Middle-East Journal of Scientific Research 21 (8): 1333-1340, 2014
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2014.21.08.84136

Study of Prooxidant Action of Ultraviolet Radiation with Wavelength 258 Nm Using Bacterial Biosensors

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Abstract: Ultraviolet light with wavelength 255 - 260 nm is known to be a DNA-damaging factor. It causes formation of several photoproducts and reactive oxygen species. An approach based on use of bacterial LUX-biosensors allows to evaluate the role of ROS in the biological effects of ultraviolet radiation. Experiments shows that exposure of *E. coli* culture to the ultraviolet light with wavelength 258 nm leads to formation of hydrogen peroxide within cells and in a culture medium.

Key words: Ultraviolet · Biosensors · Prooxidants · Oxidative Stress · Hydrogen Peroxide

INTRODUCTION

Absorption of ultraviolet light with wavelength 255 - 260 nm by DNA bases leads to formation of several photoproducts, mostly cyclobutane dimers. It is believed that this process is the basis for much of the genetic effects caused by this type of radiation. Meanwhile, the energy of photons with wavelength 258 nm is sufficient to activate a variety of other photochemical reactions [1, 2]. These processes may be accompanied by formation of highly active compounds that can react with DNA. One such substance is hydrogen peroxide. An approach based on use of bacterial biosensors allows to register both the formation of hydrogen peroxide and DNA damage in bacterial cells. Luminescent biosensors created by G.B. Zavilgelsky *et al* (2007) that have the lux-operon of luminous bacteria placed under control of necessary promoters allow us to monitor these processes in real time. This methodology was successfully applied to study the mechanism of action of 1,1-dimethyl hydrazine (Heptyl) [3]. In this paper, it was used to study the biological effects of ultraviolet radiation with wavelength 258 nm.

MATERIALS AND METHODS

In this study *E.coli* strains MG1655 (pKatG-lux) and AB1157 (pRecA-lux), containing the plasmid carrying the operon luxCDABE, under control of promoters of katG and RecA, were used. Biosensor with the promoter PkatG registers formation of hydrogen peroxide in the cell; biosensor with the promoter PrecA registers DNA damage [3].

Cultures of strains were grown on complete medium Luria-Bertani (LB), with addition of ampicillin (100 µg / ml) for 18-20 hours at 36°C; then 50 µl of culture were transferred into 5 ml of fresh medium and cultured for 2 hours. Aliquots of this culture by 45 - 50 µl were transferred to the cells (Diameter 7 mm) of a micro plate, adding 5 µl of solution required for positive control.

Barrier discharge Cl_2-excilamp BD_P (Barrier Discharge, Portable), developed at the Institute of High Current Electronics SB RAS (Tomsk, Russia) and produced by LLC "Excilamps" (Tomsk, Russia) was used as a source of ultraviolet radiation with wavelength 258 nm. Barrier discharge excilamps is a new class of mercury-free, gas-discharge sources of ultraviolet radiation based on excimer and exciplex molecules [4].

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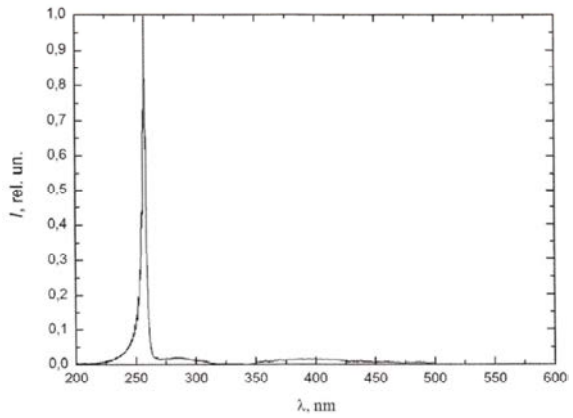

Fig. 1: The emission spectrum of Cl_2-excilamp.

The emission spectrum of an excilamp is shown in Fig. 1. To remove ozone from the working area, the lamp is equipped with a fan.

Micro plates were disposed so that the distance between the surface of the lamp and the surface layer of the irradiated culture was 50 mm. Irradiance at a given distance was 1.59 mW/cm^{2}s.

The thickness of the layer of the treated suspension was 1.3 mm. Doses given in the article are the doses received by the surface of the bacterial suspension during irradiation.

In the study of patterns of absorption of ultraviolet light by LB medium, we proceeded from the fact that dilution of the chromophore with an optically transparent liquid is equivalent to reducing the optical path in undiluted chromophore. Measurements were made with a spectrophotometer Beckman DU 800, using a cuvette with optical path of 10 mm. The absorption of the medium layer of 1 mm was simulated by tenfold dilution of the LB medium-distilled water, the other values- with proportional dilution.

After irradiation, the plates with samples were put into a micro plate luminometer LM-01A (Immunotech) and incubated at 36°S. The intensity of bioluminescence was measured every 10 - 15 min for 120-140 min.

In the experiments on the effect of UV-treated medium on *E.coli* culture, aliquots of 50 µl medium were exposed to radiation in the cell plate and then added to the bacterial culture.

To estimate the influence of investigated factors on the expression of operons we calculated the induction coefficient (Is) as follows:

$$I^S = \frac{L_e}{L_k} - 1 \quad (1)$$

where:

L_k = Luminescence intensity in a control sample;
L_e = Luminescence intensity in the test sample.

A statistically significant excess of L_e over L_k, estimated on t-test, was considered an indication of the reliability of the effect of induction.

All experiments were performed in 12 independent repeats.

Hydrogen peroxide («Ferrain») was used as a standard oxidative stress-inducer; the exact concentration of hydrogen peroxide was determined by titration with potassium permanganate [5].

In the experiment with catalase, 5µl (2 units) of catalase (Sigma), dissolved in 0,9% NaCl, were added to 45 µl medium or culture.

To determine the survival of the overnight culture suspension of the test strain grown in medium LB, series of ten-fold dilutions with saline were prepared. Dilutions from 4 to10 were seeded by 0.1 ml on plates with LB medium. Plates were incubated at 37 ° C for 24 hours. For each plate the average number of colonies (CFU / ml) grown on three plates was calculated. Survival rate and survival rate error were determined by standard formulas.

RESULTS

Weakening of the Power of Radiation Passing Through the Culture Medium: In accordance with the law of Lambert-Beer, the transmission of light with a wavelength of 258 nm by LB medium is described by an exponential curve; in this case it has the form $y = 99{,}15e^{-44{,}8x}$, where y is transmission percentage, x is concentration of the medium (Percentage of original). The total dose of radiation absorbed by the culture is described by the integral of this function and is equal to the dose absorbed by a medium with constant effective transmission coefficient 17%. An adequate model could have been made with a medium layer of equal thickness, transmitting 17% of the light, with all of the bacterial mass distributed along the bottom of the cell. Thus, the force of light passing through the medium and acting on the bacteria is weakened 5.88 times by average.

Survival Rate: Experiments have shown that after the irradiation of biosensor strain culture with UV light with wavelength 258 nm, the survival rate of bacteria decreases with increasing doses of UV. Survival rate curve for one of the biosensor strains is shown in Figure 2.

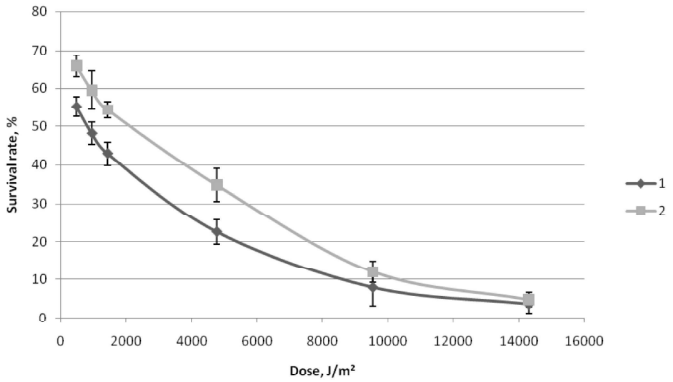

Fig. 2: Survival rate curve of E. coli AB1157 (pRecA-lux). 1 - survival rate after UV irradiation, 2 - survival rate after UV irradiation in the presence of catalase (10 U/ml

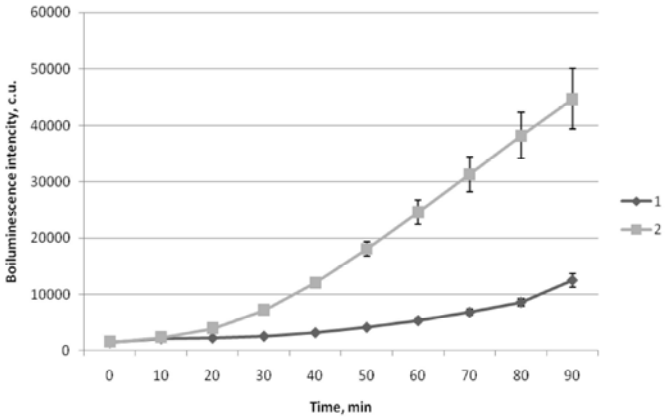

Fig. 3: Bioluminescent response of E. coli MG1655 (pKatG-lux) on exposure to UV with wavelength 258 nm. 1 - bioluminescence of the control sample, 2 - irradiated sample bioluminescence. Radiation dose was 1400 J/m^2.

Survival rate in the smallest of studied doses is 55.1%; it decreases with increasing of the dose (Fig.2). Adding catalase (10 U/ml) before irradiation increases survival rate by 10-12%.

Induction of KatG: It was shown that exposure of culture of *E. coli* MG1655 (pKatG-lux) to 258 nm UV light leads to the development of bioluminescent response (Fig. 3). The maximum response developed within 70 minutes from the beginning of the measurements. Statistically significant induction is recorded for doses of 500 - 14 300 J/m^2. Maximum value of induction factor (3.60) was reached after irradiation at a dose of 1431 J/m^2. Dose-response graph has a specific arched shape, characteristic for prooxidants (Fig. 4).

Described effects can be compared with the effects of exposure of this strain to hydrogen peroxide. On the calibration curve for the effects of hydrogen peroxide exposure specific to this strain, it is clear that the effect of the maximum rate of UV induction (3.6) may corresponds to the effect of hydrogen peroxide at a concentration of 10^{-4} M (Fig. 5).

Induction of RecA: Bioluminescence of biosensor strain *E. coli* AB1157 (pRecA-lux), which is a detector of DNA damage, increased by 3-6 times after exposure to doses of 500 - 28 600 J/m^2 (Fig. 6). The maximum induction factor developed 60-70 minutes after the beginnng of the measurements. I^s reached its highest value (5.25) after irradiation with 4800 J/m^2. In the studied dose range, the dose dependence was observed, with an UVC-induced SOS-response-specific form with continuous growth and a sharp drop after reaching the maximum effect [6].

From the calibration curve for the effects of hydrogen peroxide exposure specific to this strain, it is clear that induction factor 5.25 may correspond to the effects of hydrogen peroxide at a concentration of 10^{-4} M (Fig. 7).

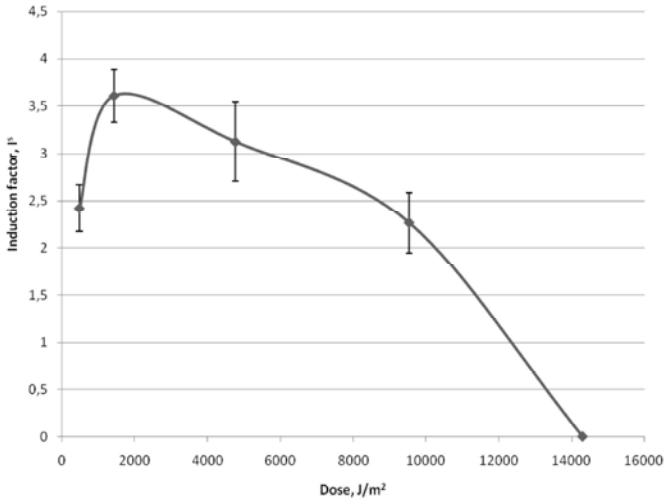

Fig. 4: Induction factor of bioluminescence of E. coli MG1655 (pKatG-lux) after UV irradiation with wavelength 258 nm. (Maximum values are shown. Maximum response time is 70 minutes from the beginning of the measurement.)

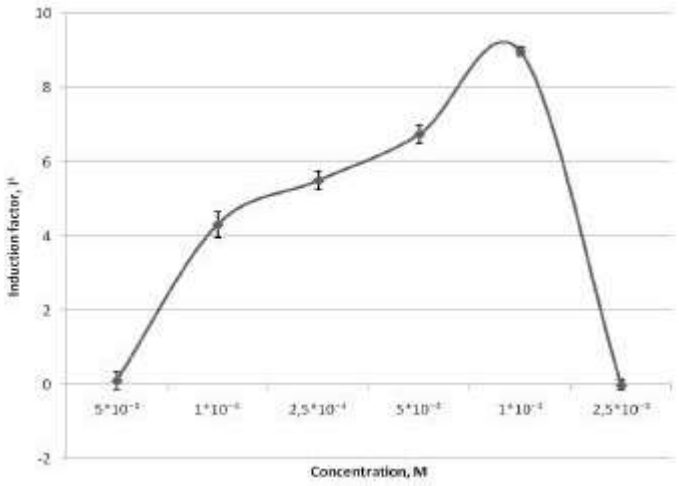

Fig. 5: Calibration curve of bioluminescence induction of E. coli MG1655 (pKatG-lux) exposed to hydrogen peroxide.

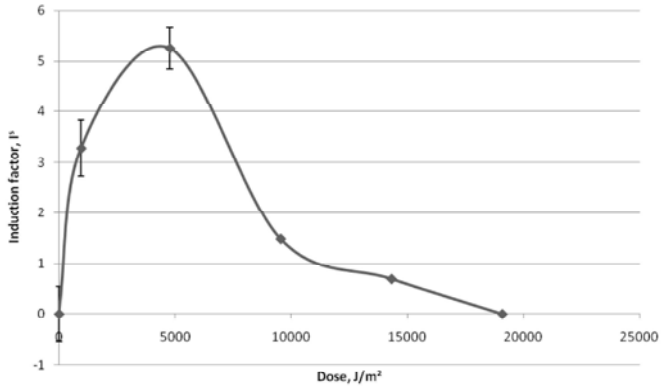

Fig. 6: Induction of bioluminescence of E. coli AB1157 (pRecA-lux) after UV irradiation with wavelength 258 nm. (Maximum values are shown. Maximum response time is 70 minutes from the beginning of the measurement.)

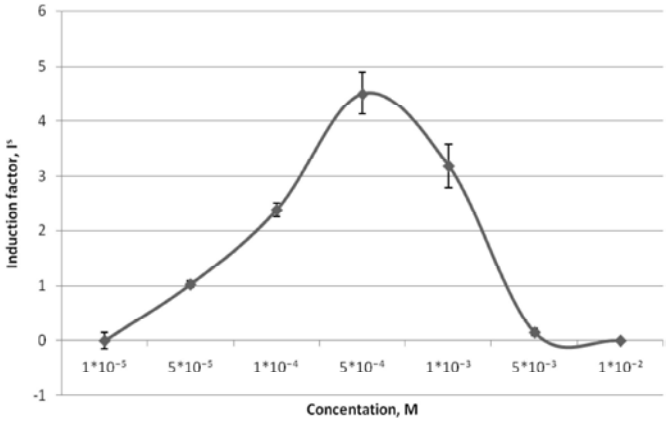

Fig. 7: Calibration curve of induction factor E. coli AB1157 (pRecA-lux) exposed to hydrogen peroxide.

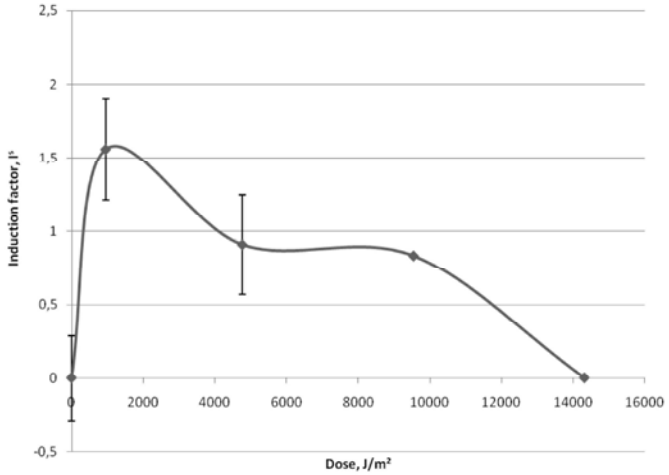

Fig. 8: Induction factor of bioluminescence of E. coli MG1655(pKatG-lux) after UV irradiation with wavelength 258 nm. (Maximum values are shown. Maximum response time is 90 minutes from the beginning of the measurement.)

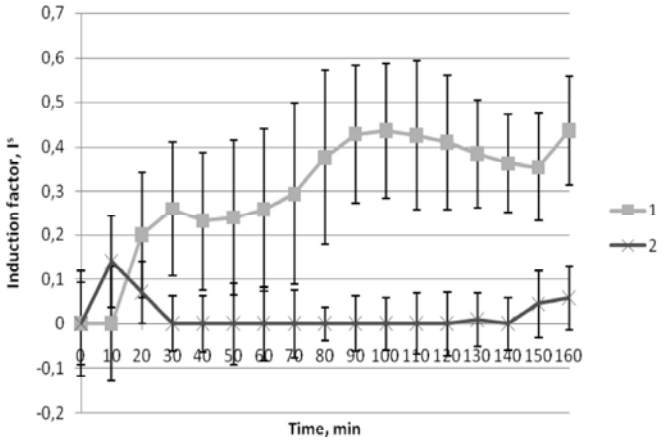

Fig. 9: Bioluminescence induction coefficient of E. coli MG1655 (pKatG-lux) under effect of the LB medium irradiated with UV with wavelength 258 nm, in presence of 10 U/ml catalase. The radiation dose was 2400 J/m^2 1 - UV 2 - UV in presence of catalase.

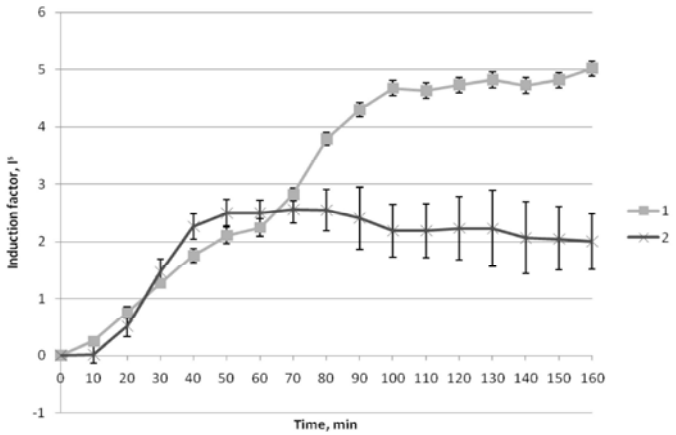

Fig. 10: Induction factor of bioluminescence of E. coli AB1157 (pRecA-lux) after UV irradiation with wavelength 258 nm in presence of 10 U/ml catalase. The radiation dose was 2400 J/m^2. 1 - UV 2 - UV in presence of catalase.

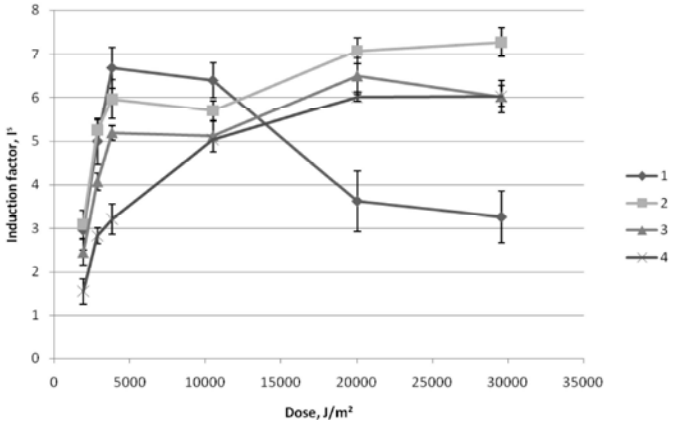

Fig. 11: Induction factor of bioluminescence of E. coli MG1655 (pKatG-lux) after UV irradiation with wavelength 258 nm in the presence of different concentrations of catalase. 1 - control (exposure to UV only), 2 - in the presence of catalase 1 u/ml, 3 - in the presence of catalase 10 u/ml, 4 - in the presence of catalase 100 u/ml.

Medium Irradiation: To study the contribution of the formation of hydrogen peroxide in the culture medium to the observed effects, experiments were performed on pre-irradiated medium added to the biosensor culture. It was found that exposure of the medium in this dose range causes a statistically significant increase in bioluminescence of *E. coli* MG1655 (pKatG-lux) (Fig. 8). The maximum effect developed within 90 minutes from the beginning of the measurements. Induction factor reached the largest value (1.56, which is 17% of the maximal effect of direct irradiation of bacterial culture) by irradiation at a dose of 1000 J/m^2. In this dose range, a dependence of the induction from the radiation dose was observed; the curve had an arched shape.

To confirm the hypothesis that the above effects were caused by the action of hydrogen peroxide, the experiments on irradiation of strains culture and effects of pre-irradiated medium on bacteria were repeated in the presence of catalase (10 U/ml). From the data presented in Figures 9 and 10, we can see that the addition of catalase reduces the effects of UV. In experiments with pre-irradiated medium induction is suppressed to zero values; in experiments with direct irradiation of culture suppression is in the range of 43.9-60.1%.

Dose/effect curves have an arched shape because the UV range under consideration causes the death of some cells; the dynamics of the observed emission reflects the combined effect of two processes: destruction of some of the bacteria and induction of SOS-operons of surviving cells. Induction processes tend to dominate when the radiation dose is small, whereas the bactericidal effects of the UV increase as the dose increases, resulting in the total luminescence of culture decreasing.

As can be seen from the data presented in Figure 2, the observed effects are masked by more than 50% due to the bactericidal effect of UV radiation.

The data presented in Figure 11 also shows that our values of the induction factor are under-reported due to bactericidal effect.

As shown in Figure 11, with UV irradiation doses of up to 10,000 J/m^2, catalase reduces the bioluminescence induction (Maximum values are 10.8%, 22.3%, 52.0% for concentrations of 1, 10 and 100 unit/ml catalase, respectively), whereas with higher doses, when the process of cell death begins to prevail over the SOS-induction, the luminescence of culture in the presence of catalase increases relative to controls, apparently, due to reduction of the bactericidal effect of UV.

Similar results were obtained for the RecA-lux strain - 39.3%, 52.7% and 71.4% inhibition effect of UV by catalase at concentrations of catalase 1, 10 and 100 units/ml, respectively.

DISCUSSION

Interpreting the results on survival rate, one should bear in mind that the handling of bacteria in the complete culture medium is a prerequisite for correct setting of toxicity tests with biosensors, because only in these conditions the highest level of expression of the operons is provided. It is difficult to calculate the exact dose of cell irradiation, as some part of the light gets absorbed by the medium. According to our calculations, even in an 1.3 mm-thick layer, provided that bacteria are distributed evenly in the environment, the power of light acting on the bacteria is reduced by more than five times. As the experiments with catalase showed, the death of bacteria irradiated in the complete medium is caused not only by photo damage of biomolecules, but also by formation of hydrogen peroxide outside the cell.

Photochemical reaction of pyrimidine bases dimerization underlying the lethal and mutagenic action of UVC, was discovered in 1961 by Berends and Bakers [6] in experiments with frozen solutions of thymine [7]. The photo dimerization reaction is a covalent combination of two molecules with double bonds in 5th and 6th carbon atoms forming a cyclobutane ring. Dimerization of the pyrimidine bases at physiological temperatures is theoretically possible for the convergence of thymine or cytosine, which occurs after its local denaturation strengthened by photo dimerization [8].

Currently there are three groups of methods for determining the content of pyrimidine dimers. The first group of methods is based on the use of T4 endonuclease-V - an enzyme with N-glycosylase activity and AP-liase activity. The enzyme hydrolyzes the bond between desoxyribose and a damaged base and then cuts the DNA strand at the site of formed pirimidine-deficient site. The subsequent separation of DNA fragments by gel electrophoresis allows to evaluate the amount of dimers formed. There are also methods using oligonucleotide chips, which have immobilized T4-endonuclease V as the basis [9]. But in terms of specific determination of pyrimidine dimers the use of this enzyme has a significant drawback, because AP sites generated as a result of other factors besides cutting pyrimidine dimmers also are substrates for the action of T4 endonuclease-V [10].

The second group includes the physical and chemical methods based on chromatographic analysis and mass spectrometry [11,12]. These methods allow to accurately identify synthetic photoproducts, in particular, thymine dimers, but when dealing with cellular genetic material, they require quite tough pre-treatment methods, which can themselves cause damage to DNA molecules, which significantly reduces the resolution [10].

Apparently, the most accurate is the third group of methods which are based on the creation of specific antibodies that react with pyrimidine dimers. Changes in the topology of DNA treated with such antibodies, are estimated with an atomic force microscope. Results obtained using this technique show that only about 60% of sites exposed to cutting with T4 -endonuclease V, are indeed pyrimidine dimers [8]. Thus, we conclude that the role of pyrimidine dimers in the implementation of the effects of UV irradiation is still significantly overestimated.

The absorption of light with wavelength 258 nm is not specific to DNA; it is also typical for a number of other chromophores. The complexity of developing photoreactions in this system makes the transfer of patterns found in one system to another unreasonable. Each experimental system requires an independent experimental evaluation of the most important destructive factors. As shown by our experiments, exposure of culture E. coli to the ultraviolet light with wavelength 258 nm leads to the induction of catalase operon (Fig. 3, 4). The value of the induction factor is reduced by 50-60% after irradiation in the presence of catalase. Decrease in both the induction of Rec- and Kat-operons and UV bactericidal effect after adding catalase shows the significant role of hydrogen peroxide in the development of two above-mentioned effects.

Kat-induction is also observed during the treatment of the irradiated medium. Thus, we can conclude that the induction of catalase operon is caused by hydrogen

peroxide produced within cells and in culture medium. The maximum level of induction factor registered under ultraviolet irradiation is 3.6. To obtain an equivalent induction factor by the action of hydrogen peroxide we have to add 10^{-4} M solution of it to the culture. The level of Rec-induction with the preliminary introduction of the hydrogen peroxide in this concentration in the medium is 2.4 which makes 45.7% of the maximum induction factor detected after UV irradiation. The difference of 50.3% may be caused both by contribution of non-peroxide processes (Dimerization of pyrimidines and the formation of other photoproducts) and the fact that after UV irradiation peroxide is not comes through the outer membrane, but is generated directly in the cell.

Thus, our data allows to assert that formation of hydrogen peroxide makes a significant contribution to the development of SOS-response in bacteria irradiated with ultraviolet light with wavelength 258 nm.

Ultraviolet C gives only a fraction of a percent of the energy of sunlight at the earth's surface. However, in the highlands and areas of "ozone holes" power of this type of radiation is much higher. People can receive appreciable doses of UVC using bactericidal lamps and during some technical procedures. Therefore the study of mechanisms of genotoxicity of UV with wavelength 258 nm has both theoretical and practical importance. Recognition of the "responsibility" of not only pyrimidine dimers but also hydrogen peroxide for the most of these effects opens the ways to search for UV-protectors based on the antioxidant mechanism. Such preparations may be an important addition to the sunscreen-products traditionally used to protect the skin from ultraviolet radiation.

ACKNOWLEDGEMENTS

This research was sponsored by the Ministry of Education and Science of the Russian Federation, projects No 213.01-11/2014-32 and No 6.1202.2014/K. We thank Dr. I.V. Manukhov for critical reading of the manuscript.

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