 445-452.
16. Chen, G. Z., Patten, C. L., and Schellhorn, H. E. (2003) *Antimicrob. Agents Chemother.*, **47**, 3485-3493.
17. Miller, J. H. (1992) *Experiments in Molecular Genetics*, Cold Spring Harbor, N. Y.
18. Chattopadhyay, M. K., Tabor, C. W., and Tabor, H. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 2261-2265.
19. Nikaido, H. (2003) *Microbiol. Mol. Biol. Rev.*, **67**, 593-656.
20. Szybalsky, W., and Bryson, V. (1952) *J. Bacteriol.*, **64**, 489-499.
21. *Determination of the Microorganisms' Sensitivity to Bacterial Preparations (Methodical Instructions of MUK 4.2.1890-04)* (2004) *Klin. Mikrobiol. Antimikrob. Khimioter.*, **6**, 306-359.
22. Wiese, A., Reiners, J. O., Brandenburg, K., Kawahara, K., Zahringer, U., and Seydel, U. (1996) *Biophys. J.*, **70**, 321-329.
23. Foster, J. W. (2004) *Nat. Rev. Microbiol.*, **2**, 898-907.
24. Rami, A., Toutain, C. M., and Jacq, A. (2005) *Res. Microbiol.*, **156**, 356-360.
25. Rosner, J. L., and Slonczewski, J. L. (1994) *J. Bacteriol.*, **176**, 6262-6269.
26. Harder, K. J., Nikaido, H. F., and Matsuhashi, M. (1981) *Antimicrob. Agents Chemother.*, **20**, 549-552.
27. Grkovic, S., Brown, M. H., and Skurray, R. A. (2003) *Microbiol. Mol. Biol. Rev.*, **66**, 671-701.
28. Cohen, S. P., Levy, S. B., Foulds, J., and Rosner, J. L. (1993) *J. Bacteriol.*, **175**, 7856-7862.
29. Aumercier, M., Murray, D., and Rosner, J. L. (1990) *Antimicrob. Agents Chemother.*, **34**, 786-191.
30. Diniello, G. B., Algranati, I. D., and Goldemberg, S. H. (1997) *Cell. Mol. Biol.*, **44**, 521-526.
31. Petropoulos, A. D., Xaplanteri, M. A., Dinos, G. P., Wilson, D. N., and Kalpaxis, D. L. (2004) *J. Biol. Chem.*, **279**, 26518-26525.
32. Soksawatmaekhin, W., Kuraishi, A., Sakata, K., Kashiwagi, K., and Igarashi, K. (2004) *Mol. Microbiol.*, **51**, 1401-1412.
33. Aldsworth, T. G., Sharman, R. L., and Dodd, C. E. (1999) *Cell Mol. Life Sci.*, **56**, 378-383.
34. Pomposiello, P. J., Bennik, M. H., and Demple, B. (2001) *J. Bacteriol.*, **183**, 3890-3902.
35. Goswami, M., Mangoli, S. H., and Jawali, N. (2006) *Antimicrob. Agents Chemother.*, **50**, 949-954.
36. Kikuchi, Y., Kurahashi, O., Nagano, T., and Kamio, Y. (1997) *Biosci. Biotechnol. Biochem.*, **62**, 1267-1270.
37. Hengge-Aronis, R. (2002) *Microbiol. Mol. Biol. Rev.*, **66**, 373-395.