Development of a Biosensor Test System with GFP Reporter Protein for Detection of DNA Damages

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A sensitive biosensor test system was developed for evaluation of premutation effects of propellants on the cell genome.

Key Words: *propellant components; mutagens; reparation; biosensor test system*

Environmental pollution with space industry waste is a key problem of ecological physiology. For example, Proton rocket launch can be accompanied by settling-out of hundreds liters of fuel. Negative effects of soil, water, and air pollution with propellant components (PC) in regions where primary rocket stages are separated are a risk factor for human health. The major ecotoxicant components of rocket propellant are 1,1-dimethylhydrazine and its degradation products, which exert mutagenic and carcinogenic effects [1,3,6,8]. By penetrating the "first defense barrier" (homeostatic detoxication mechanisms), chemical PC damage some organs, inhibit basic biochemical cell reactions, and change various parameters of lipid metabolism and blood system [1,3]. However, little is known on the mutagenic effects of hydrazines, which can be the key elements of PC action on human and animal cells [2,4-7].

Our aim was to develop a biosensor test system based on fluorescent GFP protein for evaluation of the effects of PC and other mutagens on the genome in living cell.

MATERIALS AND METHODS

Experiments were carried out on *E. coli* cells (XL1 Blue strain, recA$^-$) transformed by *pGreenTyr* plasmid or *E. coli* cells (BL-21DE3, recA$^+$) transformed by *pRebGFP-DHFR-6His* plasmid developed in our laboratory. The structure region of fluorescent GFP gene was cloned into these plasmids under the *taq*- and *recA*-promoters, respectively. Transformed cells were cultured for 4-5 h in LB medium up to optical density of 0.6-0.7 at λ=600 nm. After attaining this density, the following mutagens were added to LB medium: 4-nitroquinoline oxide (70 ng/ml), bromodeoxyuracil (10 μg/ml), actinomycin D (10 μg/ml), mitomycin C (0.25 μg/ml), and nalidixic acid (50 μg/ml). In addition, the following mutagenic PC were used: 1,1-dymethylhydrazine (10 μg/ml), tetramethyltetrazine (100 μg/ml) and nitrosodimethylamine (300 μg/ml). Synthesis of GFP in *pGreenTyr* plasmid was induced with isopropyl-β-D-thiogalactoside (IPTG). The cells were cultured for 3-4 h, thereafter GFP synthesis was assayed at λ_{exc}=480 nm and λ_{emis}=520 nm. The values of fluorescent intensity was normalized to the optical density of the cell suspension (1.0 at λ=600 nm).

The data were processed statistically.

RESULTS

In bacteria, inducible reparation of DNA abnormalities is performed by a number of systems including *recA*-depended SOS-reparation. During SOS-induction, recA protein inactivates repressor protein LexA, which leads to derepression of *uvrA*, *recA*, and *sulA* genes and reparation of DNA damages. Activation of the reparation system is a mani-

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festation of the mutagenic action of chemical and physical factors on DNA. The prokaryotic *E. coli* cells transformed with *pGreenTyr* or *pRebGFP-DHFR-6His* plasmids were used as the basis of highly sensitive biosensor system for detection of the effects of mutagens on DNA. In *pGreenTyr* plasmid, the structure region of GFP-coding gene (first isolated from *Aequorea Victoria* mollusk) was cloned into *lac*-operon under *taq* promoter. Similarly, in *pRebGFP-DHFR-6His* plasmid the GFP gene was cloned under *recA* of *Proteus Mirabilis* promoter. Induction of GFP synthesis in *pRebGFP-DHFR-6His* plasmid resulted from the action of many chemical and physical damaging factors on DNA, because *gfp* gene was cloned into this plasmid under recA promoter incorporated into the SOS-system of cell reparation. Damage to DNA can induce *recA* promoter. In *pRebGFP-DHFR-6His* plasmid, GFP synthesis was also triggered by the action of various chemical and physical damaging factors on DNA, since *gfp* gene was cloned into this plasmid under *recA* promoter (up-regulation). In *pGreenTyr* plasmid, the *taq*-promoter can be induced by IPTG, which promotes synthesis of GFP *de novo*. By contrast to *pRebGFP-DHFR-6His* plasmid, GFP synthesis will be down-regulated when DNA of this plasmid is damaged, because this gene is not controlled by the cell reparation system (down-regulation). We present the data to assess the effect of the mutagens on inducible and non-inducible synthesis of GFP in *pGreenTyr* plasmid (Fig. 1). The mutagen doses were chosen with consideration of optimal concentrations of the compounds that exert no toxic effect on the bacterial cells [3]. Evidently, significant constitutive synthesis of GFP proceeds in the *E. coli* clones transformed with *pGreenTyr* plasmid, which is characteristic of many genes induced by IPTG ("promoter flow").

Constitutive synthesis of GFP is significantly down-regulated by the mutagens (including PC), which indicates either damage to plasmid DNA caused by these mutagens or their effect on reparation (or replication) processes in the cell. The combined effect of these actions cannot be excluded. Probably, changes in the synthesis of fluorescent protein do not depend on the mode of mutagen action. At the same time, the ability to up-regulate synthesis of the fluorescent protein in response to inductor after the application of damaging agents is mostly preserved. For example, IPTG inductor up-regulated GFP synthesis by 1.5-2.5 times despite methylation or other damage to DNA. Probably, the reparative and protein synthesis induction mechanisms are mutually independent to a large degree, so the system of induced protein synthesis

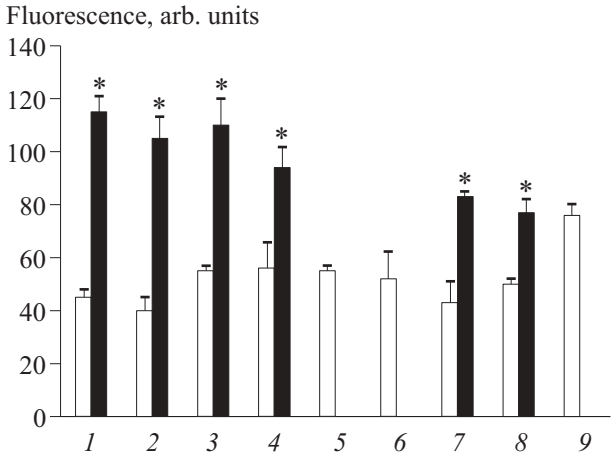

Fig. 1. Effect of mutagens on GFP synthesis in *pGreenTyr* plasmid. *1*) bromo-deoxy-uridine (10 μg/ml); *2*) actinomycin D (10 μg/ml); *3*) tetramethyltetrazine (100 μg/ml); *4*) 1,1-dimethylhydrazine (10 μg/ml); *5*) nalidixic acid (50 μg/ml); *6*) mitomycin C (0.25 μg/ml); *7*) nitrosodimethylamine (300 μg/ml); *8*) 4-nitroquinoline oxide (70 ng/ml); *9*) cultured *E. coli* XL 1 Blue cells without inductors or mutagens. The data are normalized to 1.5×10^8 cell/ml. Open and solid bars represent not induced (control) and induced GFP synthesis, respectively. Here and in Fig. 2: *$p<0.01$ compared to the control.

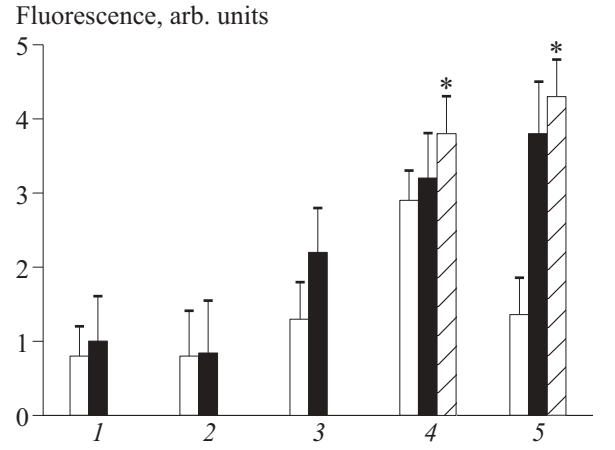

Fig. 2. Effect of mutagens on GFP synthesis in *pRebGFP-DHFR-6His* plasmid. *1*) nitrosodimethylamine (300 μg/ml); *2*) tetramethyltetrazine (100 μg/ml); *3*) 1,1-dimethylhydrazine (10 μg/ml); *4*) mitomycin C (0.12 and 0.25 μg/ml); *5*) nalidixic acid (30 and 50 μg/ml). The open bars represent the not induced (control) synthesis of GFP. The solid and hatched bars show GFP synthesis under the action of mutagens in increasing concentrations (averaged in 5 experiments).

have a certain "safety reserve" even when DNA is damaged. We also examined the effects of some mutagens and PC on induction of protein synthesis in *pRebGFP-DHFR-6His* plasmid (Fig. 2).

Nalidixic acid, 1,1-dimethylhydrazine, and mitomycin C up-regulate GFP synthesis, which indicates a damage to DNA structure or to its replication mechanisms. Nitrosodimethylamine and tetramethyltetrazine produced no significant effect on GFP

synthesis. There are data on toxicity of 1,1-dimethylhydrazine to bacteria [3-5]. However, the concentration of this agent, which down-regulates bacterial metabolism by 50%, is 20 mg/liter. In our experiments, the concentration of 1,1-dimethylhydrazine did not exceed 10 mg/liter, which is a half of the value needed to inhibit the growth of *E. coli* cells by 50%. In the presence of bacterial cells, more than 90% hydrazines are metabolized at a concentration of 10 μg/ml, although these agents do not degrade without the cells. Probably, the cells remain intact and actively metabolize hydrazines, which contradicts the hypothesis on pronounced toxicity of these compounds. In our experiments, culture growth was not inhibited by mutagens except nalidixic acid.

Thus, the reported effects are specific. The biosensor test-system developed to detect the damages to genomic DNA can be used as a tool to study the direct action of hydrazines on the cell genome [4].

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