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REVIEW

Bacterial NO Synthases

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Abstract—Unlike mammalian NO synthases, bacterial NO synthases do not contain a reductase domain. The only exception from this rule is the NO synthase from myxobacterium *Sorangium cellulosum*, but its reductase domain has unusual structure and location in the enzyme molecule. Recent achievements in bacterial genome sequencing have revealed the gene coding NO synthase (represented as an oxygenase domain) in some bacteria and have advanced the study of structure and functions of bacterial NO synthases. Important features of structure, sources of reducing equivalents, evolutionary connections, and functions of bacterial NO synthases (i.e. participation in nitration of the indole ring of Trp, in reparation of UV-radiation damage, role in adaptation of bacteria to oxidative stress, participation in the synthesis of cGMP, and resistance of bacteria against antibiotics) are described.

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Key words: NO synthase, nitric oxide, oxygenase domain, bacteria

Nitric oxide is produced by NO synthase (EC 1.14.13.39). This enzyme catalyzes the reaction of L-arginine with oxygen that results in formation of L-citrulline and NO (Fig. 1). The process occurs in two stages with *N*-hydroxy-L-arginine as a stable intermediate.

Nitric oxide functions as a cytotoxic agent or as a signal molecule participating in transformation of external signals into a specific response of a cell in higher plants and animals [1-3]. Interest in research on bacterial and lower plant NO synthases has sharply increased recently. The main difficulty in studying them is that in these lower organisms an enzyme completely homologous to the best studied mammalian NO synthase (mNOS) has not been found. For this reason, the term "NO synthase-like (NOS-like) activity" is sometimes used to denote an enzyme carrying out the functions of NO synthase in bacteria and lower plants.

Abbreviations: baNOS, bsNOS, deiNOS, gsNOS, saNOS, scNOS, stNOS, and NOS_{NOC} are NO synthases of *Bacillus anthracis*, *B. subtilis*, *Deinococcus radiodurans*, *Geobacillus stearothermophilus*, *Staphylococcus aureus*, *Sorangium cellulosum*, *Streptomyces turgidiscabies*, and *Nocardia*, accordingly; cPTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidasolin-1-oxyl-3-oxide; H_4B, 6R-tetrahydrobiopterin; mNOS, mammalian NO synthase; NOS_{ox}, oxygenase domain; NOS_{red}, reductase domain; THF, tetrahydrofolate; TrpRS, tryptophan-tRNA-synthase.

Recent progress in studying bacterial NO synthases stems from the fact that the number of cloned and purified NO synthases is growing as bacterial genomes are being decoded. Also, studies on mutants without a *nos* gene are an important source of information concerning the structure and functions of bacterial NO synthases.

STRUCTURE OF BACTERIAL NO SYNTHASES

Basic features of structure of bacterial NO synthases. Many attempts to isolate and characterize NO synthases from bacteria have been made in the last 10 years. By now NO synthases have been identified for ~20 species of bacteria including those of *Bacillus* [4, 5], *Deinococcus* [6, 7], *Geobacillus* [8, 9], *Lactobacillus* [10-12], *Nocardia* [13], *Sorangium* [14], *Staphylococcus* [15], and *Streptomyces* [16] genera. It is natural that the studied properties of these enzymes were compared, first of all, to characteristics of the best investigated mammalian NO synthases. It is known that the mammalian enzyme is a homodimer containing an *N*-terminal catalytic or oxygenase domain (NOS_{ox}), a *C*-terminal reductase domain (NOS_{red}), and a regulatory calmodulin-binding region join NOS_{ox} and NOS_{red} into a united complex. The NOS_{ox} domain binds substrate L-arginine and cofactors—protoporphyrin IX (heme) and 6R-tetrahydrobiopterin (H_4B). Besides participation in catalysis, H_4B stabilizes the dimer structure

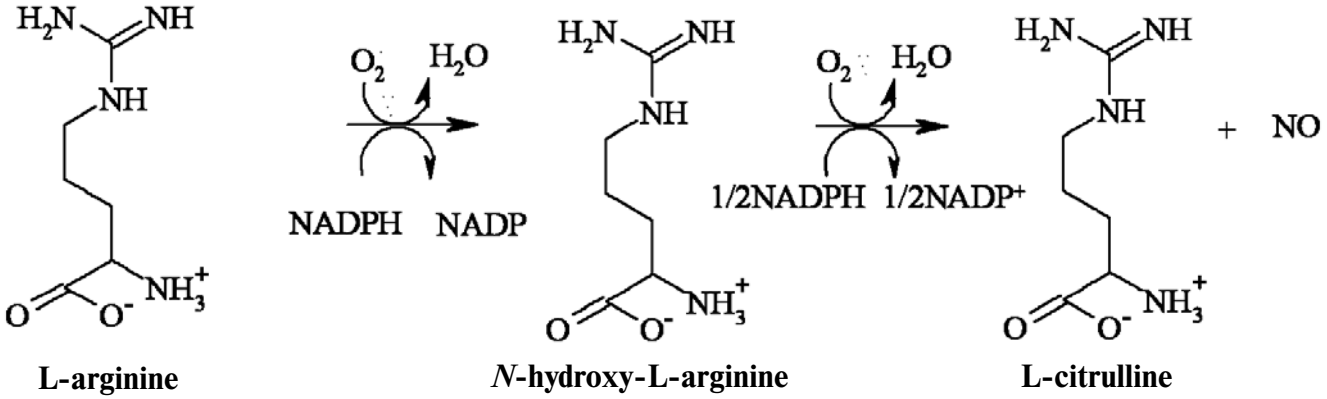

Fig. 1. Reaction catalyzed by NO synthase.

of NO synthase. The NOS_{red} domain has sites for binding of cofactors FMN, FAD, and NADPH. It transfers electrons from NADPH through flavins and H_4B to Fe(III)-heme of the NOS_{ox} domain for activation of O_2. Thus, mNOS is a bimodal multifactorial enzyme.

In contrast to mammalian enzyme, the reductase domain is absent in bacterial NO synthases; therefore, the presence of a suitable reducing agent is necessary for its functioning. Besides, β-loop and zinc-binding region interacting with pterin and stabilizing the $mNOS_{ox}$ dimer were not found in the *N*-terminal domain in the prokaryote enzyme. The central calmodulin-binding domain is also absent in them. Enzyme of such structure is found, basically, in Gram-positive bacteria, but it is also encountered in some Gram-negative bacteria and archaebacteria [8, 17, 18].

Despite the distinctions noted above, the catalytic properties of bacterial NO synthases are similar to those for the $mNOS_{ox}$ domain with the exception of prokaryotic enzymes having slower rate of nitric oxide dissociation from a heme iron atom, which may obstruct freeing and detection of produced NO [4, 8, 17]. Similarly to mammalian enzyme, the catalytically active form of bacterial NO synthase is a homodimer capable of binding substrate L-arginine and producing NO after introduction of cofactor H_4B or tetrahydrofolate (THF) into the incubating medium.

Many species of bacteria do not have the genes responsible for synthesis of the pterin cofactor H_4B, but they have the genes coding synthesis of THF, which can bind to the pterin segment of bacterial NO synthases and can substitute for H_4B as a redox active cofactor [4, 5]. Reece et al. [7] showed that the dissociation constant for the complex of NO synthase from *Deinococcus radiodurans* with L-arginine is 0.9 ± 0.1 mM, but it decreases in the presence of THF to 7.4 ± 0.1 μM. It is assumed that the lack of an *N*-terminal zinc-binding region in bacterial NO synthases facilitates the possibility of THF binding, the molecule of which has greater size than H_4B [6, 17].

Genome sequence analysis of prokaryotes has revealed for many of them the existence of genes homologous to those coding the oxygenase domain of mNOS. Genes for bacterial NOS_{ox} domains for *Bacillus anthracis* (baNOS) [5], *B. subtilis* (bsNOS) [4], *Deinococcus radiodurans* (deiNOS) [5], *Geobacillus stearothermophilus* (gsNOS) [8], and *Staphylococcus aureus* (saNOS) [18] have been successfully expressed in *Escherichia coli*.

Spectroscopic analysis reveals subtle differences in structures of heme centers between NO synthases of *S. aureus*, *B. subtilis*, and *B. anthracis* concerning states of the iron atom and the environment of the heme, which may cause distinctions in their reaction capabilities [19-21]. Oxygenated complexes with the iron atom of the enzymatic heme, formed by catalysis, can also be analyzed using spectroscopy [22-24]. For saNOS [25], bsNOS [4], and deiNOS [5] the specified complexes are characterized by appearance of the Soret band at 430 nm that corresponds to heme-oxy(II) complex of mammalian NO synthase containing ($Fe^{III}O_2^-$) [26]. For more detailed electronic characteristic of oxygenated complexes synthesized during catalysis, Raman spectra of mutants Trp56Tyr and Trp56Phe of saNOS and similar variants of endothelial $mNOS_{ox}$ forms may be useful [23]. As a result of these studies, it was shown that the σ-link of heme iron with cysteine is modulated by a hydrogen bond between Cys and Trp of the enzyme. Behavior of the oxy-ferroheme of gsNOS at both stages of the NO synthase reaction was studied by electron paramagnetic resonance (EPR) and electron–nuclear double resonance (ENDOR) [24]. It was shown that L-arginine is able to change the nature and reactive ability of intermediate compounds during catalysis.

Comparison of structures of bacterial NO synthases and the NOS_{ox} domain of the mammalian enzyme has also confirmed their significant homology [23, 27]. Their clearest resemblance is displayed on comparison of NO synthases bound to substrate and/or various cofactors or their analogs. This is true for gsNOS bound with L-argi-

nine [8], for bsNOS bound to L-arginine and THF [17], and also for saNOS and baNOS complexed with analogs of L-arginine [18, 19].

X-Ray crystallographic analysis of saNOS showed its topological similarity to the oxygenase domain of eukaryotic NO synthase and revealed the absence of the *N*-terminal region and two cysteine residues forming tetrahedrally coordinated Zn-tetrathiolate necessary for mNOS dimer stabilization [18]. Nevertheless, the structure saNOS should, obviously, possess certain compensatory changes to sustain stability of the enzyme dimer. Indeed, study of the interaction surface between subunits of saNOS led to the conclusion that dimerization of this enzyme is due to certain conservative amino acid residues that stabilize the tertiary structure of the monomer. According to the X-ray diffraction data, the ligand-binding segment of saNOS is similar to its analog in mammalian NO synthase but is more open, probably for optimum interaction with redox proteins.

Comparative crystal structure analysis of NO synthases from the thermophilic bacterium *Geobacillus stearothermophilus* (gsNOS) and other bacteria has revealed a high degree of their resemblance [8]. A found distinction is the heme pocket in which Arg365 (gsNOS) substitutes for Lys356 (bsNOS), which causes structural rearrangements in the gsNOS molecule resulting in a more compact state, thus Ile223 is moved forward ~0.6 Å closer to the heme iron atom. It is assumed that dissociation of a ligand from the heme is impeded because of compactization of the gsNOS molecule. Slower kinetics of the enzymatic reaction in gsNOS in comparison with others NO synthases put this enzyme in a favor when studying heme–oxygen intermediates, which are more stable. For example, Fe(III)–NO complex in gsNOS is five times more stable than in bsNOS and 50 times more stable than in the inducible form of mNOS. Besides, gsNOS excels by high thermostability – the temperature of its melting is 20°C higher than for bsNOS. As the melting temperature of the enzyme decreases after removal of 13 amino acid residues from the *N*-end of gsNOS, it is believed that exactly this site defines its thermostability.

Because of the absence of the *N*-terminal hook region and Cys_4-zinc site in NO synthases of prokaryotes, these enzymes can serve as a convenient model for studying of their folding process accompanying association of subunits and also binding of substrates and cofactors. Addition of ligand transforms NO synthase from conformationally destabilized "loose" dimer form into compactly packed "tight" dimer that participates in catalysis [28, 29]. Also, it was determined that the crystallographic structure of loose-dimer enzyme from *B. subtilis* is characterized by the presence of destabilized domains and change in degree of association of subunits [29]. After ligand addition or heme reduction loose-dimer turns into tight-dimer; this has been shown for both soluble and crystal forms of the enzyme. During this conformational metamorphosis of bsNOS dimer the distance between iron atoms in heme considerably decreases (from 37.1 to 34.7 Å). It should be noted that the loose-dimer structure of NO synthase from *B. subtilis* is similar in properties to recombinant mNOS excreted in the absence of pterin. Therefore, it is obvious that availability of pterin can control activity of NO synthase.

When studying conformational transformations of NO synthases, it is necessary to consider that regulation of monomer–dimer equilibrium can be mediated by the product of the NO synthase reaction – nitric oxide [30]. For example, the inducible form of mNOS in the absence of H_4B is a mixture of monomer and loose-dimer forms. It was shown that NO initiates loose-dimer dissociation into monomers for this enzyme and that the disulfide bond between Cys104 and Cys109, localised in the Zn-binding site, is formed during monomerization. Whether nitric oxide is able to control monomerization and dimerization in bacterial NO synthases and how this process may be conducted in the absence of the zinc domain is still unknown.

Sources of reducing equivalents for bacterial NO synthases. The question of the nature of the reducing equivalent supply for bacterial NO synthases is extremely important due to the absence of the flavoprotein redox domain in their structure. Some authors have shown [4] that bsNOS can accept electrons from $mNOS_{red}$, producing nitrite in the presence of NADPH. However, observable activity of bacterial enzyme and speed of electron transfer were much lower than in mammalian NO synthases, which led to the suggestion of significant difference between natural redox partners of bsNOS and $mNOS_{red}$.

In 2007 it has reported that there are two low molecular weight FMN-containing proteins in *B. subtilis* that are suitable candidates for the role of native donors of reduction equivalents for NO synthase; these flavodoxins, YkuP and YkuN, especially YkuN, can effectively participate in catalysis of the enzyme from this organism [31]. It was shown that YkuN also actively reduces NO synthase heme from another bacterial species (*D. radiodurans*), but it is not able to do this with NOS_{ox} of mammals and insects. This study is important because bacterial flavoprotein, which can transport electrons in bsNOS *in vitro*, was identified for the first time, and it can participate in nitric oxide synthesis from L-arginine or *N*-hydroxy-L-arginine in a complex with flavooxyreductase. However it remained unclear whether bacterial NO synthases lacking the reductase domain possess the ability to synthesize NO *in vivo*. Recently, on the basis of a combination of genetic and biochemical approaches, it was determined that the enzymes from *B. subtilis* and *B. anthracis* are actually able to produce nitric oxide in living cells [32]. However, deletion of the *ykuN* gene did not affect the sensitivity of the test measuring NO synthase activity *in vivo*, and therefore it is premature to attribute

YkuN to specific bacterial reductases participating in bsNOS catalysis.

It is known that bacterial genome codes many reductases, but the function of many of them is still unknown. It is possible that various nonspecific reductases can be used for catalysis by NO synthases. For example, it is known that the Gram-negative bacterium *E. coli* does not contain a *nos* gene, but nevertheless it was possible to successfully carry out $mNOS_{ox}$ expression with the assistance of reductases from this microorganism [32]. Successful use of endocellular reductases for the activity of oxygenase domains of NO synthases from the *Bacillus* genus was also shown (species *B. anthracis* and *B. subtilis*) [32].

Domain structure of bacterial NO synthases. As of now, the only studied representative of prokaryotes containing the reductase domain of NO synthase is the myxobacterium *Sorangium cellulosum* [14, 33]. Unlike the mammalian enzyme, the reductase domain of scNOS is located in the *N*-terminal part after a domain with undetermined function (Fig. 2). Besides an unusual location in the NO synthase molecule, it has unique composition — it contains an Fe_2S_2 subdomain not found in other NO synthases, as well as FAD and NAD(P)H. It is also necessary to emphasize the importance of the nature of the pterin component in the NO synthase reaction in *S. cellulosum* — nitric oxide synthesis in scNOS is 2.5 times faster in the presence of H_4B in comparison with THF [14]. According to the authors, the existence of such flexibility concerning pterin cofactor structure may be important for control of NO formation under various conditions. A prominent feature of $scNOS_{ox}$ is absence of an Fe(III)–NO-containing intermediate [14]. This is probably caused by change in amino acid composition of the oxygenase domain in comparison with others NO synthases, which leads to faster release of NO from the heme-binding pocket. Some data for the enzyme from *B. subtilis* also stress the importance of amino acids replacements in NO synthase structure. It was shown that dissociation of NO from Fe(III)–NO complex in bsNOS occurs 20 times slower than for mNOS [34]. Some data indicate that this kinetic feature of NO synthase from *B. subtilis* is caused by the change from Val to Ile in the heme pocket. The structure of the heme pocket during this process changes in such a manner that steric boundaries hindering dissociation and diffusion of nitric oxide from the heme are formed. The Ile residue in this region of NO synthase is also conservative in other bacteria, whereas the Val residue is conservative in mammalian enzyme. Therefore, it is assumed that the steric structure of the regarded region of the enzyme is universal for each of these phyla of organisms.

The enzyme from *Streptomyces turgidiscabies* (stNOS) differs from others bacterial NO synthases as it contains a characteristic for mNOS zinc-binding domain in the *N*-terminus [35].

Evolutionary aspects of bacterial NO synthases. In spite of the fact that NO synthase activity has been found in many bacteria, the enzyme is genetically characterized only for a few. It is significant that high degree of homology manifested in ~45% identity and 50-60% similarity of amino acids is revealed between bacterial and eukaryotic NO synthases [32].

Sudhamsu and Crane [36] suggested a phylogenetic tree for NO synthases of different origin based on their sequence analysis of 32 NO synthases (27 bacterial, four eukaryotic, and one archaebacterial). Four different groups of organisms are presented on the scheme: 1) *Rhodococcus*, *Streptomyces*, and *Deinococcus*; 2) *Sorangium* and *Eukaryotes*; 3) *Natronomonas*, *Staphylococcus*, *Geobacillus*, and *Bacillus*; 4) *Exiguobacterium*. It is assumed that the first group is the most ancient, the second one is derived from the first, and the fourth comes from the second and the third through an unknown intermediate. The authors suggest the possibility of horizontal gene transfer for the genetic information concerning NO synthases. Gusarov et al. [32], having analyzed an even greater number of NO synthases genes, proposed a hypothesis that bacterial enzymes are early precursors of eukaryotic NOS, the latter having obtained a reductase domain at later stages of evolution. There are recent data supporting this hypothe-

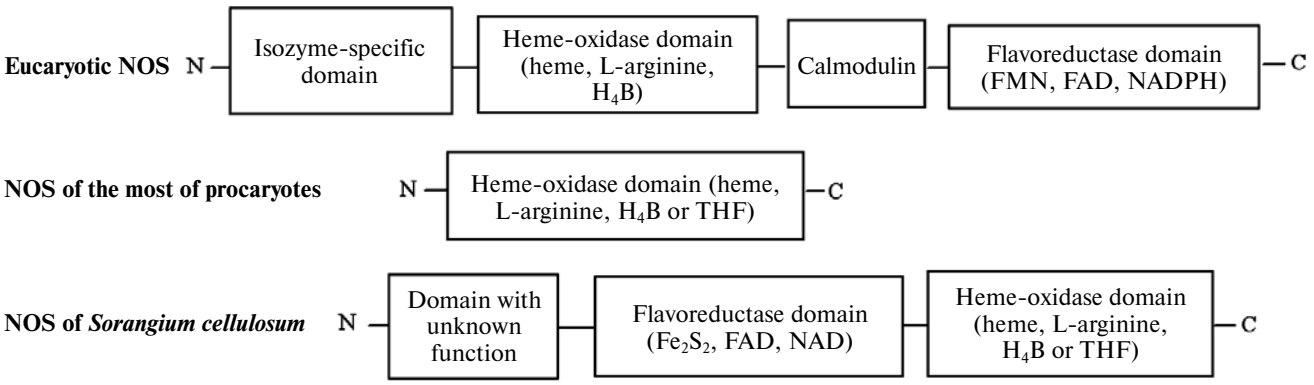

Fig. 2. Domain structure of NO synthases from different sources.

sis that concerns the structure of NO synthase from the Gram-negative bacterium *Sorangium cellulosum* [14] that has, besides an oxygenase domain, an altered reductase domain that differs from $mNOS_{red}$ one by iron-containing component, and also by ability to bind not only H_4B, but also THF. In the course of evolution the fusion of two genes probably occurred, the genes existing independently before — a gene coding the oxygenase domain (bacterial NO synthase) and a gene coding reductase domain (for example, enzymes of the *P-450* cytochrome family) [37]. It is believed that such fusion took place in the time of formation of basic groups of eukaryotic organisms in the Proterozoic Era in response to increase in oxygen concentration in the atmosphere. An adaptive reaction to toxic action of oxygen could be one of the prospective causes of the appearance of the NO synthase reaction.

FUNCTIONS OF BACTERIAL NO SYNTHASES

It is known that the basic target of NO in eukaryotes is guanylate cyclase containing a heme. Among physiological receptors of nitric oxide in prokaryotes also prevail proteins having iron, which can be present as nonheme atoms (or [Fe-S] clusters) or heme [38]. However, whether NO synthases of bacteria can serve as NO suppliers for these targets is currently unknown.

There is little data concerning the functional role of prokaryotic NO synthases, but facts that are known are extremely interesting.

Participation of bacterial NO synthases in tryptophan indole ring nitration. There are recent data concerning functions of NO synthase from streptomycetes, inducers of ordinary scab of potato and root vegetables [16, 35, 39]. Nitric oxide formation *in vivo* by *Streptomyces* cells was shown by electron paramagnetic resonance using dithiocarboxy-sarcosine in a complex with iron as a spin trap for NO and by chemiluminescent detection of NO [16]. It should be noted that the synthesis of NO is most intense in elongation regions of streptomyces hyphae, which are connected to root tips of a host plant. Results of addition of NOS inhibitors and nitric oxide donors [39] and also genetic and isotope data [35] prove that formed nitric oxide is used (at the stage of tryptophan residue nitration) in synthesis of taxtomine — a phytotoxin of cyclic dipeptide nature (Fig. 3). This compound is considered to be one of the main pathogenicity factors of streptomycetes, causing specific adhesion of the phytopathogen to a host plant. Damage to plant cell wall is caused mainly by disturbance of its synthesis because of inhibition of cellulose biosynthesis. The so-called "pathogenicity islet", a DNA segment of *S. turgidiscabies*, *S. acidiscabies*, and *S. scabies*, besides genes of two nonribosomal peptide synthases coding taxtomine synthesis, also contains an NO synthase gene. It is also important that stNOS-dependent production of nitric oxide

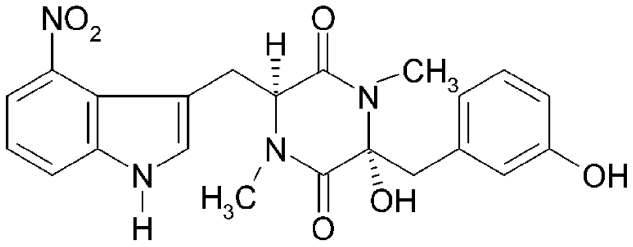

Fig. 3. Structure of taxtomine.

increases in response to induction by cellobiose, which is a component of host plant cell wall, and is also observed in the site of "host–pathogen" contact [16]. However, it is necessary to consider that nitric oxide produced by streptomycetes is capable, in turn, of affecting different metabolic and, ultimately, physiological and also protective processes of the host plant [3, 40].

Discovery of the involvement of prokaryotic NO synthase in "host plant–pathogen" interaction gives impetus for the further research of the role of this enzyme and also of NO as signal molecule in different human pathogens of bacterial origin.

Besides the participation of prokaryotic NO synthase in synthesis of the 4-nitro-tryptophanyl residue of taxtomine already mentioned above, one more connection between tryptophan metabolism and bacterial enzyme functioning has been found [41, 42]. NO synthase (deiNOS), associated with Trp-tRNA-synthase II (TrpRS II), was discovered in cells of the radiation- and drying-resistant bacterium *Deinococcus radiodurans* [41]. The complex is quite strong, with dissociation constant of 6-30 μM. TrpRS II has only 29% identity of genetic sequence in comparison with typical TrpRS, and it is enzymatically five times less active than TrpRS I. Binding with deiNOS in stoichiometric ratio 1 : 1 increases TrpRS II solubility, affinity to substrate (L-arginine), and the activity of this enzyme. In turn, binding of TrpRS II to NO synthase of *D. radiodurans* affects its activity — fluorescence quenching of ATP analog 2,4,6-trinitrophenyl-ATP bound with TrpRS II is observed under these conditions. (TrpRS II is dimer, which cooperatively binds two molecules of ATP when functioning, and fluorescence augmentation is noted after addition of the specified ATP analog to TrpRS II). Thus, it is obvious that each protein of the complex is able to affect the active site properties of the other [41].

It was shown that the deiNOS–TrpRS complex catalyzes selective nitration of tryptophan at the fourth position [42]. Nitration of tryptophan by NO synthase of *D. radiodurans* has saturation kinetics, which shows that the synthesis of 4-nitro-tryptophan contains a stage of tryptophan binding with deiNOS. The ability of H_4B to inhibit tryptophan nitration, but not nitrite formation (the ultimate product of NO degradation) hints that tryptophan

binding occurs at the pterin region of deiNOS and that it does not stem from diffusive interaction between free tryptophan and the nitro-product of the NO synthase reaction. It is possible that the role of deiNOS during tryptophan binding consists in protection of the other (excluding the fourth position) positions of the indole ring from nitration. The physiological role of 4-nitrotryptophan for *D. radiodurans* has not yet been determined. It is not known whether the compound is a moiety of proteins. It might be that it has a signal function or is a precursor of some yet unidentified product of secondary metabolism of this bacterium.

It is interesting that the activities of the two enzymes of the complex change in opposite direction after radiation exposure of *D. radiodurans* cells: for TrpRS II it increases 2.2 times, and for deiNOS it decreases more than 10 times. These facts indicate that under certain conditions, for example, under radiation exposure, the deiNOS–TrpRS complex is apparently not formed.

Role of NO synthases in adaptation of bacteria to oxidative stress. It is known that phagocytes of mammals intensively produce active forms of nitrogen (including NO) and oxygen for their defense against absorbed pathogenic microorganisms [43, 44]. However, the phenomenon of NO-mediated cytoprotection providing their adaptation to oxidative immunologic stress was revealed for such Gram-positive bacteria, as *S. aureus*, *B. anthracis*, and *B. subtilis* [45-47]. The generation of nitric oxide by *B. anthracis* is necessary for virulence and survival in macrophages [46]. It was shown in *in vivo* experiments that survival of these bacteria depends on baNOS activity. For example, spores of *B. anthracis* deficient in NO synthase lost their virulence for cells of mice used as a model for studying anthrax. When discussing the paradoxical effect of nitric oxide production both by pathogenic bacterium cells and macrophages for struggle with each other, it is necessary to consider the time when it is produced. This process is separated in time for the microorganism and the host macroorganism. The main synthesis of NO by *B. anthracis* starts from the very beginning of infection and proceeds for approximately 2 h. In contrast to this, the inducible form of mammalian NO synthase is activated only 8-12 h after the infection [44] when the main formation of reactive oxygen species is already finished.

According to a number of authors [45-47], there are two basic mechanisms of NO-dependent protection of bacterial cells against oxidative stress. The first consists of the inhibition of the Fenton reaction by nitric oxide: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$.

It is apparent from this equation that this reaction is a source of hydroxyl radicals, which, interacting with the residues of nitrogen bases and sugars of DNA, can lead to its damage in bacteria. NO can decrease the Fenton reaction by inhibition of free cysteine reduction, which is used as the donor of reducing equivalents for continuous transformation to Fe^{2+} from Fe^{3+}. The second mechanism consists of nitric oxide activation of catalase—an enzyme that can remove surplus toxic hydrogen peroxide from cells. It is necessary to note that NO-dependent protection of bacteria against oxidative stress is a quick response – resistance of *B. anthracis* cells to H_2O_2 increases ~100 times over only 5 sec after NO treatment [46]. It should be noted that this effect is not observed if nitric oxide and H_2O_2 are added to bacterial cells simultaneously or NO is introduced after H_2O_2.

However, the above-described participation of bacterial NO synthase in protection against oxidative stress is not universal. For example, a *D. radiodurans* mutant with deletion in the *nos* gene does not possess, as it would be possible to expect, greater sensitivity to oxidative damage in comparison with wild type [48]. Besides, H_2O_2 does not induce expression of the deiNOS-coding gene. However, as increased level of nitric oxide inducing growth of bacteria is observed even 8 h after UV-radiation exposure (time when oxygen radicals are already not present), the authors assume that NO not only participates in protection against oxidative stress in *D. radiodurans*. Apparently, the sensitivity of bacterial NO synthase to oxidative stress may vary depending on carbon source during cultivation. For the *apg1* strain of *Rhodococcus* living on fronds of the aqueous fern *Azolla pinnata*, the augmentation of resistance to hydrogen dioxide due to increase in nitric oxide formation [49, 50] is observed in the presence of sucrose (but not other carbohydrates or glycerol).

Participation of bacterial NO synthases in repair of UV radiation-caused damage. Patel et al. [48] applied a genetic approach to establish the role of NO synthase in elimination of damage caused during UV irradiation of the radiation-resistant bacterium *D. radiodurans*. It was shown that the nitric oxide produced by deiNOS participates in this repair. The authors observed resumption of growth of a Δ*nos* mutant, lacking the NO synthase coding gene, by introducing recombinant deiNOS or adding NO using an NO donor or NO gas. It is remarkable that resumption of growth of the Δ*nos*-mutant of *D. radiodurans* was noted 5-10 min after addition of exogenous NO, during the procedure, and during an 8 h period after UV radiation. However, degradation of nitric oxide generated by wild type cells by a selective scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-l-oxyl-3-oxide (cPTIO), caused delay in their growth. After the UV irradiation the deiNOS gene was induced as expressed by mRNA level and nitric oxide increase in the bacterial cells. It has been determined that NO participates in transcription regulation of the *obgE* gene coding GTPase and responsible for cells proliferation. Induction of this gene in the Δ*nos* mutant of the bacterium accelerates normalization of growth.

Considering all of the data, the authors assumed that regulation of *nos* expression and the level of NO partici-

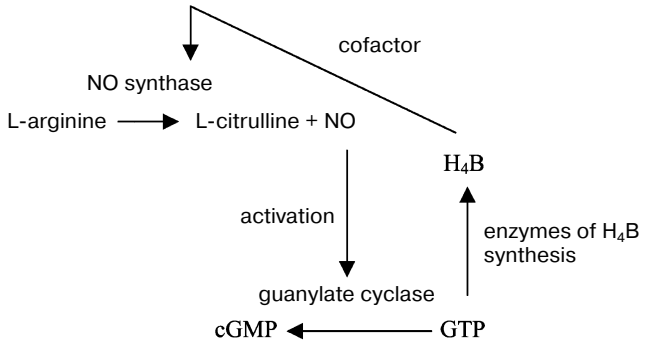

Fig. 4. Effect of GTP on NO synthase of *Nocardia* as precursor of H_4B and cGMP.

pating in reparation and regulation of growth of *D. radiodurans* occurs in response to damaging action of UV radiation. Thus, it is improbable that nitric oxide is involved in direct protection against the UV irradiation or prevents the damage of cells by it, but most likely is involved in processes of reparation and growth resumption.

Bacterial NO synthase and cGMP synthesis. *Nocardia* NRRL 5646 was the first microorganism for which NO synthase (NOS_{NOC}) was isolated and biochemically characterized in 1995 [13]. Structural resemblance of the oxygenase domain of the enzyme from mammals and NOS of bacteria suggests the idea of their functional analogy. Considering that functioning of NO synthase, guanylate cyclase, and enzymes of H_4B synthesis are interdependent in mammals [51, 52], an attempt to establish the existence of similar relationships in microorganisms was undertaken for *Nocardia* [53]. Addition of L-arginine, H_4B, or the NO donor sodium nitroprusside into the cultivation medium of the bacterium led to an increase in cGMP level. In turn, exclusion of H_4B or introduction of the NO synthase reaction inhibitor *N*-nitro-L-arginine caused almost complete inhibition of guanylate cyclase activity. The authors suggest that the nitric oxide synthesized by NOS_{NOC} stimulates guanylate cyclase of the microorganism [53]. On the basis of newly established data, the assumption that GTP and a product of its metabolism, H_4B, affect the activity NO synthase and guanylate cyclase in *Nocardia* (Fig. 4) has been made. Besides, it has been determined that GTP cyclohydrolase, catalyzing the first stage of pteridin synthesis, has an inhibiting effect on NOS_{NOC} [54].

NO synthase and bacterial resistance to antibiotics. It was recently revealed that the nitric oxide produced by bacterial NO synthases increases the resistance of bacteria to different antibiotics [47]. It is believed that due to this many bacteria can exist in associations of microorganisms that are producers of antibiotics. More than 20 antibiotics whose target is DNA, protein synthesis, or metabolism of bacterial cell wall induced sharp growth inhibition of a *nos*-deficient mutant of *B. subtilis*. For some direct modification of the antibiotic structure by oxidation products of nitric oxide and consequent reduction of its toxicity was shown. For example, the interaction of nitrosonium ion, NO^+, with the arylamine part of the acriflavine molecule has been shown. It is known that one mechanism of the effects of antibiotics on bacteria is formation of reactive oxygen species. Therefore, besides the possibility of structural change in antibiotics, it is possible to explain NO-dependent resistance to them by already discussed above mechanisms of protection of bacteria from oxidative stress via inhibition of the Fenton reaction and activation of catalase. Obviously, fine regulation of nitric oxide level in cells is needed as it is vital that the concentration of NO produced by bacterial cells is sufficient for repressing the Fenton reaction, but at the same time does not reach a level influencing the functioning of the respiratory chain and glycolysis.

The results about the influence of NO synthases on bacterial resistance to antibiotics suggest the idea of intensifying the therapeutic efficacy of bactericidal antibiotics by inhibition of these enzymes. However, at present the problem of selective inhibition of bacterial NOS without damage to similar enzymes of the host is still far from solved.

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