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The New NO-Signaling Functions in *E. coli* Cells Cultured Under Anaerobic Conditions

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ABSTRACT

Hypoxia and related genetic instability, NO-accumulation, tumor progression, and resistance to medical treatments are the common characteristics of the solid tumors. Hypoxia-inducible factor (HIF-1α) appears to be the key regulator in mammalian cell response to hypoxic conditions: its regulation activity induced by O_2 and NO. In facultative anaerobic *E. coli* cells, the Fnr[4Fe-4S]$^{2+}$ protein regulates more than 100 genes as a response to hypoxia as well as NO treatment. Similarities between the transcription active dimer forms and functions in *E. coli* protein Fnr and mammalian transcription factor HIF-1α, as well as the chemical principles of NO interaction with the biological targets, led us to use the *E. coli* cells as a genetic model to examine the new pathways of NO-signaling transduction under hypoxic conditions.

INTRODUCTION

PCR-testing of human cells has established the expression of at least 84 genes included in signaling pathways related to hypoxia. They include the dimeric transcription factor HIF-1α which functions as the key regulator of the mammalian cellular response to hypoxia. Its presence was monitored in different kinds of tumors and there is some evidence to suggest that it is HIF-1α which related to the malignization and resistance of cells to the chemotherapeutic agents. In 2006 S. Moncada described NO as one of the stabilization factors of HIF-1α in hypoxic cancer cells.

In facultative anaerobic *E. coli* cells, the universal anaerobic regulator iron-sulfur protein Fnr[4Fe-4S]$^{2-}$ performs the same functions as the dimeric factor HIF-1α. The protein controls the transcription of more than 100 genes encoding the individual components of the transcription chains during the anaerobic respiration and the other alternative functions including DNA-reparation processes. Under anaerobic conditions, the regulator binds on the iron-sulfur cluster and forms the transcription-active dimer [1]. Single-electron cluster oxidation or its nitrosylation by NO leads to the formation of transcription-inert monomer.

In the absence of oxygen, nitrosylation of DNA bases with nitric oxide produces apurinic sites, which form the precursors of single-stranded DNA through the deamination of purines and pyrimidines and their depurination. They are the obligatory components in the "complex" of compounds for the SOS DNA-repair signal in *E. coli* [2, 3]. This allowed us to assume that the similar signals for the SOS-induction can be caused by NO in *E. coli* under anaerobic conditions. Experimental confirmation of this assumption was the goal of this work.

Unlike the mammalian cells, where NO synthesis occurs enzymatically with NO-synthases due to the oxidation of L-arginine, nitric oxide is selectively accumulated in *E. coli* during anaerobic cultivation in a process that depends on a number of cellular structures and metabolites, including the polyfunctional anaerobiosis protein Fnr[4Fe-4S]$^{2+}$. Presence of the iron-sulfur cluster in the structure of Fnr protein as NO-target leads to enhancement of the *aidB* gene expression. While a NO-accumulation becomes the basis of NO-signaling in hypoxic conditions.

MATERIALS AND METHODS

The goal of our work was to obtain the experimental evidence for the new signaling functions of nitric oxide in *E. coli* cells under anaerobic conditions of cultivation.

We studied the levels of the expression of the *aidB* (the *Ada*-regulon) and the *sfiA* (the *SOS*-regulon) genes expression using the mutant *E. coli* cells with the operon fusions [*aidB*::*lacZ*] and [*sfiA*::*lacZ*] in the gene promoters.

Dinitrosyl iron complex with thiol ligand glutathione (DNICglu, 0.001–0.5 mM) [4] as well as 4-nitroquinoline-1-oxide (4NQO, 2.63 nM – the positive control) were studied in the experiments with the *sfiA* and the *aidB* genes expression.

The methodology used made it possible to quantify the expression based on the colorimetric detection of the activity of the β-galactosidase enzyme in the *E. coli* strains PQ37 [*sfiA*::*lacZ*] [5] and MV2176 [*aidB*::*lacZ*] [6].

The results are present in Miller Units as the mean values of 3 experiments and a 95% confidence interval.

RESULTS

In Fig. 1 we show the comparative kinetic curves of *sfiA* gene expression under aerobic and anaerobic conditions. SOS DNA-repair inducible response developed independently of the presence of O_2. The maximum expression values were established in the experiments with 4NQO +O_2; under anaerobic conditions, they were significantly lower.

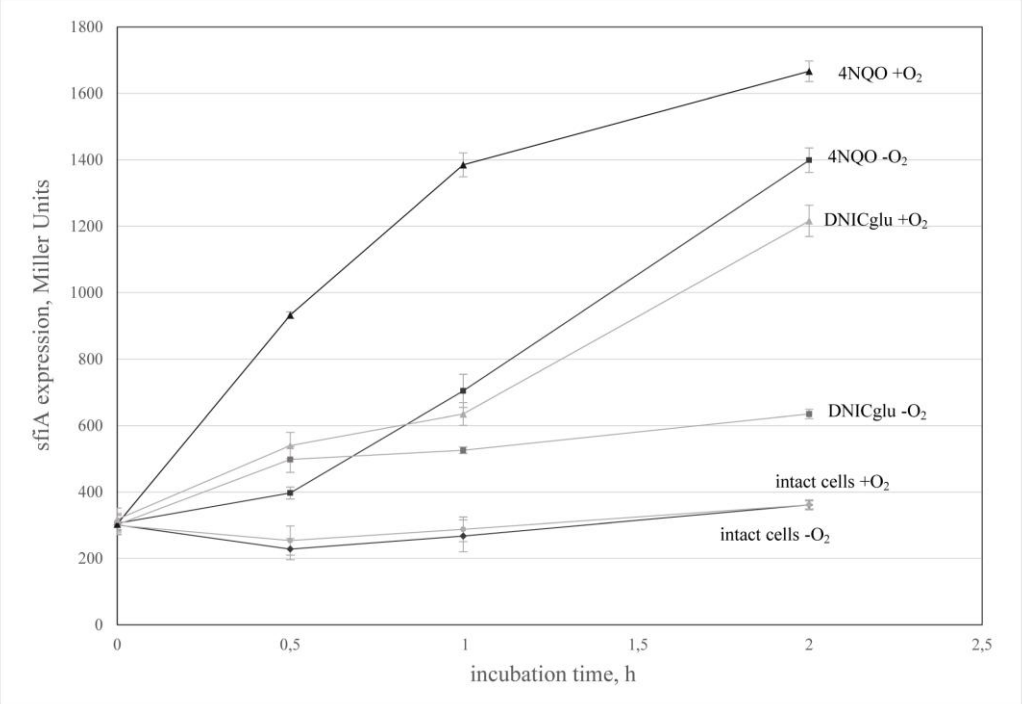

Fig. 1. The kinetics of *sfiA* expression in *E. coli* PQ37 [*sfiA*::*lacZ*], induced by NO-donor DNICglu and 4NQO (positive control): depending on the O_2 presence.

The experimental confirmation of our hypothesis on the new NO-signaling function in *aidB* gene expression in hypoxia was obtained for the first time (Fig. 2). The *aidB* expression increased sharply (4-7 times) in untreated *E. coli* cells during anaerobic cultivation due to activation of a single-regulator of anaerobic metabolism Fnr. Fnr[4Fe-4S]$^{2+}$ is an iron-dependent protein, and an excess of free iron enhanced its activity. On the contrary, the chelator of free Fe o-phenanthroline reduced the levels of *aidB* expression in a linear dependence on the concentration. The NO-donors studied decreased the *aidB* expression in anaerobic incubation (the effect of down-regulation) due to the iron nitrosylation in the Fnr[4Fe-4S]$^{2+}$ protein and subsequent destruction of its dimeric structure.

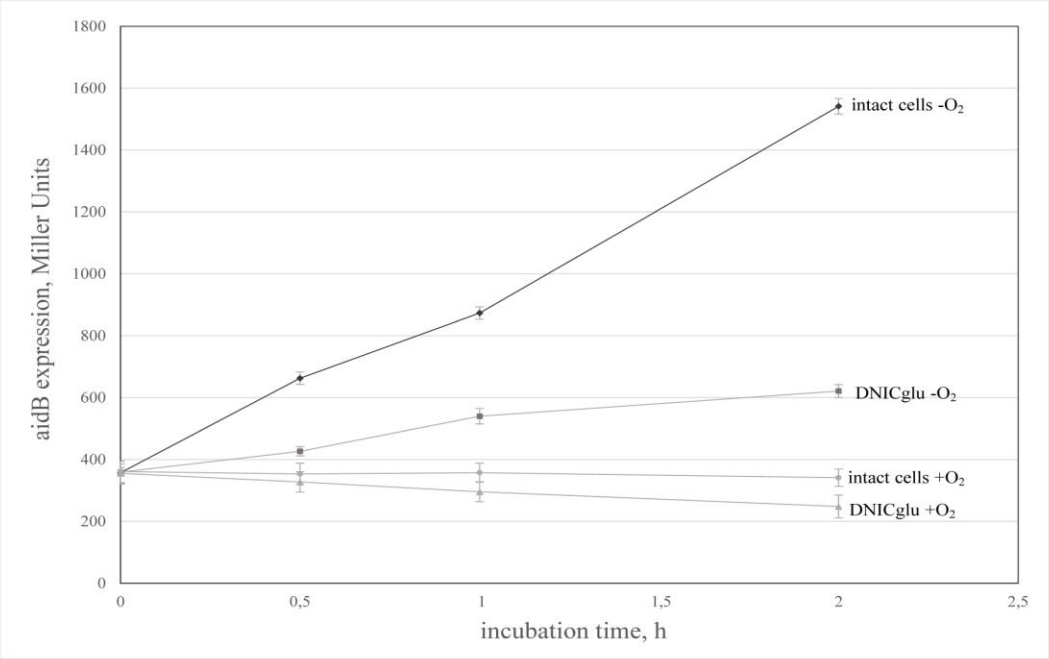

Fig. 2. The kinetics of *aidB* expression in *E. coli* MV2176 [*aidB*::*lacZ*], induced by DNICglu: depending on the O_2 presence.

DISCUSSION

The potentially mutagenic alkylating agents are present in the environment and inside the living organisms, being the metabolic intermediates. One of the main sources of endogenous alkylation is bacterial nitrosation of amino acids with endogenous NO [7].

In the course of evolution, the complex of DNA protecting systems has been formed against the alkylation injuries [2, 6].

In *E. coli* these protective mechanisms are regulated by the constitutive *tag* and *ogt* genes, and the *ada*, *alkA*, *alkB*, and *aid* genes, expression of which is associated with induction of the DNA adaptive response (the Ada-response) [8]. The products of the tag and *alkA* genes are the glycosylases and they both excise N^3-meA from DNA to yield apurinic sites [9].

The *alkB* gene belongs to the same Ada regulation response and its expression is similarly controlled by the *ada* promoter [10]. Nevertheless, the AlkB protein is iron-dependent and utilizes a unique mechanism of oxidative demethylation to direct repair N^1-meA and N^3-meC, eliminating the methyl group in the form of formaldehyde [11].

The multifunctional AidB protein is regulated via the two pathways: one is Ada-dependent, while the other is Ada-independent and function in response to acidification of the medium [12]. The *aidB* gene is an "alarm gene" inside the *ada* operon: it is expressed by a lower level of alkylation as compared with the other genes and it is the only gene providing for repair of interstrand crosslinks induced in DNA by chlorine-substituted nitrosoalkylurea (ACNU, the potent anticancer drug) [13].

In contrasts to the other Ada genes, the *aidB* gene expression is induced by acidification of the medium up to pH 6.0-6.5 or by oxygen limitation and does not restrict by the Ada regulation. Oxygen deficiency in growth medium, which causes the facultative anaerobic bacterium *E. coli* to the stationary growth phase, leads to activation of a specific form of genome protection, associated with *aidB*. In this growth phase, the *aidB* expression is regulated by meAda together with the RpoS protein [8].

In the absence of aeration and exogenous alkylation, the *aidB* gene expression in *E. coli* cells is enhanced; however, the mechanisms underlying this process remain obscure.

The *aidB* expression was suppressed in *E. coli* cells cultured under anaerobic conditions with a NO-donor. This process is accompanied by the appearance in the cells of protein-bound mononuclear dinitrosyl-iron complexes with thiol ligands which characterized by an EPR signal with g_{aver} = 2.03 ($g_{\perp}$ = 2.04, $g_{||}$ = 2.014). We supposed that the same complexes would be also formed in the reaction of NO with the Fe–S cluster of the Fnr[4Fe–4S]$^{2+}$ the anaerobic protein, a transcription factor of *aidB* gene and led to the destruction of this cluster and, suppression of the *aidB* gene expression.

This is accompanied by the "narrow" EPR-signal with g_{aver} = 2.03, $g_{\perp}$ = 2.032 and $g_{||}$ =2.02, which are the characteristics of the DNICs with persulfide ligands [14]. This phenomenon is associated with the accumulation of sulfide dianions due to activation of the special enzyme cysteine desulfurase. This protein produces anions of inorganic sulfur from cysteine and is usually involved in the reconstruction of the Fe–S cluster of the iron-sulfur proteins. Thus, NO ensures destruction and then reconstruction of the [4Fe–4S]$^{2+}$ clusters and involves in the regulation of the *aidB* gene expression in *E. coli* cells cultured under anaerobic conditions.

CONCLUSION

What is becoming clear is that NO is an important molecule, which may downregulate the *aidB* gene expression, as well as the SOS DNA-repair signal transduction in *E. coli* cells cultured under anaerobic conditions.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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