Biochemistry (Moscow), Vol. 69, No. 8, 2004, pp. 883-889. Translated from Biokhimiya, Vol. 69, No. 8, 2004, pp. 1088-1095.

Genetic Signal Transduction by Nitrosyl-Iron Complexes in *Escherichia coli*

S. V. Vasilieva[1]*, E. Yu. Moshkovskaya[1], N. A. Sanina[2], S. M. Aldoshin[2], and A. F. Vanin[3]

[1]Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow 117977, Russia; fax: (7-095) 137-4101; E-mail: svasilieva@polymer.chph.ras.ru
[2]Institute of Problems of Chemical Physics, Russian Academy of Sciences, Chernogolovka 142432, Moscow Region, Russia; fax: (7-095) 785-7048; E-mail: sanina@icp.ac.ru
[3]Semenov Institute of Chemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow 117977; fax: (7-095) 938-2156; E-mail: mikoyan@center.chph.ras.ru

Received July 8, 2003
Revision received November 13, 2003

Abstract—Nitrosyl-iron complexes used as aqueous preparations of binuclear dinitrosyl-iron complex with glutathione ($DNIC_{glu}$), initially polycrystalline preparations of binuclear tetranitrosyl-iron complex with thiosulfate ($TNIC_{thio}$), and also binuclear tetranitrosyl-iron complex with aminotriazole ($TNIC_{atria}$) and mononuclear dinitrosyl-iron complex with triazole ($DNIC_{tria}$) in the concentration to 0.1 mM activated expression of the *soxS* and *sfiA* genes in *Escherichia coli*. Higher concentrations of polycrystalline preparations of low stability in aqueous solutions were cytotoxic, whereas $DNIC_{glu}$, which is more stable in water (up to two days), increased the gene expression on increase in its concentration to 0.5 mM. The iron chelating agent *o*-phenanthroline completely inhibited the gene expression induced by all compounds studied. The genetic signal transduction seemed to be realized not by nitric oxide molecules and/or iron ions released in solutions but directly by the complexes themselves, which activate transcriptional proteins by transfer onto them of nitrosyl-iron groups $[Fe^{+}(NO^{+})_2]$.

Key words: nitric oxide, SOS regulon, SoxRS regulon, nitrosyl-iron complexes, *Escherichia coli*

Nitric oxide enzymatically generated in many biological systems has been shown to function as a signal molecule in various physiological and biochemical processes [1], including transcription and translation of genetic material [2-7]. The low stability of this agent in the cells and tissues mainly caused by effect of superoxide anions O_2^- is overcome by inclusion of NO molecules into S-nitrosothiols (RS-NO) and dinitrosyl-iron complexes (DNIC) with natural thiol-containing ligands $(RS^-)_2Fe^+(NO^+)_2$ that function as NO depots and carriers in cells and tissues [8].

RS-NO and DNICs can act not only as donors of neutral NO molecules but also as donors of nitrosonium ions (NO^+) and groups $Fe^+(NO^+)_2$ and provide the functioning of RS-NO and DNICs as special signal molecules. Binding of NO^+ and $Fe^+(NO^+)_2$ to thiol groups of proteins and enzymes causes, respectively, S-nitrosylation of these biomolecules or production of DNIC bound to them and, as a result, changes in the functional activities of proteins and enzymes [9, 10].

We have earlier studied the effect of exogenous nitric oxide and some of RS-NO and DNICs, such as S-nitrosoglutathione (GS-NO) and DNICs with glutathione and cysteine ($DNIC_{glu}$ and $DNIC_{cys}$), on genetic systems responsible for resistance of *Escherichia coli* to oxidative stress: the regulons SOS and SoxRS [11-13]. Expression of the genes *sfiA* and *soxS* (the regulons SOS and SoxRS, respectively) was suppressed by the known iron chelator *o*-phenanthroline, which destroyed DNICs. Based on the totality of our data and that in the literature, we supposed that NO and RS-NO should initiate expression of these genes on their conversion to DNICs, which directly interact with molecular structures responsible for expression of these genes.

To additionally support this hypothesis experimentally, we studied the genetic activity of a number of polycrystalline nitrosyl-iron complexes with synthetic ligands,

Abbreviations: $DNIC_{glu}$) dinitrosyl-iron complex with glutathione; $DNIC_{cys}$) dinitrosyl-iron complex with cysteine; $TNIC_{thio}$) tetranitrosyl-iron complex with thiosulfate; $TNIC_{atria}$) tetranitrosyl-iron complex with aminotriazole; $DNIC_{tria}$) dinitrosyl-iron complex with triazole; RS-NO) S-nitrosothiol; GS-NO) S-nitrosoglutathione; ONPG) *o*-nitrophenyl-β-D-galactopyranoside; 4NQO) 4-nitroquinoline-1-oxide; OPh) *o*-phenanthroline; EPR-spectroscopy) electron paramagnetic resonance spectroscopy.

* To whom correspondence should be addressed.

0006-2979/04/6908-0883 ©2004 MAIK "Nauka/Interperiodica"

which were for the first time synthesized in the Institute of Problems of Chemical Physics, Russian Academy of Sciences (Chernogolovka) [14]. This work presents the results of comparative study of the SOS- and SoxRS-inducing activity of mononuclear $[Fe(SR)_2(NO)_2]^-$ and binuclear $[Fe_2(SR)_2(NO)_4]^-$ nitrosyl-iron complexes with natural and synthetic ligands. Attention was mainly paid to elucidate the chemical nature of initiators of the genetic signal transduction in the abovementioned regulons.

MATERIALS AND METHODS

Reagents. Cysteine, glutathione, HEPES, a chromogen *o*-nitrophenyl-β-D-galactopyranoside (ONPG), and 4-nitroquinoline-1-oxide (4NQO) were from Sigma (USA), and iron sulfate was from Fluka (Switzerland). Dinitrosyl-iron complex with glutathione ($DNIC_{glu}$) was used as dimer which was prepared by treatment of a mixture of 5.4 mM $FeSO_4$ and 10.8 mM glutathione (at the iron/thiol ratio of 1 : 2) with a gaseous nitric oxide in a Thunberg vessel at the pressure of 200-300 mm Hg in 15 mM Hepes (pH 7.6) previously degassed in vacuum. Crystalline binuclear tetranitrosyl-iron complex with thiosulfate ($TNIC_{thio}$) was prepared by passing gaseous nitric oxide through a mixture of aqueous solutions of $FeSO_4 \cdot 7H_2O$ and $Na_2S_2O_3 \cdot 5H_2O$ [14]. Mononuclear dinitrosyl-iron complex with triazole ($DNIC_{tria}$) was prepared by exchange reaction of $TNIC_{thio}$ with triazole and its subsequent crystallization from aqueous alkaline solution in an atmosphere of nitrogen. On synthesis of binuclear tetranitrosyl-iron complex with aminotriazole ($TNIC_{atria}$, Fig. 1c), triazole was replaced by aminotriazole. The synthesis and features of crystalline nitrosyl-iron complexes are described in detail in [14]. Purity of the compounds synthesized was monitored by IR spectroscopy. Spectra of the specimens were recorded with a Perkin Elmer 1720X Fourier transform spectrophotometer. To study IR spectra, the specimens of the compounds were prepared as tablets with KBr (1 mg compound under study per 300 mg KBr) [14]. In absorption spectra of $TNIC_{thio}$ in polar solvents by UV spectroscopy, two maximums were revealed, at 320 and 355 nm. Crystalline structures of $TNIC_{thio}$, $DNIC_{tria}$, and $TNIC_{atria}$ are presented in Figs. 1a, 1b, and 1c, respectively.

Bacterial strains. Expression of the gene *sfiA* was studied using isogenic *E. coli* strains PQ65 uvr+ (F^-, thr, his, pyrD, galE, galY, rpoB, ΔlacU169, trp::MuC^+, Pho^c, sfiA::Mud(Aplac)C^{ts}) and PQ66 uvrA mutant PQ65 [15, 16]. Expression of the gene *SoxS* was studied using the *E. coli* strain TN530 (F^-, Δ(lacU-argF) U169, rpsL179, $soxRS^+$, λϕ (ΔsoxS′::lacZ). These strains were presented by Prof. M. Hofnung and Dr. P. Quillardet (Pasteur Institute, Paris). In these strains, the structural gene *lacZ* of β-galactosidase is under the control of promotor of the gene *sfiA* (SOS)- or *soxS* (SoxRS)-regulons, and the

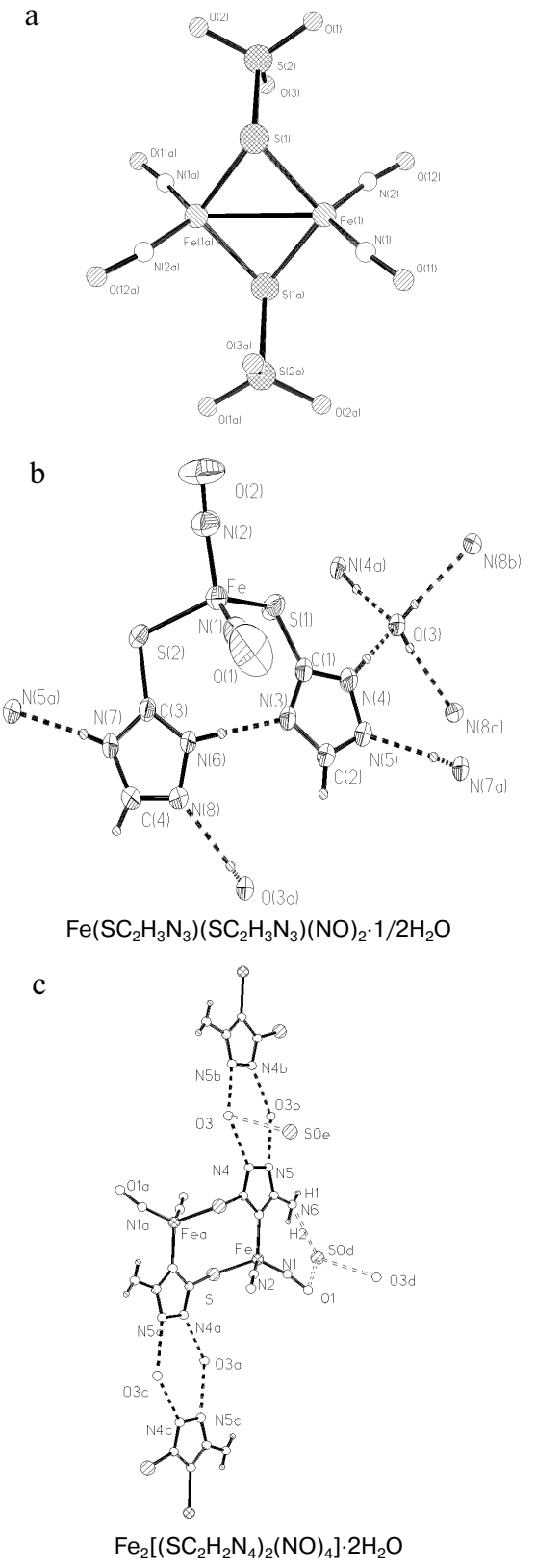

Fig. 1. Structures of stable NO donors: a) dinitrosyl-iron complex with thiosulfate ($DNIC_{thio}$); b) dinitrosyl-iron complex with triazole ($DNIC_{tria}$); c) tetranitrosyl-iron complex with aminotriazole ($TNIC_{atria}$).

genome has a deletion of the chromosomal *lac*-operon. Thus, expression of the genes under study was determined indirectly colorimetrically by activity of β-galactosidase.

Expression of the genes *sfiA* and *soxS* was studied according to the protocol presented in [17, 18]. An overnight culture of *E. coli* was grown for 3 h in L-medium to the titer of 10^8 cells/ml. After treatment with inducers for 60 min, 0.3 ml of the suspension was supplemented with 2.7 ml buffer (see below) to determine the activity of β-galactosidase and 0.6 ml of *o*-nitrophenyl-β-D-galactopyranoside, a chromogen for β-galactosidase. The cells were incubated at 37°C for 1 h. The coloring was stopped by addition of 2 ml of 1 M Na_2CO_3 to pH value of 11.0. The absorption was determined at $\lambda = 420$ nm using a Specord-UV-VIS spectrophotometer (Germany). The activity of β-galactosidase was calculated by the equation $(\beta\text{-Gal}) = D_{420}/t$, where D is the absorption at 420 nm and t is the time of incubation with the chromogen. The relative activity of β-galactosidase was calculated by the equation:

$$(\beta\text{-Gal})_{rel} = [(\beta\text{-Gal})_{tr} - (\beta\text{-Gal})_c]/(\beta\text{-Gal})_c,$$

where $(\beta\text{-Gal})_{tr}$ and $(\beta\text{-Gal})_c$ are activities of β-galactosidase in the cells treated with the agents mentioned and in the untreated control, respectively. For positive control in experiments on the gene expression, 4HQO was used at the concentration of 2.63 nM [5, 7]. The β-galactosidase activity was determined in the following buffer [18]: Na_2HPO_4 (32.2 g), $NaH_2PO_4 \cdot H_2O$ (11.0 g), KCl (1.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), SDS (2.0 g), β-mercaptoethanol (5.4 ml), distilled water (700 ml). At pH 7.7, the volume was adjusted to 1000 ml.

Survival test. After treatment with NO-containing compounds for 60 min, the suspension of bacteria was suspended in L-medium and plated by successive dilutions onto Petri dishes with L-agar. The colonies grown from the surviving cells were counted after incubation of the dishes for 24 h at 37°C.

EPR studies. Bacteria were grown aerobically in 1 liter of L-medium to optical density of 0.4 at 600 nm. To prepare a specimen, 250 ml of the culture was centrifuged at 7000*g* and concentrated to 5 ml after preincubation of the cells for 30 min with NO-containing compounds and with *o*-phenanthroline where indicated. Then the cells were centrifuged again, resuspended in 0.3 ml of L-medium, and rapidly frozen in calibrated ampules for studies by EPR. EPR spectra were recorded in the X-band with a Radiopan spectrometer (Poland) at 77 K, microwave power of 5 mW, modulation amplitude of 0.5 mT.

RESULTS AND DISCUSSION

Stability of DNIC preparations in aqueous solutions. Unlike dimeric $DNIC_{glu}$, crystalline preparations $TNIC_{thio}$, $DNIC_{tria}$, and $TNIC_{atria}$ displayed a low stability on dissolving in water. This was manifested by rapid disappearance (in 20-30 min) of their specific EPR signal at g = 2.03 in aqueous solution at neutral pH. Paramagnetism of these complexes was not recovered by subsequent additions into the solution of thiosulfate, triazole, or aminotriazole, respectively. The dimer $DNIC_{glu}$ in aqueous solution had no noticeable EPR signal because of specific for this form spin-pairing of molecular DNICs which form the dimer [8]. The EPR signal of this dimer sharply increased on addition of a 30-fold excess glutathione and increase in pH of the solution to 10-11. This feature of dimeric $DNIC_{glu}$ was retained on storage of its aqueous solution for 5-6 h. Decomposition of crystalline DNIC and TNIC preparations in aqueous solutions was accompanied by precipitation of iron hydroxide which was released from the complexes during their decomposition and by appearance of nitric oxide in the solution. The latter was detected by its vasodilating effect on isolated rat coronary vessels. This activity was inhibited with an inhibitor of guanylate cyclase, Methylene Blue. Just the activation of this enzyme with nitric oxide is known to initiate vasodilatation.

Cytotoxic effects of DNIC and TNIC preparations. The cytotoxic effect of DNIC and TNIC preparations studied on *E. coli* isogenic strains inversely depended on their stability in aqueous solutions. The more stable $DNIC_{glu}$ was cytotoxic for the cells in the concentration more than 100 μM, whereas initially crystalline DNIC and TNIC preparations noticeably decreased the cell survival even in the concentration of 10 μM (Fig. 2). Significant toxic effects of these DNICs and TNICs under aerobic conditions seemed to be due to generation

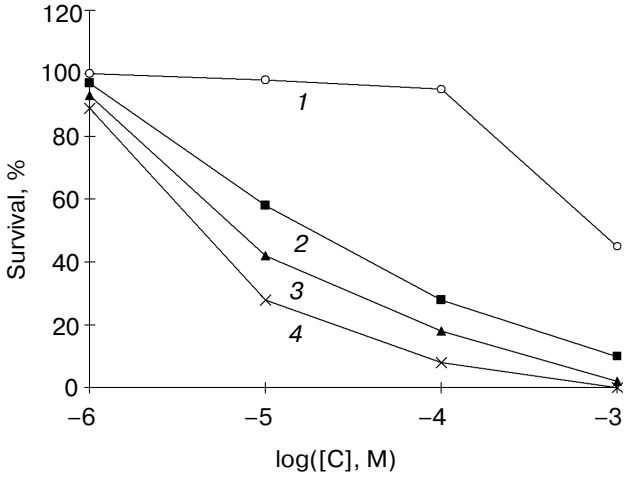

Fig. 2. Comparative cytotoxicity of NO donors. Survival of *E. coli* PQ66 in the presence of $DNIC_{glu}$ (*1*), $TNIC_{thio}$ (*2*), $DNIC_{tria}$ (*3*), and $TNIC_{atria}$ (*4*) was determined as described in "Materials and Methods". Results are presented as the means of three determinations.

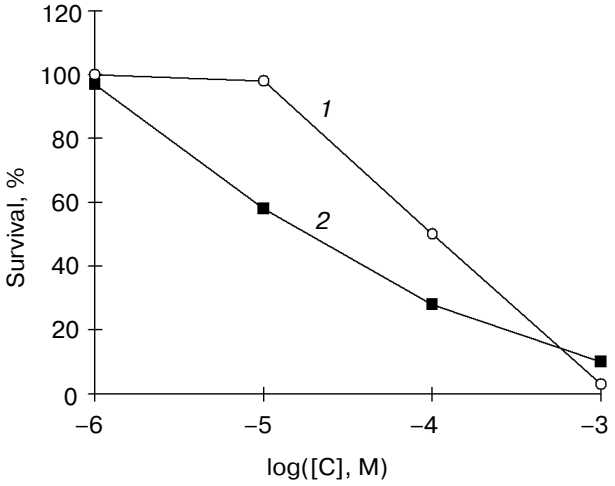

Fig. 3. Comparative cytotoxicity of $TNIC_{thio}$. The survival of *E. coli* PQ65 uvr+ (*1*) and PQ66 uvrA (*2*) was determined as described in "Materials and Methods". Results are presented as the means of three determinations.

of superoxide O_2^- ions during the reaction with oxygen molecules of bivalent iron released from the complexes. The subsequent interaction of superoxide with NO molecules resulted in appearance of toxic peroxynitrite in the solution [19, 20].

It should be noted that the mutant *E. coli* strain PQ66 uvrA was more sensitive to the cytotoxic effect of $TNIC_{thio}$ than the isogenic strain PQ65 uvr+ (Fig. 3), and this suggested a contribution of the UvrABC-excision DNA repair induced by products of $TNIC_{thio}$ decomposition to elimination of prelethal damages. The UvrABC complex was found [21] *in vitro* to eliminate oxidative damage of DNA, in particular, thymine glycols and AP-sites produced in DNA by highly specific DNA glycosylases. In principle, nitric oxide released from $TNIC_{thio}$ can suppress the activity of *E. coli* UvrA protein by destruction of [ZnS] clusters among its factor of transcription, the so-called "zinc fingers" [22].

Induction of expression of the genes *sfiA* and *soxS* in *E. coli* cells by DNICs and TNICs. Depending on concentration, all DNICs and TNICs studied in the present work induced the regulons SOS and SoxRS in *E. coli* (Figs. 4-6). Activation of the regulon SOS was recorded by expression of the gene *sfiA* in *E. coli* PQ66 uvrA (Fig. 4) and *E. coli* PG65 uvr+ cells (Fig. 5). Activation of the regulon SoxRS was followed by expression of the gene *soxS* (Fig. 6).

The expression level of the gene *sfiA* was two-four-fold higher in the mutant strain PQ66 uvrA than in the isogenic strain PQ65 uvr+, independently of the structure of nitrosyl-iron complexes studied. At low concentrations, all compounds studied induced in PQ66 uvrA cells nearly the same expression of the gene *sfiA* (Fig. 4), whereas in the PQ65 uvr+ cells the expression was initiated only by polycrystalline preparations of the complexes. However, on increase in the concentration of inducers to 80 μM and more, the activity of these preparations dramatically decreased, obviously because of their cytotoxicity. The more stable in solution and less cytotoxic $DNIC_{glu}$ activated expression of the gene *sfiA* at higher concentrations. Thus, at the concentration of 0.5 mM, this expression was two and more times higher than maximal levels of expression characteristic for polycrystalline complexes in the strain PQ66 uvrA (Fig. 4), whereas in the strain PQ65 uvr+ it was sharply increased, virtually from zero values to the level recorded for polycrystalline TNICs and DNICs (Fig. 5). A similar pattern was observed for activation of expression of the gene *soxS* (Fig. 6).

Consistent with earlier data on the inhibitory effect of a chelator of bivalent iron, *o*-phenanthroline, on expression of the genes *sfiA* and *soxS* induced by $DNIC_{glu}$ or $DNIC_{cys}$ [11, 13], this chelator dramatically inhibited (to the basal level) expression of these genes induced by polycrystalline DNICs and TNICs. This effect is exemplified in Fig. 7 by data on expression of the gene *soxS* induced by $TNIC_{thio}$ in *E. coli* TN530 cells in the absence of 0.5 mM phenanthroline or on its addition. Control experiments showed that this concentration of *o*-phenanthroline had virtually no toxic effect on the *E. coli* strains studied.

It should be noted that incubation of bacteria with all DNICs and TNICs studied resulted in appearance of paramagnetic DNICs which in the frozen state were characterized by an EPR signal with $g_\perp = 2.04$ and $g_\parallel =$

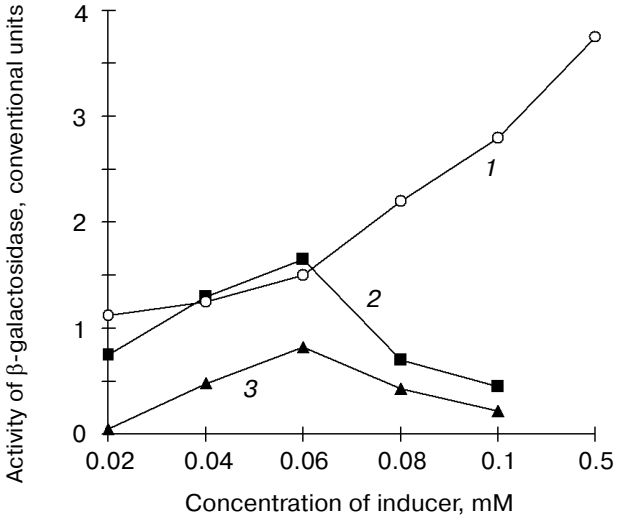

Fig. 4. Induction of expression of the gene *sfiA* in *E. coli* PQ66 uvrA cells by NO donors. Dependence of the β-galactosidase activity on concentration of $DNIC_{glu}$ (*1*), $TNIC_{thio}$ (*2*), and $DNIC_{tria}$ (*3*). The activity of β-galactosidase was determined as described in "Materials and Methods". Results are presented as the means of three determinations.

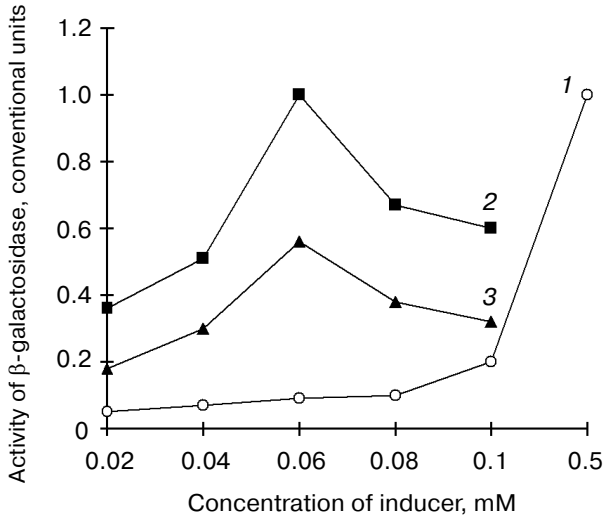

Fig. 5. Induction of expression of the gene *sfiA* in *E. coli* PQ65 uvr+ cells by NO donors. Dependence of the β-galactosidase activity on concentration of $DNIC_{glu}$ (*1*), $TNIC_{thio}$ (*2*), and $DNIC_{tria}$ (*3*). The activity of β-galactosidase was determined as described in "Materials and Methods". Results are presented as the means of three determinations.

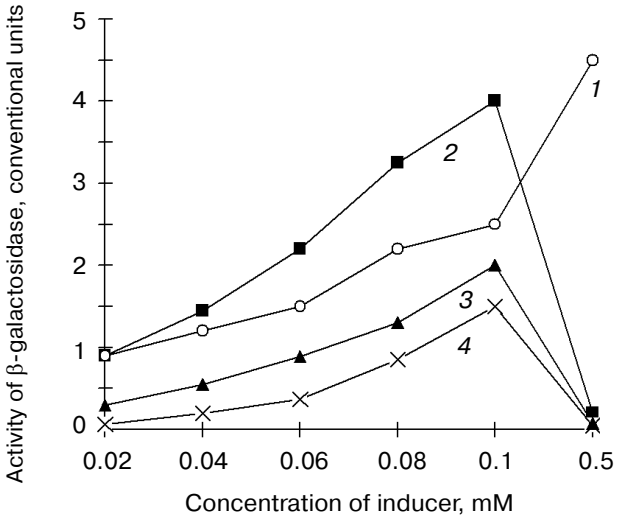

Fig. 6. Induction of expression of the gene *soxS* in *E. coli* TN530 cells by NO donors. Dependence of the β-galactosidase activity on concentration of $DNIC_{glu}$ (*1*), $TNIC_{thio}$ (*2*), $DNIC_{tria}$ (*3*), and $TNIC_{atria}$ (*4*). The activity of β-galactosidase was determined as described in "Materials and Methods". Results are presented as the means of three determinations.

0.14 [8] (Fig. 8). Double washing of the cell suspension had no effect on content of these complexes in the cells and did not change their maximums in EPR spectra. On increase in the temperature of recording to room temperature, the shape of the signal also did not change. This finding suggests a protein nature of these complexes [23]. Obviously, their formation was due to transfer of $Fe^{+}(NO^{+})_2$ groups from low-molecular-weight DNICs and TNICs onto thiol groups of proteins. The addition of *o*-phenanthroline to the cell suspension resulted in disappearance of the EPR signal and red staining of the suspension. This staining was caused by *o*-phenanthroline

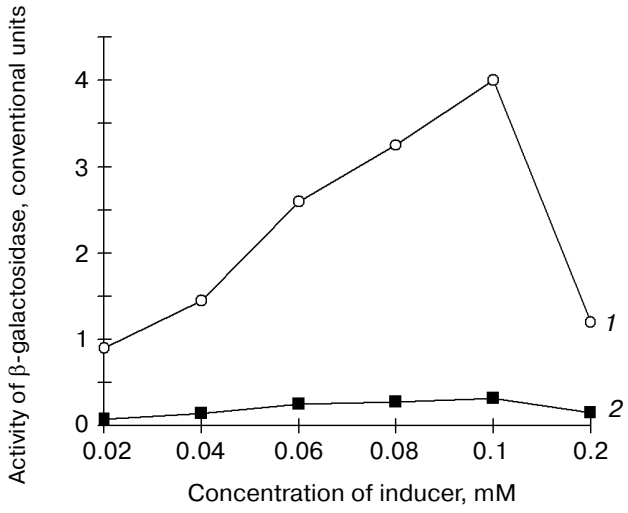

Fig. 7. Inhibition with 0.5 mM *o*-phenanthroline (*2*) of expression of the gene *soxS* in *E. coli* TN530 cells in the presence of $TNIC_{thio}$ and without pretreatment with *o*-phenanthroline (*1*). The activity of β-galactosidase was determined as described in "Materials and Methods". Results are presented as the means of three determinations.

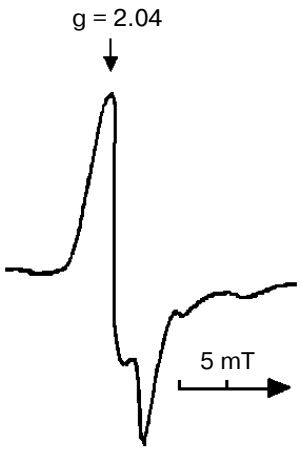

Fig. 8. EPR spectrum of suspension of *E. coli* TN530 cells washed twice after incubation with 150 μM $TNIC_{thio}$. The spectra were recorded at 77 K, microwave power of 5 mW, modulation amplitude of 0.5 mT, and amplification of $0.5 \cdot 10^4$.

complexing with bivalent iron released during decomposition of DNICs.

Thus, results of the present work allow us to conclude that just nitrosyl-iron complexes (DNICs and TNICs) as they are and not their components nitric oxide and bivalent iron, which are in solution in chemical equilibrium with the complexes, are responsible for activation of the regulons SOS and SoxRS in *E. coli*. The low stability of polycrystalline DNIC and TNIC preparations in aqueous solution compared to $DNIC_{glu}$ suggests that this equilibrium for the first type complexes should be shifted to their components. Therefore, if free NO molecules and bivalent iron ions themselves could induce expression of the genes *sfiA* and *soxS*, polycrystalline TNIC and DNIC preparations in aqueous solution would be more effective inducers than $DNIC_{glu}$, but this has not been observed experimentally. On the contrary, accumulation of these free components in solution with increase in the concentration of the complexes had no inducing effect but displayed a toxic effect on the cells by the above-described mechanism.

This has been also confirmed by our comparative data on expression of the gene *soxS* by aqueous solution of $TNIC_{thio}$ or by a mixture of $TNIC_{thio}$ with excess ligand sodium thiosulfate (1 : 5). The excess ligand did not affect expression of the gene *soxS*, and the relative activity of β-galactosidase was 0.8 and 1.0, respectively. And no changes were recorded in pH values of the reaction mixture in the presence of excess sodium thiosulfate.

Incubation of cells with $DNIC_{glu}$ solution, which was more stable and, consequently, less toxic gave another picture: increase in its concentration in the cell suspension was associated with increase in expression of both genes *sfiA* and *soxS* (Figs. 4-6). This indicated that the expression was initiated by the complex itself. The decomposition of this complex and also of its polycrystalline analogs under the influence of *o*-phenanthroline caused a dramatic decrease in the activation of expression of the genes, whereas nitric oxide that was released displayed no such activity (Fig. 7).

In spite of intensive efforts of researchers during 30 years, molecular mechanisms triggering the polyfunctional regulon SOS in *E. coli* are not yet established in detail [24]. However, most researchers think the activating effect of NO-containing compounds on the regulon SoxRS to be associated with destruction of the iron-sulfur site in the transcriptional protein sensor SoxR [2Fe-2S] and appearance of DNIC in it [2]. It was suggested that NO molecules as they are could replace in iron-sulfur clusters of this protein the bridge atoms of inorganic sulfur, and this should directly result in production of DNICs bound to apoprotein by thiol groups [2]. However, experiments with adrenodoxin, which similarly to the SoxR protein of *E. coli* is characterized by the presence of two iron-sulfur sites, did not support this hypothesis [23]. Nitric oxide destroyed iron-sulfur sites in this protein with production of DNICs bound to the protein only in the presence of exogenous bivalent iron. But exogenous iron was included initially into these complexes, whereas endogenous iron released from iron-sulfur sites was included later. Similar data were obtained on addition of low-molecular-weight DNICs into adrenodoxin solution, with a subsequent treatment of the solution with nitric oxide. Thus, just the low-molecular-weight DNICs, exogenous or produced during the reaction of exogenous iron with NO, initiated by an autocatalytic mechanism the decomposition of iron-sulfur sites. In the first stage, low-molecular-weight DNICs bound to thiol groups inside iron-sulfur sites of the protein. This resulted in the release of endogenous iron which produced DNICs with nitric oxide, and these DNICs attacked other iron-sulfur sites, etc., and this provided a self-accelerated decomposition of iron-sulfur sites with production of protein DNICs. It is reasonably to suggest that just such a mechanism should be responsible for the effect of DNICs on the sensor protein SoxR of the regulon SoxRS in *E. coli*. Our findings allow us to suggest that during activation of the protective regulons studied, NO-containing complexes act not as donors of NO but as donors of $Fe^{+}(NO^{+})_2$ groups which destroy iron-sulfur sites of protein sensors and transcriptional proteins and, thus, initiate expression of the genes.

The ability of various RS-NO to activate many genes of mammals has been shown in recent publications [7]. And these compounds are supposed to induce expression by direct S-nitrosylation of inducers of transcription. It is not improbable that this process can also be mediated by DNICs, because RS-NO have been recently shown to easily change to DNICs in the presence of free iron and thiols.

This work was supported by the Russian Foundation for Basic Research (project Nos. 02-03-33344 and 02-04-48456).

REFERENCES

1. Ignarro, L. J. (2000) *Nitric Oxide: Biology and Pharmacology*, 1st Ed., Academic Press, San Diego.
2. Ding, H., and Demple, B. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 5146-5150.
3. Bogdan, C. (2001) *Trends Cell Biol.*, **11**, 66-75.
4. Turpaev, K., Litvinov, D., Dubovaya, V., Panasyuk, A., Ivanov, D., and Prassolov, V. (2001) *Biochimie*, **83**, 1-8.
5. Hutchings, M. I., Crack, J. C., Shearer, N., Thompson, B. J., Thompson, A. J., and Spiro, S. (2002) *J. Bacteriol.*, **184**, 503-508.
6. D'Autreaux, B., Tonats, D., Bersch, D., Latour, J.-M., and Michaud-Soret, I. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 16619-16624.
7. Hemish, J., Nakaya, N., Mittal, V., and Enikolopov, G. (2003) *J. Biol. Chem.*, **278**, 42321-42329.

8. Vanin, A. F. (1998) *Biochemistry (Moscow)*, **63**, 782-793.
9. Gaston, B. (1999) *Biochim. Biophys. Acta*, **1411**, 323-333.
10. Muller, B., Kleschyov, A., Alencar, J. L., Vanin, A., and Stoclet, J.-C. (2002) *Ann. N. Y. Acad. Sci.*, **962**, 131-139.
11. Lobysheva, I. I., Stupakova, M., Mikoyan, V. D., Vasilieva, S. V., and Vanin, A. F. (1999) *FEBS Lett.*, **454**, 177-180.
12. Stupakova, M. V., Lobysheva, I. I., Mikoyan, V. D., Vanin, A. F., and Vasilieva, S. V. (2000) *Biochemistry (Moscow)*, **65**, 690-695.
13. Vasilieva, S. V., Stupakova, M. V., Lobysheva, I. I., Mikoyan, V. D., and Vanin, A. F. (2001) *Biochemistry (Moscow)*, **66**, 984-988.
14. Sanina, N. A., Rakova, O. A., Aldoshin, S. M., Atovmyan, E. G., and Ovanesyan, N. S. (2002) *Koord. Khim.*, **27b**, 198-202.
15. Gaudi, P., Moon, N., and Weiss, B. (1997) *J. Biol. Chem.*, **272**, 5082-5086.
16. Goerlich, O., Quillardet, P., and Hofnung, M. (1989) *J. Bacteriol.*, **171**, 6141-6147.
17. Quillardet, P., and Hofnung, M. (1988) *Mutat. Res.*, **205**, 107-118.
18. Quillardet, P., Huisman, O. D., Ari, R., and Hofnung, M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 5971-5975.
19. Vanin, A. F., Stukan, R. A., and Manukhina, E. B. (1996) *Biochim. Biophys. Acta*, **1295**, 5-12.
20. Denningen, J. W., and Marletta, M. A. (1999) *Biochim. Biophys. Acta*, **1411**, 334-350.
21. Lin, J. J., and Sancar, A. (1989) *Biochemistry*, **28**, 7884-7909.
22. Tarchevskii, I. A. (2002) *Signal Systems of Plant Cells* [in Russian], Nauka, Moscow, pp. 46-47.
23. Voevodskaya, N. V., Serezhenkov, V. A., Kubrina, L. N., and Vanin, A. F. (2002) *Biochem. J.*, **368**, 633-639.
24. Sutton, M. D., Guzzo, A., Narumi, I., Costanzo, M., Altenbach, A., Ferentz, A., Hubbell, W., and Walker, G. (2002) *DNA Repair*, **1**, 77-93.