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Research Article

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Vitamin Para-Aminobenzoic Acid (PABA) Controls Generation of Nitric Oxide (NO) *In Vitro* and Its Biological Functions in the Bacterial Cells

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Abstract

Nitric oxide (NO) serves either a universal signaling molecule or extremely toxic agent, depending on the dose. Up to date there have been a very limited number of natural compounds serving as effective regulators of NO signaling and toxic potencies. NO acts in concert with H_2S to coordinate cell responses; however, how exactly this interaction is achieved is not known. Both agents have an effect on the accumulation of both reactive chemical species, ROS and RNS and can give rise to other reactive species. Para-aminobenzoic acid (PABA) is an essential metabolite for certain organisms. Once considered a vitamin, PABA, functions as an effective inhibitor of inducible SOS DNA repair processes in *E. coli*.

In the present study we focus on the genetic and physiological evidence in favor of interference of NO-donors and PABA in bacterial cells with DNA repair gene expression and biofilm formation, depending on the rate of NO-donating *In Vitro* and intracellular ROS/RNS accumulation in the cells. The crystalline dinitrosyl iron complexes (NO-29 and NO-33) with thiourea as the ligands and 3 crystalline tetranitrosyl iron complexes with thiosulfate ($TNIC_{thio}$) - and with sulfur-containing aliphatic ligands – cysteamine and penicillamine were studied first as the NO-donors in pure solutions and in the combination with PABA.

In *E. coli* cells with the combined action of PABA (0.01-5 mM) with nitric oxide donors we observed an inhibition of NO-signaling potency in the SOS (sfiA gene)- and the SoxRS (soxS gene) DNA repair pathway up to 3.5 fold, depending on the dose of PABA. PABA tested at 0.5 mM afforded 24% protection against the level of biofilm formation induced by $TNIC_{thio}$.

Using the antioxidant-capacity assay, we observed a many-fold decrease in the ROS/RNS level production in the samples of *E. coli* cells with PABA and NO-donor-$TNIC_{thio}$.

Keywords: Nitric oxide (NO); p-aminobenzoic acid (PABA); *E. coli*; Oxygen reactive species

Introduction

In the aerobic environment, all structures of a cell - DNA, proteins and lipids of membranes - are constantly influenced by reactive species of oxygen (ROS) and nitrogen (RNS). In mammals and humans their generation induces the systems of molecular and genetic protection from stress, just as regulators of aging processes and development of major pathologies do.

Para-aminobenzoic acid (PABA), being a B vitamin, is a cofactor and precursor in the synthesis of folic acid, purines and thymine in most species of bacteria, algae and higher plants [1].

It was established, that vitamins of B group, incl. PABA and beta-carotene (provitamin A) and D-alpha-tocopherol (vitamin E), possess the UV-absorbing property and are widely used to protect from solar radiation, photocarcinogenic exposures and inflammatory agents [1-9]. Molecular and genetic mechanisms of these processes in mammalian cells are poorly understood.

In 1984, S.V. Vasilieva was the first who experimentally substantiated the hypothesis about a selective inhibition of PABA-inducible SOS DNA repair response in *E. coli* bacterial cells, -regulation of the error-prone" DNA mutagenesis, W-reactivation and W-mutagenesis, prophage induction [10], sfiA-dependent filament formation and other functions [11-13]. The results obtained in a study *In Vitro* of the molecular mechanisms of these phenomena using methods of differential spectrophotometry and thermal denaturation of DNA, optical activity (circular dichroism) and fluorescence became the basis for the hypothesis about a direct chemical interaction of PABA and DNA. Experiments *In Vitro* proved the formation of electrostatic bonds between the components [14].

In 2004 the PABA/NO complex was synthesized and proposed for use as a targeted catalyst of glutathione-S transferase P1 [15]. This enzyme selectively super-expresses in cancer cells, leads to changes in their redox balance in the cell and launches many potentially lethal cellular responses [16]. PABA functions as a protector of cells against ROS radicals, directly interacting with OH hydroxyl radicals and inhibiting oxidation of deoxyribose in Fenton reaction [7,8]. It stimulates biosynthesis of endogenous interferon in animals and humans; the antiviral drug "AKTIPOL" was developed on the basis of PABA and introduced into practice for treatment of herpes and adenovirus eye infections [17].

On the whole, vitamins are widely used in complex therapy for

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inhibition of oxygen free radicals ROS; however, much less is known about the role of vitamins in regulation of reactive nitrogen species (RNS) and their generation in various biological systems.

The aim of our work is to study the mechanisms of regulation by para-aminobenzoic acid of DNA-repair systems of oxidative and nitrosative stress as well as biofilm antibiotic resistance induced by nitric oxide donors in E. coli cells.

Materials and Methods

NO donors

Water-soluble nitrosyl iron-sulfur complexes – crystalline NO donors with various ligands, were first synthesized at the Institute of Problems of Chemical Physics RAS: tetranitrosyl iron complex with thiosulfate $Na_2[Fe_2(S_2O_3)_2(NO)_4]^{2-}\cdot 4H_2O$ ($TNIC_{thio}$), nitrosyl iron complexes with aliphatic ligands of natural origin cysteine – cisaconite: (CysA) - $[Fe_2(S(CH_2)_2NH_3)_2(NO)_4]SO_4$ 2.5 H_2O and penicillamine – penaconite (PenA) $[Fe_2(S(C(CH_3)_2CH(NH_3)COOH))_2(NO)_4]SO_4$ $5H_2O$ and two cationic dinitrosyl iron complexes (DNICs) with thiourea - $Fe(SC(NH_2)_2)_2(NO)_2]_2SO_4\cdot H_2O$ (NO-29) and $[Fe(SC(NH_2)_2)_2(NO)_2]$ $Cl\cdot H_2O$ (NO-33). Structure and physico-chemical characteristics of the donors were studied in [18-21].

NO donors were dissolved immediately before each experiment in distilled water or in phosphate-buffer saline (PBS), pH=7.4.

Para-aminobenzoic acid (PABA) was recrystallized from the hot aqueous solution and dried in the air. Water/PBS solutions with pH=7.4 were studied.

Quantification of NO (+/-PABA) releasing

NO generated by the NO donors ($TNIC_{thio}$ and PenA) was quantitated using the sensor electrode amiNO-700 of the INNO NITRIC OXIDE MEASURING SYSTEM (Innovative Instruments, United States) [22]. The concentration of NO was detected for maximum 4000 s at 2 s intervals. The electrochemical sensor was calibrated using the standard 100 μM $NaNO_2$ aqueous solution. All experiments were performed under aerobic or anaerobic conditions at 23°C; pH of solutions was measured with an HI 8314 membrane pH-meter (HANNA Instruments, Germany).

Bacterial strains

The bacterial strains of *Escherichia coli* were studied: *E. coli* PQ37 sfiA::Mud (APlac) cts lac ΔU169 mal^+ uvrA galE galY PhoG rda F^- thr leu his pyrD thi trp::Muc^+ srl300::Tn10 was kindly provided by Hofnung (Pasteur Institute, Paris) [23];

***E.coli* MC4100** F- [*araD139*]B/r Δ(*argF-lac*)169 & lambda-e14-*flhD5301*

Δ(*fruK-yeiR*)725 (*fruA25*) *relA1 rpsL150(strR) rbsR22* Δ(*fimB-fimE*)632(::IS1) *deoC1* was a gift of Ding, USA [24];

***E.coli* TN530** F2 Δ(*lacZYA-argF)U169 rpsL λ(soxS'-lacZ) soxRS*+ was a gift of Nunoshiba, Japan [25].

P. aeruginosa PAO1 (the clinical isolate, lab. collection) was used in the biofilm experiments [26].

β-galactosidase (β-gal) assay

The level of the sfiA-gene expression (the SOS-regulon) was studied in *E. coli* PQ37 with the [sfiA::lacZ] operon fusion and a deletion in the chromosomal lac operon, so that β-galactosidase activity was strictly dependent on the *sfiA* expression. sfiA gene expression was monitored as described by Quillardet et al. [23]. *E. coli* TN530 wt [soxS::lacZ] strain was used for the soxS gene expression according to [25]. The quantitative level of β-gal activity in the cells was determined according to Miller [27]. Briefly, an overnight *E. coli* culture was diluted 1:50 into Luria-Bertani broth (LB) medium and grown for 3,5 h to OD600=0.3-0.4, which corresponded to the early log-phase of growth. Cells were treated with NO donors and/or PABA for 30 min at 37°C and further incubated in the presence of the chromogen *o*-nitrophenyl-β-*D*-galactopyranoside (ONPG). The β-gal activity was measured by PD-303UV digital spectrophotometer (APEL Co. Ltd., Japan), at 420 nm. To calculate the β-gal activity (E), an equation E=1000•OD420/t, where OD420 is the optical density at 420 nm and t is the time of incubation with the chromogen, was used. As the positive controls, 26.3 μM 4-nitroquinoline oxide, 4NQO, was used for *E. coli* PQ37 and 0.5 mM menadione for *E. coli* TN530.

Plankton cell culture and biofilm processing

The basic protocol of the method described in [28]. The plankton cell growth was determined by OD_{600} value. 0.1% (wt/v) crystal violet was used for 10 min to stain the attached cells. Unattached dye was rinsed away by washing two times with distilled water and the stained biomass was dissolved with 1:4 (v/v) mixtures of acetone and ethanol. After 15 min, the OD_{570} was measured to quantify the biofilm biomass. Antibiotic ciprofloxacin (CF) was used as a positive control.

Antioxidant-capacity assay and quantitative study of ROS/RNS production in *E. coli* cells

The sum of reactive oxygen (ROS) and nitrogen RNS species [being formed in the cells treated with iron-sulfur nitrosative agents (NO-donors) in aerobic conditions] was measured using antioxidant capacity assay, that employed the reactive oxygen species (ROS)-sensitive probe 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA). Because of the dominance of oxidative processes caused by RNS, the term nitroxidative stress is proposed, emphasizing the oxidative (as opposed to nitrosative or nitrative) stress that dominates RNS actions under biological conditions, in the cells [29]. The antioxidant-capacity assay was used to examine the intracellular ROS in *E. coli* TN530.

E. coli TN530 cells were grown to logarithmic growth phase in LB medium in accordance with Wu and Outten [28], washed twice in phosphate saline buffer pH 7.4 (PBS) and resuspended therein. PABA solution aliquots in PBS were added to the cell suspension with $1\bullet 10^6$ titer. The concentration of NO donor $TNIC_{thio}$ in all variants - 0.05 mM. 198 ml of the $TNIC_{thio}$ +/- PABA treated cell suspension were transferred to a 2 ml Eppendorf with radical probe, 5-(and-6)-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA) (Molecular Probes, Eugene, OR, USA) at the conc. of 100 mM and incubated at 37°C. ROS production was measured by fluorescence intensity (conv) on QUBIT PORTABLE FLUOROMETER (INVITROGEN, Turner BioSystems, USA) at 488 nm (excitation) and 525-530 nm (emission) every 15 min during 1 h.

CM-H_2DCFDA (inactive for ROS) is converted to DCFH (dichlorofluorescein diacetate, active for ROS), by being taken into the cell and acted upon by an intracellular enzyme esterase. The $H_2O_2^{\bullet}$ or $O_2^{\bullet -}$ oxidizes intracellular DCFH (non-fluorescent) to DCF (fluorescent) [30].

Statistical Analysis

Results are presented as the mean values of at least three experiments

and SEM, confidence intervals are shown for P value=0.05. Statistical analysis of the experimental results was performed using the Microsoft Excel and OriginPro 7.0 software packages.

Results

In Figure 1 we summarized the experimental data of DNA repair gene expression obtained in *E. coli* and PABA (0-5.0 mM) influence in the samples with 0.1 mM NO-33 and 0.05 mM NO-29. Pure aqueous PABA solutions did not influence the gene expression. PABA tested at 5 mM decreased the expression of the soxS and the sfiA gene expression by 2.5-3.5 folds. The low PABA doses were much less effective. PABA afforded 36% protection against PenA damages in the sample with PABA: PenA (1:10) in *E. coli* PQ37; but in the experiment with $TNIC_{thio}$:PABA (1:100) PABA influence was less effective in *E. coli* TN530 (Figures 2a and 2b).

In *P. aeruginosa*, the non-lethal dose of NO donor ($TNIC_{thio}$ 0.02 mM) inhibited the level of biofilm formation with and without of PABA application.

In the sample of ($TNIC_{thio}$ 0.02 mM+PABA 0.5 mM) the level of the biofilm formation inhibition corresponded to 45% as compared to the control and it was higher than that in the sample with CF (Figure 3). PABA inhibited the levels of bacterial biofilm formation in *E. coli* MC4100 induced by NO donors CysA, PenA and $TNIC_{thio}$ (Figure 4). CysA was the most effective inhibitor in the biofilm formation process.

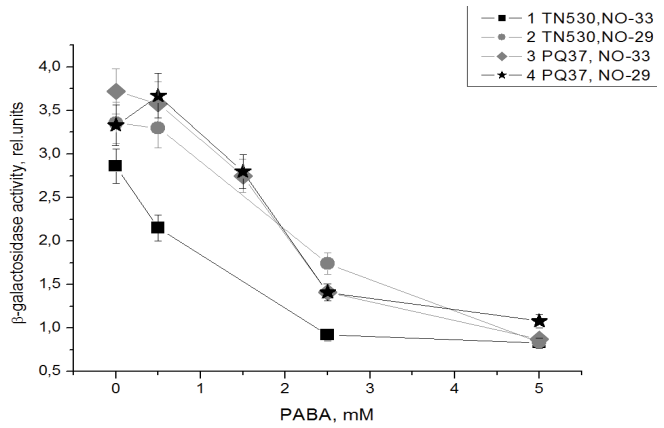

Figure 1: Expression of the soxS gene (SoxRS regulon) in *E. coli* TN530 (1,2) and the sfiA gene (SOS regulon) in *E. coli* PQ37 (3,4), induced by 0.1 mM NO-33 and 0.05 mM NO-29 and PABA influence.

The kinetic curves of NO donating by NO- donors (nM) and PABA influence were shown in Figures 5 and 6. We studied the process in the aerobic and anaerobic conditions in the experiments with PenA, exclusively. On the initial stage of the donor incubation (up to 1350 s) the level of NO donating by $TNIC_{thio}$ decreased in the sample with PABA, but then it was higher. On Figure 6 we observed the same dependence (samples 1 and 3). We observed the highest level of NO-donating in anaerobic conditions (Figure 6, sample 2). In common, the process of NO-donating depended on PABA concentration. The growth in PABA dose promoted the reduction of NO-generation in aerobic conditions. And the maximum level of NO-donating inhibition was observed in the sample of [PenA+PABA (1:10)] in aerobic conditions.

Using the antioxidant-capacity methodology Nakajima et al. [30] tested an influence of PABA on the ROS\RNS production in *E. coli* cells treated with $TNIC_{thio}$ as NO-donor, (DCFDA fluorescence, in relative units) (Figure 7). The NO donor was tested in the constant conc. of 0.05 mM, while the doses of PABA were variable from 0.05 mM to 5 mM. We observed the significant inhibition of the ROS\RNS production in all samples with $TNIC_{thio}$ in the combination with PABA. The change in NO:PABA=1:0 to NO:PABA=1:100 the level of ROS\RNS was decreased five-fold.

Discussion

Our work is the first to study the function of para-aminobenzoic acid vitamin in the regulation of protective reactions of bacterial cells to cyto- and genotoxic stress caused by mono- and binuclear iron-sulphur-nitrosyl complexes - crystalline NO donors. All studied donors easily penetrate through the bacterial cell membrane [31-33] and are thought to form intracellularly, on the basis of donated nitric oxide and cellular iron, main NO transport and functional structures – dinitrosyl iron complexes – S-nitrosothiols and dinitrosyl iron complexes (DNICs), which are more stable and ensure NO transport *in vivo* [34-36].

Such complexes were first observed and identified in all biological systems by their specific EPR signals [37,38]. The mechanisms of DNIC appearance in the bacterial cells are not quite clear. These NO compounds

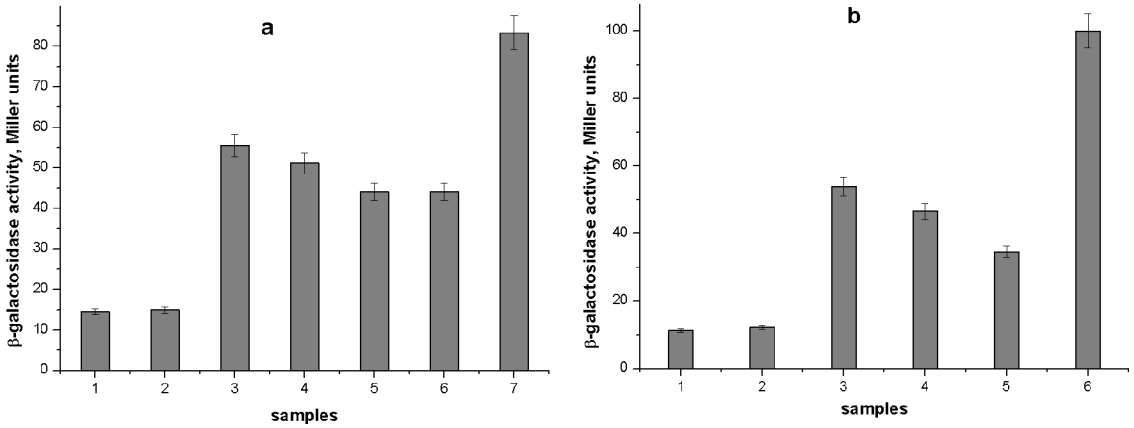

Figure 2: Expression of the soxS gene (SoxRS regulon) in *E. coli* TN530 (a) and the sfiA gene (SOS regulon) in *E. coli* PQ37 (b), induced by NO donors and PABA influence (a): 1-control; 2-PABA 0.01-5 mM; 3-$TNIC_{thio}$ 0.01 mM; 4-$TNIC_{thio}$+PABA (1:10); 5-$TNIC_{thio}$+PABA (1:100); 6-$TNIC_{thio}$+PABA (1:500); 7-menadione 0.5 mM (positive control); (b): 1-control; 2-PABA 0.5 mM; 3-PenA 0.05 mM; 4-PenA+PABA (1:1); 5-PenA+PABA (1:10); 6-4NQO (positive control).

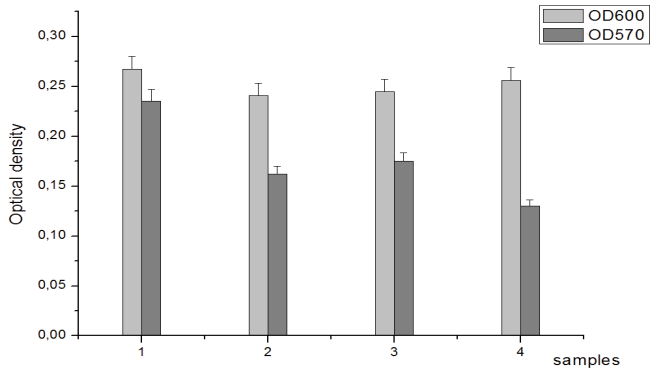

Figure 3: Biofilm formation in *P. aeruginosa:* 1-control; 2-CF 0.05 μM; 3-$TNIC_{thio}$ 0.02 mM; 4-$TNIC_{thio}$ 0.02 mM+PABA 0.5 mM.

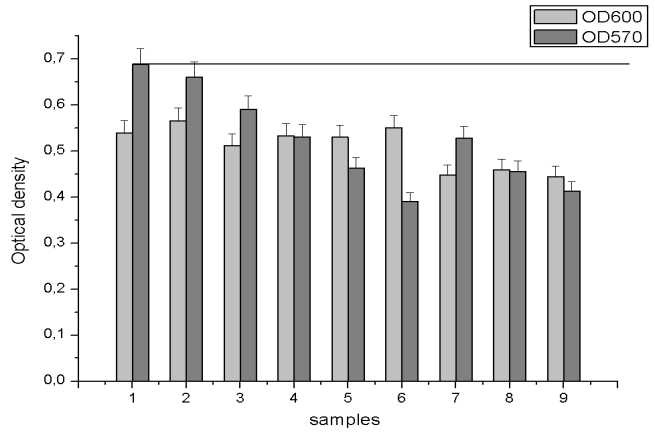

Figure 4: Biofilm formation in *E. coli* MC4100: 1-control; 2-PABA 0.01 mM-PABA 0.05 mM; 3-$TNIC_{thio}$ 0.01 mM; 4-$TNIC_{thio}$+PABA (1:1); 5-CysA 0.01 mM; 6-CysA+PABA (1:1); 7-PenA 0.05 mM; 8-PenA+PABA (1:1); 9-CF 0.07 μg/ml.

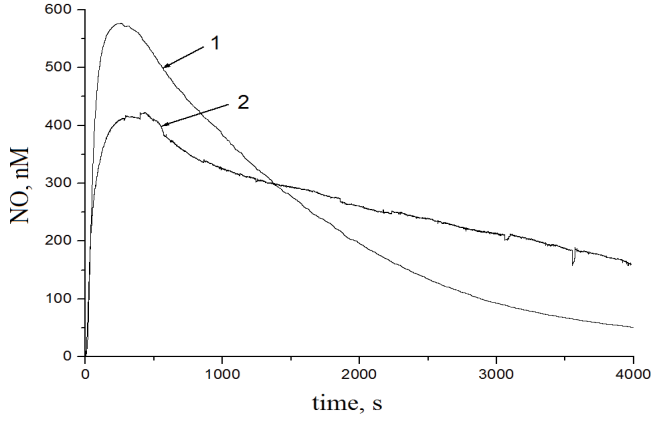

Figure 5: Time dependence of NO (nM) generated by $TNIC_{thio}$ ($1•10^{-5}$M) in water solution at 25°C: 1-$TNIC_{thio}$; 2-$TNIC_{thio}$+PABA (1:1).

trigger the signal pathways of transduction associated with physiological and pathological responses of cells to different types of stress. Despite a characteristic for all DNICs EPR signal with anisotropic factor g=2.03, the reactivity and functions of DNICs are different, depending on physiological conditions and the genotype of cells.

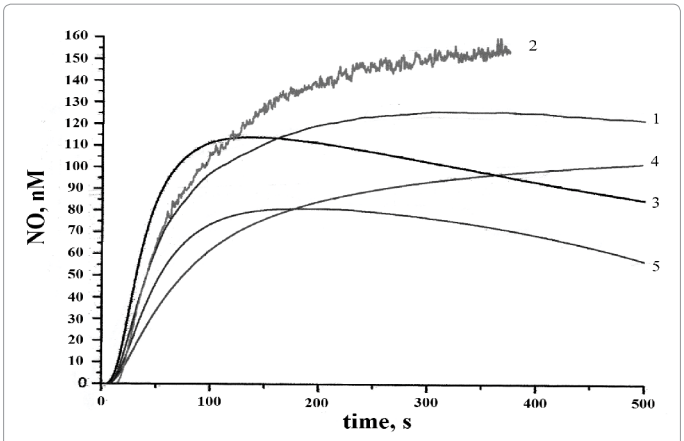

Figure 6: Time dependence of NO (nM) generated by PenA ($1•10^{-5}$M) in buffer pH=7.4 under aerobic ($+O_2$) and anaerobic ($-O_2$) conditions at 25°C: 1-PenA ($+O_2$), without PABA; 2-PenA ($-O_2$), without PABA; 3-PenA ($+O_2$), with PABA (1:1); 4-PenA ($-O_2$), with PABA (1:1); 5-PenA ($+O_2$), with PABA (1:10).

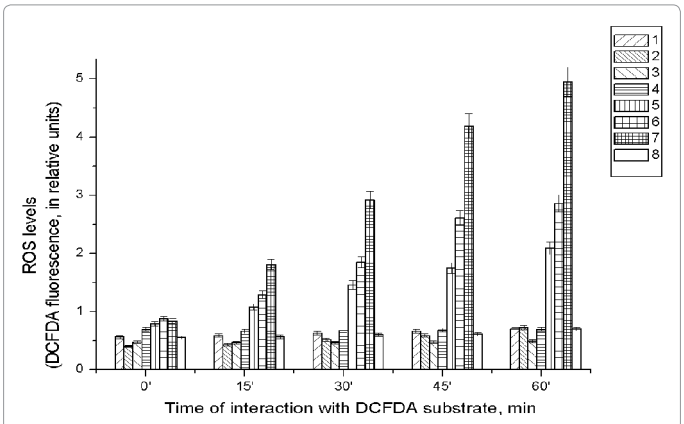

Figure 7: The level of ROS/RNS production in *E. coli* TN530 cells treated with 0.05 mM $TNIC_{thio}$ and PABA influence: 1-control; 2-menadione 0.5 mM; 3-PABA 5 mM; 4-$TNIC_{thio}$+PABA 5 mM; 5-$TNIC_{thio}$+PABA 0.5 mM; 6-NIC_{thio}+PABA 0.05 mM; 7-$TNIC_{thio}$ 0.05 mM; 8-CF 0.05 mM. The levels of all samples are indicated by deducing «0» for PBS fluorescence.

In enteric bacteria, nitric oxide is produced mostly as a byproduct of anaerobic metabolism; NO resembles superoxide - a natural agent of the respiratory process.

In 1999, Vasilieva et al. were the first to substantiate and experimentally confirm a SOS-inducing activity of NO-donors [31]. The authors used the method of quantitative assessment of the sfiA gene expression of the SOS regulon in *E. coli* strain PQ37 by S-nitrosothiols (GSNO, SNAP) and iron-sulphur-nitrosyl complexes (DNICs). It was subsequently proved that all NO-donors have a strong SOS-inducing activity and the level of sfiA gene expression is dose-dependent [22,39-41].

EPR-spectroscopic study of the cells treated with NO-donors showed characteristic for intracellular protein DNICs signal with the anisotropic factor g=2.03. The appearance of these signals correlated with the increase in the expression of the sfiA gene of the SOS response regulating the repair of DNA damage during oxidative stress. The stress was triggered by peroxynitrite which is formed as a result of interaction between NO molecules donated by the donor and superoxide anion. A direct dependence was found between the intensity of the SOS-induction and the content of intracellular iron since iron chelator-o-

phenanthroline-inhibited NO-induced expression of the sfiA gene in the cells; also the decrease in the intensity of signal g=2.03 was observed in the EPR spectra of these cells as a result of a lower level of intracellular protein DNICs.

These data have become direct evidence in favor of interconnection between formation in the cells of two signals: 1) EPR signal g=2.03 of the DNIC-protein complex and 2) the signal initiating induction of the SOS-box multi enzyme complex to launch the DNA-SOS repair system. To date, not all details of the molecular structures comprising the DNA-SOS-repair system of *E. coli* have been established. However, the presence in the DNA structure of double-strand breaks that cannot be repaired by other known DNA repair systems is undeniable [42].

Resistance of *E. coli* cells to superoxide anion and nitric oxide is selectively controlled by multi-functional SoxRS-regulon. A two-step mechanism of induction of SoxRS-regulon has been studied in detail and is associated with interaction of the iron-sulfur cluster of the sensory SoxR[2Fe-2S] protein with superoxide anion O_2•$^-$ or other redox agent (including DNICs), as well as with the changes in the redox state of the cluster of this protein and activation of the soxS structural gene with the induction of at least 15 repair enzymes of antioxidant protection, including against most antibiotics that generate ROS radicals [43-45].

According to Lancaster Jr. [29] the peak in oxidative reactions *In Vitro* with 1:1 fluxes of NO• and O_2•$^-$ does not occur under biological conditions, in the cells and 1) the quantitatively dominant (92-99.6%) process *in vivo* is oxidation, compared to nitrosation and nitration; 2) only five of the possible RNS reactions with thiol are quantitatively important biologically. Because of the dominance of oxidative processes caused by Lancaster Jr. [29] proposed the term nitroxidative stress, emphasizing the oxidative (as opposed to nitrosative or nitrative) stress that dominates RNS actions in the cells.

Thus, when protecting from stress and invasive pathogens in aerobic environment, nitrogen oxide in composition with DNICs and superoxide anion O_2•$^-$ are getting involved in the formation of the body of reactive compounds and their precursors, ROS and RNS (O_2^-•, NO$^-$•, OONO$^-$, OH$^-$, NO_2•$^-$). NO can function in 3 Redox states of NO^+, NO•, NO^- and in each of them nitric oxide has the unique chemical activity in relation to its main biological targets – SH-groups and [Fe-S] clusters.

Our *In Vitro* studies have shown that PABA significantly inhibits generation of nitric oxide by NO donors - $TNIC_{thio}$ and PenA in the buffer (pH 7.4), with a predominant drop in the indices of NO-donation in the aerobic environment (Figures 5 and 6) and with dose dependence on the concentration of PABA. A 5-fold inhibition of NO-generation was observed in a variant with the PenA+PABA ratio (1:10).

The conducted study has shown that a combined action of PABA (0.01-5 mM) and nitric oxide donors inhibits induction of the signal activity of SOS (sfiA) and SoxRS (soxS) genes of *E. coli* regulons up to 3.5 times maximum, depending on the dose of PABA (Figures 1 and 2).

Antioxidative activity of PABA towards inhibiting the levels of formation of biofilms with antibiotic resistance - as a response to oxidative stress - was first found in our experiments on a combined action of PABA and $TNIC_{thio}$ donors in *P. aeruginosa* cells (Figure 3) and $TNIC_{thio}$, PenA and CysA in *E. coli* 4100 (Figure 4). In all cases, the application of PABA resulted in the increase of the effectiveness of NO donors as inhibitors of biofilm formation.

We were the first to reveal on *E. coli* cells that the nitric oxide donor PenA modified cytotoxic effects of UVC (254 nm) radiation under aerobic and anaerobic conditions. The effect of NO on the cytotoxic action of UV radiation depended on the genotype of the cells - the activity of DNA excision repair system Uvr ABC and expressed either as a 4- or 10-fold protective effect (in aerobic environment) or as a 5-fold sensitization of cells under hypoxic conditions to the ROS/RNS radicals [19].

The obtained results on the nitric oxide donors are consistent with the conclusions made by Hu et al. [7] that PABA effectively absorbs ROS radicals (OH• radical, singlet oxygen [$^1O_2^-$] and HOCL) and protects DNA from UVC (254 nm) radiation. It is the radical damages to calf thymus DNA that is potentiated by the Fenton reaction system containing iron. We suppose that in our experiments with the iron-containing NO-donating agents we obtained exactly the same situation that has been described by Hu et al. [7].

Our study provides convincing evidence for the dependence of ROS/RNS radical products in *E. coli* cells TN530 when they are individually or in combination with PABA treated with $TNIC_{thio}$ donor (Figure 7). The total level of reactive oxygen species in the cells (in relative units) decreased with the increase of the PABA dose (at the constant $TNIC_{thio}$ concentration, 0.05 mm).

On the base of the results obtained we conclude that a combined application of PABA and iron-sulphur-nitrosyl complexes - nitric oxide donors, in which NO can be coordinated or bound with different ligands, aimed at regulating protective reactions of cells against stress, enables PABA to selectively interact with ROS/RNS radicals that are formed during hydrolysis of the donors and their metabolism. At the same time, PABA effectively absorbed NO generated by NO-donating agents in the experiments *In Vitro*.

Conclusion

For a long time para- aminobenzoic acid (PABA) considered a vitamin and a precursor of folic acid for certain bacteria, fungi and plants. On the basis of our results (the present paper and the previous priority publications) we conclude that in the bacterial cell PABA functions a potent inhibitor of DNA repair pathways induced by nitric oxide and a powerful antioxidant; an inhibitor of the bacterial biofilm formation and a regulator of nitric oxide generation *In Vitro*. Our data are of significance since the natural compounds with immense therapeutic effects and the medicine agents of the new class on the basis of the stable dinitrosyl-iron complexes (NO-donors) for the treatment of infections caused by *E. coli* and other invasive pathogens are of great concern.

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