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EXPERIMENTAL ARTICLES

Involvement of Alkylhydroxybenzenes in the *Escherichia coli* Response to the Lethal Effect of UV Irradiation

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Abstract—This work is concerned with the role of alkylhydroxybenzenes (AHBs), chemical analogs of the autoregulatory microbial d_1 factors, on the development of the stress response of bacterial cells to UV irradiation, including SOS system induction, preservation of cell viability, and S ⟶ R phase transitions of the *Escherichia coli* test strain with the bioluminescence genes cloned under the control of the *recA* gene promoter. UV irradiation, a natural stress factor, and an increase in AHB concentrations were found to elicit uniform responses in bacteria, indicating that AHBs function as alarmones, i.e., alarm signals. It was revealed that preincubating bacteria with alkylhydroxybenzenes considerably enhanced their viability upon irradiation with lethal UV doses; this was accompanied by a relative decrease in the SOS response activity and a concomitant increase in the frequency of phase transitions. The efficiency of the protective action of AHBs increased with an increase in their hydrophobicity degree. The probable mechanism of the protective effect of AHBs is discussed, based on their capacity for the interaction with biopolymers, which results in changing their structural organization and conferring resistance to a broad spectrum of stress factors. Such a "passive" protective mechanism reduces the susceptibility of DNA to UV irradiation, causing a decrease in the parameters related to the SOS system induction that is responsible for the "active" protective mechanism in bacterial cells. As a result, viability retention under the lethal influence of UV irradiation is possible at minimal values of repair activity and is accompanied by an increase in the phenotypic variability of the surviving part of a bacterial population.

Keywords: *Escherichia coli*, UV irradiation, alkylhydroxybenzene homologues, SOS system, *recA* gene, bioluminescence, S ⟶ R phase transitions, cell survival, passive and active DNA protection.

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A recent major scientific achievement is the elucidation of the molecular mechanisms of the biological activity of the extracellular autoregulatory factors that control many aspects of the functional activity and cell differentiation of prokaryotes [1]. This is especially true in the case of nonspecies-specific extracellular autoregulators (d_1 factors). In a number of bacteria and yeasts, these factors belong to alkylhydroxybenzenes (AHBs). They control the transition of recipient cells to the hypometabolic and anabiotic state [2]. Another aspect of the biological activity of AHBs is their functioning as adaptogens involved in developing of microbial resistance to a wide variety of deleterious factors [3]. In a number of studies, the protective effects of AHBs on the cells of bacterial and yeast cultures under various kinds of stress [4, 5], including UV irradiation [6], were demonstrated. This provided the foundation for more detailed research on the mechanisms of action of the above group of microbial autoregulators.

Previous studies on this subject revealed that the molecular mechanisms of the regulatory effects of AHBs were based on their capacity for nonspecific binding to cell biopolymers such as proteins and the DNA, as well as to membrane lipids, due to the formation of hydrogen bonds and hydrophobic and electrostatic interactions [7–10]. Formation of AHBs complexes with enzyme proteins caused changes in their conformation and, accordingly, in their functional activity and stability [7, 10, 11]. In addition, our earlier works demonstrated a direct interaction between AHBs and the DNA accompanied by alterations in its topology and physicochemical properties [8]. Importantly, the resulting DNA + AHB complexes were characterized by enhanced resistance to UV irradiation [9].

Under ordinary conditions, DNA intensely absorbs UV light within the 240–300 nm wavelength range with a maximum at 254 nm. This accounts for the serious damage of this biopolymer, with its extreme manifestations resulting in single- or double-strand breaks in the DNA. An essential role in terms of

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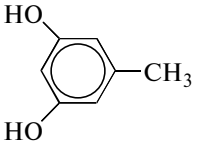
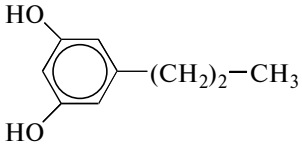
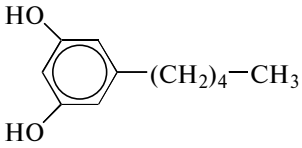
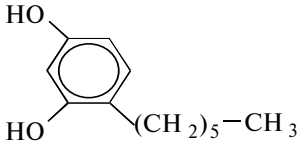
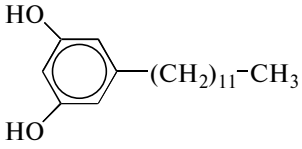
Table 1. Chemical analogs of alkylhydroxybenzenes used in the work

Designation	Structural formula	Molecular mass	Manufacturer
C7-AHB		124	Sigma, United States
C9-AHB		152	Enamine, Ukraine
C11-AHB		180	Sigma, United States
C12-AHB		194	Sigma, United States
C18-AHB		278	Enamine, Ukraine

protecting the cells from UV radiation is therefore assigned to the DNA repair system. Of paramount importance for this system is the operation of the SOS response genes [12]. The mechanism of SOS regulon induction is based on the interaction between the products of the *recA* and *lexA* genes. By binding to impaired single-stranded DNA areas, the RecA protein facilitates the cleavage of the LexA protein dimers and thereby abolishes the LexA-induced repression of about 40 RecA-dependent genes involved in repairing the DNA, including the *recA* gene [13].

Initial research on alkylhydroxybenzene effects on repair activity suggested the possibility that the stress genes of the bacterial SOS system were subject to both positive and negative regulation, due to the involvement of AHB homologues with different hydrophobicity [6]. However, cellular protective systems use also a "passive" mechanism involving AHBs as (i) traps for reactive oxygen species (ROS) [10] and (ii) biopolymer molecule stabilizers [7–11].

Of special interest in the light of the above was the whole coherent pattern of the interrelationships between the "active" and "passive" mechanisms of DNA protection from UV radiation involving alkylhydroxybenzenes. An additional intriguing issue was to develop the experimental system simulating the impact of environmental factors characterized by lethal intensity that occur in natural ecosystems. This system should be different from our earlier models of short-term sublethal stresses including those caused by UV irradiation [4–6].

Therefore, the goal of this work was to investigate the involvement of alkylhydroxybenzenes, natural adaptogens, in the stress response of *Escherichia coli* to lethal intensity of UV irradiation with special emphasis on elucidating the effects of AHB homologues with different hydrophobicity with respect to the "active" and "passive" mechanisms of DNA protection in this system.

MATERIALS AND METHODS

We used a number of alkylhydroxybenzene homologues differing in the length and location of the hydrophobic alkyl radical, including the commercially available preparations C7-AHB, C11-AHB, and C12-AHB (Sigma, United States) as well as C9-AHB and C18-AHB (Enamine, Ukraine), with a purity degree of 99.9%, which were synthesized for this study (Table 1).

The recombinant strain *Escherichia coli recA'::lux*, kindly provided by I.V. Manukhov (State Research Institute of Genetics, Russia) was used as the model microorganism. Its special feature is that it contains the p*recA'::luxCDABE*-Amp^R plasmid with the *lux* gene cassette from the luminescent soil bacterium *Photorhabdus luminescence* Zm 1, cloned using the promoter of the *E. coli recA* gene and the marker gene responsible for resistance to ampicillin. This genetic system secures a sufficiently low background luminescence level of carrier cells. The luminescence level is drastically increased upon damaging the DNA, due to a synchronous induction of the *recA* gene and the bioluminescence gene promoter it controls. Accordingly, measuring the luminescence intensity of the tested strain provides for the real-time quantitative assessment of the SOS system activation level, particularly upon UV irradiation [14], without carrying out any additional procedures.

Prior to the study, the *E. coli recA'::lux* strain was grown for 16–18 h at 37°C in LB broth (Sigma, United States) in the presence of 20 μg/mL ampicillin. Shortly before the experiment, the culture was diluted, at a ratio of 1 : 20, with fresh medium of the same composition and incubated for 3–5 h in order to achieve an optical density of 0.2 U (λ = 640 nm, l = 1 cm, Fluorat-02 Panorama, Lumex R&D Company, Russia). This corresponded to the early phase of the exponential growth of the batch culture. In the experimental systems, aliquots of this culture were mixed with AHB solutions (1 : 1), so that the final AHB concentrations were 10^{-5}, 10^{-4}, and 10^{-3} M. In the control systems, the AHB solutions were replaced with distilled water. The mixtures were preincubated for 60 min. Thereupon, 1 mL samples were dispensed into the wells of polystyrene plates.

The samples were UV-irradiated with a broadband mercury–quartz lamp (Osram, Germany) at a distance of 10 cm, using an interference light filter with a transmission peak around 254 nm. This enabled us to selectively impair the DNA of bacterial cells, while only minimally affecting other intracellular structures. The irradiance of the samples measured with a TKA-PKM UV radiometer (Russia) was 6.7 W/cm^2, the exposure time varied from 0 to 180 min with 60 min increments, which corresponded to total UV doses of 1.21, 2.43, and 3.64 J/m^2, respectively.

After the intervals specified above (60, 120, and 180 min), 100 μL aliquots were taken from the tested samples in order to determine the bioluminescence intensity using an LM 01T bioluminometer (Immunotech, Czech Republic).

In the tested samples, we determined the number of viable cells from the colony-forming unit number (CFU) by inoculating 10 μL aliquots on LB agar with a subsequent 24 h incubation at 37°C. We established whether the resulting colonies belonged to the S, R, or RS type using a magnifying glass (magnification 5×).

The quantitative assessment of the SOS response induction was carried out using the formula $F_i = \mathrm{lux}A_i \times B_0/\mathrm{lux}A_0 \times B_i$ where $\mathrm{lux}A_0$ is the light emission of the control cell suspension, $\mathrm{lux}A_i$ is the light emission of the irradiated cell suspension, B_0 the viable cell number in the control sample, and B_i the viable cell number in the irradiated sample [15].

At least three repeats of each experiment were carried out. The results were statistically processed using the Statistica software package. Shannon's entropy test [16] was employed to calculate the phenotypic S ⟶ R phase variation index.

RESULTS

During the first stage of our study, we investigated the pattern of the responses of the test strain *E. coli recA'::lux* to various doses of UV radiation, a natural stress factor. Comparing cell viability (CFU number) and bioluminescence intensity made it possible to determine the quantitative and dynamic characteristics of the process of SOS system induction (Fig. 1). An UV radiation dose within the 0–1.21 J/m^2 range stimulated bioluminescence as a consequence of SOS system induction, which manifested itself in an increase in the absolute luminescence intensity with a maximum value 2.65 times exceeding the control level. However, further rise in the stress factor dose resulted in a progressive decrease in detectable bioluminescence that reached the initial value at the radiation dose of 3.64 J/m^2 (Fig. 1a). The most likely reason is the death of a significant portion of bacterial cells that was monitored upon the inoculation of the tested samples on the solid medium. The CFU number displayed a near-exponential dependence on the UV radiation dose (Fig. 1b). Nonetheless, the relative index F_i, which links bioluminescence intensity to the viable cell number, indicated an inverse relationship between these values: the F_i value (25.37) at the absolute bioluminescence maximum with the UV radiation dose of 1.21 J/m^2 was lower than the F_i value (282.85) by an order of magnitude at the UV radiation dose of 3.64 J/m^2 (Fig. 1c). Since the radiation dose in our studies varied depending on the exposure time, we conclude that the activation of repair processes continued in a numerically insignificant subpopulation of the cells that remained viable and metabolically active upon a long-term (180 min) stress. In terms of colony morphology, an additional effect produced on the *E. coli recA'::lux* cells that survived the lethal dose was the phenotypic phase variation of the population that manifested itself in replacing the dominant S-type colonies with R type colonies that amounted to 50% of the colonies in the experimental and 8% in the control system.

Taking account of the earlier established capacity of one of the AHB homologues, C12-AHB, to activate stress gene expression at a sublethal UV irradiation [6,

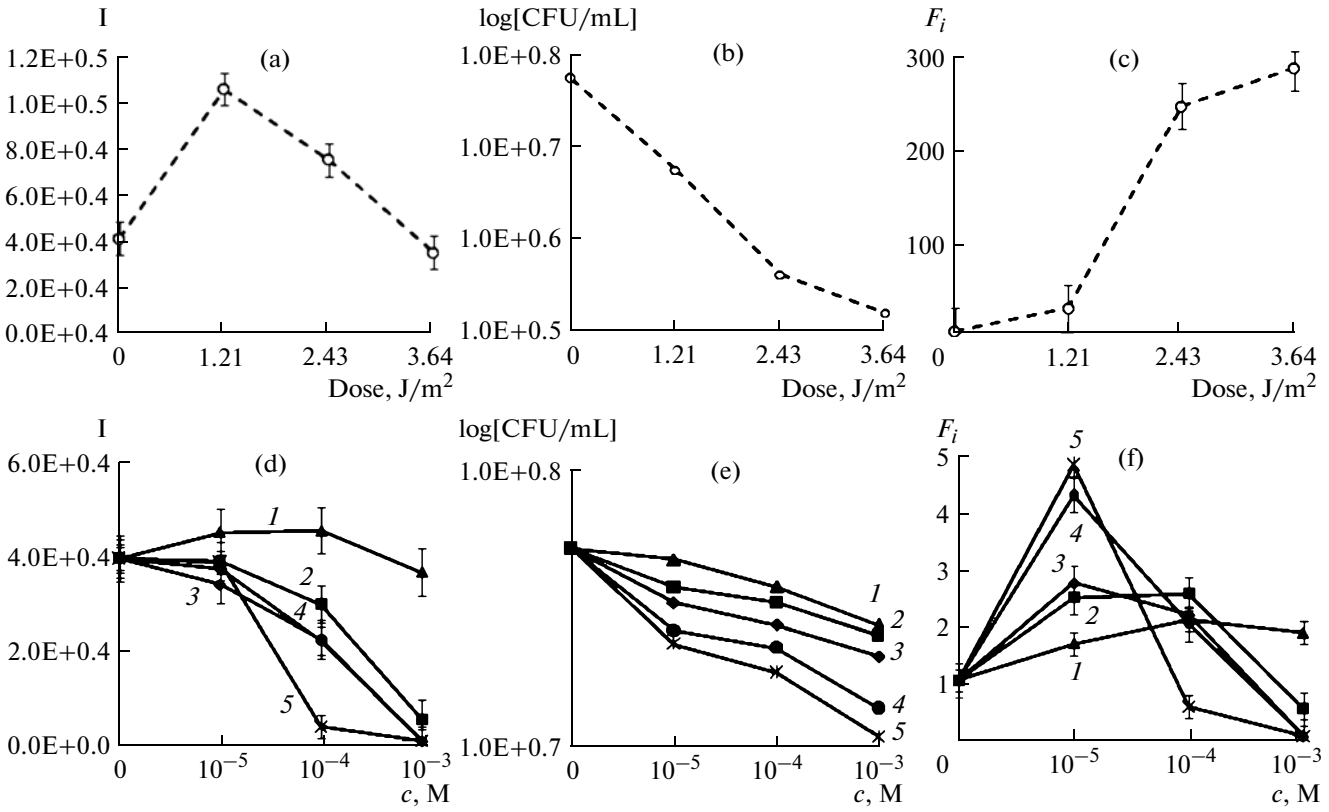


Fig. 1. Patterns of the responses of the strain *E. coli recA'::lux* to UV irradiation (a)–(c) and AHBs (d), (e): C7-AHB (*1*); C9-AHB (*2*); C11-AHB (*3*); C12-AHB (*4*); and C18-AHB (*5*). Designations: horizontal axis, radiation dose, J/m^2 (a)–(c) and AHB concentration, M (d)–(f); vertical axis, bioluminescence intensity (a), (d); viable cell number (b), (e); SOS response-inducing factor (c), (d).

17], we assessed similar regulatory properties in a representative array of autoregulatory factors. Research on AHB effects on the luminescence, viability, and phase variation enabled us to reveal a number of interlinked processes that are associated with the peculiarities of the AHB chemical structure (Table 1) and their concentrations (Fig. 1). Under the experimental conditions, none of the AHB homologues alone caused a significant increase in absolute bioluminescence; they rather brought about a significant decrease in the background fluorescence level. This inhibitory effect increased in the C9-AHB ⟶ C11-AHB ⟶ C12-AHB ⟶ C8-AHB row with increasing length of the alkyl radical and the molecules' hydrophobicity that depends thereupon. The effect was reliably observed at AHB concentrations exceeding 10^{-5} M. C7-AHB failed to affect cell bioluminescence, regardless of its concentrations (Fig. 1d). An analysis of *E. coli recA'::lux* cell viability in the presence of various AHB homologues revealed a CFU number decrease that was concomitant with the weakening of bioluminescence (Fig. 1e) and was particularly prominent with C12- and C18-AHB. The latter is due to the known growth-inhibiting activity of these long-chain AHBs [2, 3]. In terms of our work, it should be emphasized that the inhibitory action of AHBs on the growth-related characteristics of microorganisms was two orders of magnitude less significant than that associated with the lethal effect of UV radiation (see above).

Based on the data given above, we calculated the F_i value that links bioluminescence intensity to the viable microbial cell number. We established AHB concentration-dependent changes in the SOS system activity (Fig. 1f). For instance, the influence of the C12- and C18-AHB homologues at a concentration of 10^{-5} M resulted in a 4–5-fold activation of the relative fluorescence of *E. coli recA'::lux* with F_i values of 4.30 and 4.84, respectively. This is consistent with the idea that long-chain AHBs exhibit weak mutagenic activity [6, 17] and may act as alarmones, i.e. bacterial alarm signals [6]. In terms of SOS response induction, these AHB homologues were 1–2 orders of magnitude less efficient than UV radiation. High (10^{-3} M) concentrations of long-chain AHBs that induce the anabiotic state of bacterial cells [2, 18] brought about a pronounced repression of the SOS response. This was due to transcription arrest in anabiotic cells [2, 3]. It should be mentioned that the action of long-chain alkylhydroxybenzenes at the post-translational level results in inhibiting the functional activity of proteins [2, 3, 7, 11]. Since the AHB concentration of 10^{-4} M

caused minimal SOS system changes with all tested homologues, it was convenient to use it in subsequent studies on the combined action of AHBs and the natural stress factor (UV radiation). In general, the results obtained at this stage confirmed the pleiotropic effect of AHBs whose regulatory function partly depends on their interaction with cell biopolymers and the modification of their functional activity [2, 6]. This applies to the test organism *E. coli recA'::lux*.

Another effect of AHBs was their influence on the intensity of phenotypic transitions in *E. coli recA'::lux* cells. In particular, some AHB homologues at a concentration of 10^{-4} M increased the frequency of the S $\longrightarrow$ (R + RS) transition to 38%, in contrast to 8% in the control system. This effect was quite consistent with the data on the AHB influence on the frequency of phase transitions in bacteria including *Bacillus subtilis* [6], *Staphylococcus aureus* [19], *Pseudomonas aurantiaca* [20], and *Pseudomonas fluorescens* [20], which increases the likelihood of survival of the bacterial population under the influence of deleterious factors and under changing environmental conditions [21, 22].

Hence, by consecutively applying the natural stress factor (UV radiation) and alkylhydroxybenzenes, chemical analogs of bacterial autoregulators, to the test strain *E. coli recA'::lux*, we revealed principally similar changes in the following stress response-related variables: bioluminescence, viable cell number, and the level of intrapopulation phenotypic variability. This gives us grounds for assigning a signal role to AHBs in terms of protective bacterial responses, and suggesting cell preadaptation during consecutive action of AHBs and UV irradiation. This was investigated in the next stage of our work.

Monitoring the parameters of the bioluminescence intensity and cell viability and determining, from these data, the degree of SOS system activation under various UV doses in *E. coli recA'::lux* that was preincubated with AHB homologues at a concentration of 10^{-4} M revealed a number of interrelated effects (Fig. 2). The most important finding was that the absolute bioluminescence level of AHB-preincubated cells varied depending on the chemical structure and, accordingly, hydrophobicity of the AHB homologues involved. The treatment with the most amphiphilic C7-AHB yielded an absolute luminescence intensity value equal to the control one (without AHB) under the radiation dose of 1.21 J/m^2 and exceeded the control value under a higher dose (Fig. 2a). The bioluminescence level was lower than in the control system in the case of the cells preincubated with C9- and C11-AHB. As for the cells pretreated with the most hydrophobic homologues C12- and C18-AHB, their bioluminescence intensity was below the background level at all the tested radiation doses (Fig. 2a). The results obtained, therefore, did not support the conclusion that the SOS response is synergistically activated the in systems in which cells are consecutively treated with AHB and UV radiation: the number of viable cells at high radiation doses was significantly higher in the variants with long-chain homologues (Fig. 2c). Moreover, comparative analysis of F_i values that links bioluminescence intensity to the viable microbial cell number after UV irradiation revealed a relative decrease in F_i of AHB-preincubated cells (Fig. 2b). The degree of SOS response repression increased in proportion to the decrease in the hydrophobicity of AHB homologues used for the pretreatment. It was 3.7 and 7.0 with C12- and C18-AHB, respectively, at an UV irradiation dose of 3.64 J/m^2. This was 40–76 times lower than the SOS system activation level in test strain cells that were treated only with UV radiation (Fig. 2b). At the AHB concentration decreased to 10^{-5} M, statistically reliable differences between the factors of induction in the control and experimental samples remained, although repression of the SOS response was somewhat less pronounced. Increasing the tested AHB concentration to 10^{-3} M resulted in a more significant decrease in absolute bioluminescence values (I) and in a somewhat lesser reduction in relative bioluminescence (F_i, Table 2).

Analysis of the data obtained demonstrates that the influence of bacterial autoregulatory d_1 factors belonging to the AHB group on the SOS response of *E. coli recA'::lux* cells to lethal UV irradiation was chiefly inhibitory. Nonetheless, both the absolute (lgCFU/mL) and relative (percentage of the control level Figs. 2c, 2d) parameters of bacterial cell viability were significantly higher with AHB-pretreated cells than with control cells that were not treated with AHB. Taken together, the data obtained suggest that previous contact of bacterial cells with AHB results in their preadaptation, which significantly increases their resistance to lethal-intensity stress without involving the activation of the SOS repair system.

Another factor contributing to the protective effect of AHBs is their influence on the genotype stability, which can be estimated from the frequency of S $\longrightarrow$ R phase transitions in a bacterial population. If it remains at the background level, this implies genotype stability, and an increase in phenotypic variability is interpreted as a manifestation of adaptive variability [21, 22]. As shown above, AHBs without additional stress factors increased the S $\longrightarrow$ R transition frequency with the formation of a sufficiently large number of colonies with the intermediate RS phenotype, which confirms the role of AHBs in controlling the intrapopulation variability [6, 17]. The population variability index in the presence of 10^{-4} M AHB was maximum with C7- and C11-AHB (Fig. 3a), which yielded the values of Shannon's entropy test of 1.16 and 1.27, in contrast to 0.4 in the control system. UV radiation also induced transition from the dominant S type to the R type, but no intermediate RS phenotype

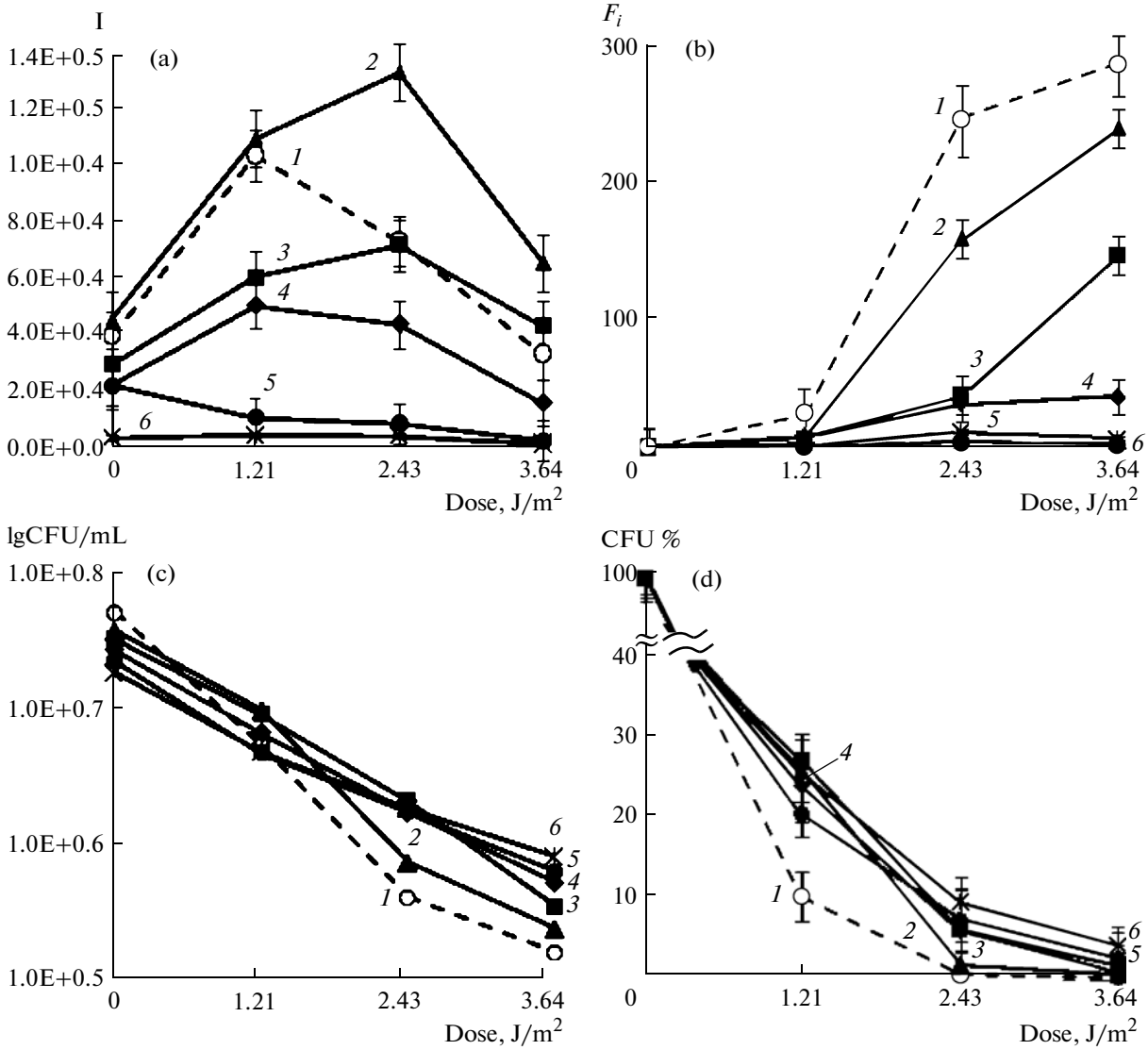


Fig. 2. Patterns of the responses of the strain *E. coli recA'::lux* to UV irradiation after pretreatment with AHBs (10^{-4} M): control (*1*), C7-AHB (*2*), C9-AHB (*3*), C11-AHB (*4*), C12-AHB (*5*), and C18-AHB (*6*). Designations: horizontal axis—radiation dose, J/m^2 vertical axis—bioluminescence intensity (a), SOS response-inducing factor (b), absolute (c) and relative (d) viable cell number.

was generated in this case, and the phenotypic variability index was 1.0 (Fig. 3b). Against this background, research on phenotypic phase variation in *E. coli recA'::lux* preincubated with AHB (10^{-4} M) and thereupon treated with an UV radiation (3.64 J/m^2) revealed a significant increase in the population phenotypic variability index within the viable population part (Fig. 3b). In particular, the highest phase variation index values occurred with the cells preincubated with the long-chain C12- and C18-AHBs. The Shannon's test values were 1.35 and 1.29, respectively. As for the variant with C9-AHB, the R type cells were completely replaced by RS type cells.

The results obtained indicate that the AHB-dependent mechanism of cell protection from lethal UV radiation doses implicating a relative suppression of the SOS system activity causes an increase in the frequency of intragenomic transitions. They increase the phenotypic variability of the viable part of the bacterial population.

DISCUSSION

Autoregulatory d_1 factors comprise various alkylhydroxybenzene isomers and homologues. They control the transition of bacterial cells to the dormant state and belong to the most evolutionarily conserved adaptogens [2] that were inherited by plants and fungi [23]. These low-molecular-weight compounds exhibit amphiphilic properties, antioxidant activity, and the

Table 2. Effect of alkylhydroxybenzenes on the relative indices of SOS system induction and the viability of *E. coli recA'::lux* upon UV irradiation (3.64 J/m^2)

Tested compound, concentration (M)	SOS system induction factor (F_i)	Remaining CFU number (% of the control)
C7-AHB, $10^{-5}/10^{-4}/10^{-3}$	238.3 ± 21.1 234.9 ± 21.0 92.15 ± 9.1*	0.39 ± 0.03 0.62 ± 0.06 0.96 ± 0.1*
C9-AHB, $10^{-5}/10^{-4}/10^{-3}$	250.0 ± 23.3 141.9 ± 13.3* 57.43 ± 5.5**	0.53 ± 0.04 1.03 ± 0.05* 1.5 ± 0.11**
C11-AHB, $10^{-5}/10^{-4}/10^{-3}$	112.0 ± 10.1 38.3 ± 3.5** 51.2 ± 4.8**	0.83 ± 0.04* 1.87 ± 0.1** 2.17 ± 0.05**
C12-AHB, $10^{-5}/10^{-4}/10^{-3}$	65.2 ± 5.0** 3.7 ± 0.3** 18.9 ± 1.7**	1.31 ± 0.05** 2.73 ± 0.2** 5.95 ± 0.43***
C18-AHB, $10^{-5}/10^{-4}/10^{-3}$	54.3 ± 5.0** 7.0 ± 0.6** 13.7 ± 1.4**	1.96 ± 0.12** 4.32 ± 0.15** 9.36 ± 0.8***
Control	282.8 ± 26.5	0.3 ± 0.07

Designations: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

capacity for nonspecific physical and chemical interactions with a wide range of biopolymers [7–11] and membrane lipids [24]. This accounts for the acquisition of enhanced resistance to diverse extreme environmental factors by these structures and whole cells [3]. Particularly extensive data on such protective activities were obtained with respect to the resistance of bacterial and yeast cells to UV and ionizing radiation [4, 6, 9] which represents an important abiotic factor in both ancient and modern ecosystems.

Another, mechanism of resistance to radiation (probably a more recent one) involves repair systems [13] that operate in the vegetative cells and actively undo the resulting damage to the genetic machinery.

Of special interest in the light of the above is unraveling the pattern of the interaction between these stress response systems. This was earlier researched in several in vitro [7–9] and in vivo [3–6] models and investigated in this work using the recombinant strain *E. coli recA'::lux*. A distinctive feature of this genetic system that facilitates attaining our goal is its capacity to respond to UV irradiation-caused DNA impairments with bioluminescence. This enables monitoring the induction (activation) of the SOS system in real time [14].

The data obtained (Figs. 2c, 2d) confirmed the earlier findings that alkylhydroxybenzenes function as adaptogens conferring enhanced resistance to environmental stress factors on bacterial and fungal cells [2, 3]. Using a novel model for investigation of the protective responses of a bacterial population under the deleterious influence of UV radiation enabled us to investigate in detail the patterns of stress response formation and to obtain data on new aspects of the adaptogenic activity of AHBs. Methodological prerequisites for our novel model included (i) the employment of an early exponential growth phase *E. coli recA'::lux* culture whose cells display the highest sensitivity to stress factors; (ii) prolonged UV irradiation that made it possible both to produce a cumulative deleterious effect enabling us to evaluate the impact of the tested

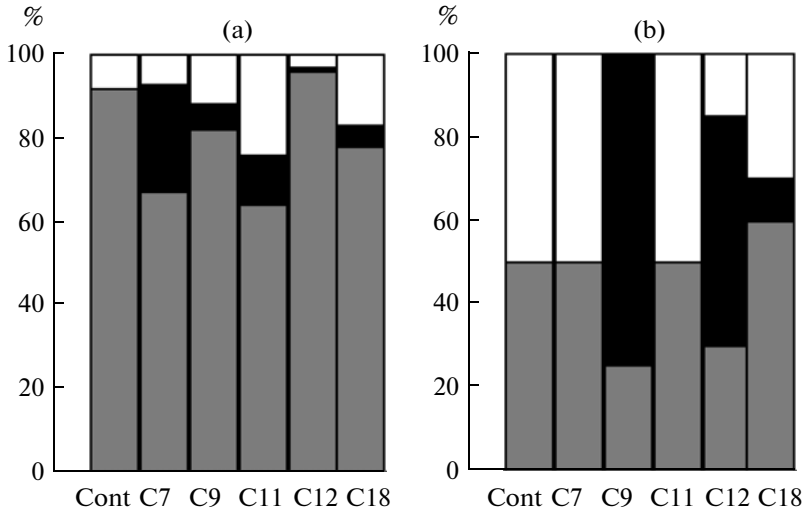


Fig. 3. Phase variation of the *E. coli recA'::lux* culture incubated for 1 h with AHB at a concentration of 10^{-4} M (a) and subsequently treated with an UV radiation dose of 3.64 J/m^2 (b). Designations: grey sectors, S type colonies' black sectors, RS type colonies; white sectors, R type sectors.

radiation dose and to analyze the dynamics of the stress response; and (iii) the monitoring of a combination of stress response-related variables at the molecular (SOS system activation), cell (proliferative activity retention), and population (changes in the phase variation spectrum of the population) levels.

Using the model developed in the present work, we consecutively investigated the stress responses of *E. coli* to the lethal influence of various UV radiation doses, to an increase in the alkylhydroxybenzene level in the culture, and to the combined effect of AHBs and subsequent lethal level of UV irradiation.

The main conclusion drawn from the first part of this work was that the test microorganism's responses to a natural stress factor (UV radiation) and to an increase in the long-chain AHB level in the culture were uniform. By assessment of the absolute and relative viability of *E. coli recA'::lux* cells, we revealed new patterns in the development of stress responses to consecutive treatment with AHBs and lethal doses of UV radiation. Lethal effects involving the death of the major part of the population (Fig. 1b) against the background of a decrease in absolute bioluminescence intensity (Fig. 1a) were established to result in an increase in the relative value of the SOS response induction factor (F_i) that was inversely proportional to the viable cell number (Fig. 1c). A similar dynamics of the stress response occurred upon increasing the concentration of long-chain AHBs in the culture; at 10^{-5} M, AHBs caused a 2–5-fold increase in the F_i value compared to the background level. This enables us to expand on our earlier concept that AHBs function as alarmones by applying it, apart from sublethal factors [6], to lethal doses of stress factors.

The results obtained also indicate that the effect of long-chain AHB is dose-dependent in terms of stress responses. With AHB concentrations exceeding 10^{-5} M, a parallel decrease in both absolute CFU number and total bioluminescence level occurred. This could be due to the fact established earlier that long-chain AHBs operate as anabiosis autoinducers. Increasing their concentrations results in the development of the hypometabolic (10^{-4} M) and then ametabolic (10^{-3} M) cell state [2, 18, 25]. This accounts for a decrease in the SOS system activity that was caused by a reduction in the general metabolic activity determined from the decrease in F_i value at AHB concentrations over 10^{-5} M. Interestingly, the short-chain C7-AHB that does not affect the development of the anabiotic state failed to induce the SOS response of the test strain, regardless of the concentration applied. This is in line with the data obtained earlier with other model organisms [6].

These results provided the foundations for our studies on the AHB effects in relation to bacterial preadaptation to the lethal influence of UV irradiation. Relative cell viability data (as CFU percentages) provided compelling evidence for a protective effect of AHBs during long-term exposure to a lethal-intensity stress factor. The magnitude of the effect significantly varied depending on the chemical structure of AHBs and their concentrations. If AHBs were added at a concentration of 10^{-4} M (Fig. 2c), their protective effect monotonously increased in the C7-AHB $\longrightarrow$ C18-AHB sequence, being 2–20 times higher than the control values at the UV dose of 2.43 to 3.64 J/m^2. Decreasing the tested AHB concentrations to 10^{-5} M or increasing them to 10^{-3} M entailed an approximately proportional decrease or increase in protective effect, respectively (Table 2). This confirms the dose-dependent adaptogenic action of AHBs.

Against this background, the results of calculating the SOS response induction factor appear to be somewhat discordant. They testify to a significant decrease in SOS system activation level in AHB-preincubated cells. However, this contradiction is eliminated by taking account of the pleiotropic effect of AHBs that involves a complex mechanism of regulating the resistance of bacterial cells to UV radiation. This mechanism implicates the influence of AHBs on the complementary and, to some extent, alternative "passive" and "active" systems of protecting cell biopolymers, especially the DNA, from harmful factors. The "active" protection is accomplished via the modulation of the expression of stress genes including the SOS system [6, 17]. Direct AHB–DNA interactions that result in changing the physical and chemical properties of the DNA and in developing resistance to a wide range of stress factors with different intensity underlies the "passive" protection system [8, 9]. The results obtained give us grounds for the suggestion that such a "passive" mechanism can secure direct DNA protection from UV radiation, resulting in lowering the activation level of the SOS system responsible for the "active" mechanism of protecting the DNA. An additional contribution to the modulation of the SOS system activity can be made by AHB effects at the transcriptional and post-translational level. As a result, the viability of the bacterial cells that assume the hypometabolic state can be retained with minimal repair activity. Of paramount importance is the fact that the preadaptive AHB effects provide for significantly higher relative and, with high UV radiation intensities, absolute numbers of viable *E. coli recA'::lux* cells than in the control system where the structural and functional integrity of the DNA is secured by the SOS system alone.

Taken together, the results obtained make it possible to regard alkylhydroxybenzene-controlled "active" and "passive" DNA protection systems as complementary. They are aimed at securing the existence of a bacterial population at various UV radiation intensities. Under stress, the biosynthetic activity (productivity) of the cells with respect to AHBs is substantially increased [10], enabling them to function as adaptogens. Long-chain AHBs activate SOS regulon

expression [6, 17], which provides for a sufficiently high repair activity level at low and medium UV radiation intensities. This secures the survival of a considerable part of a bacterial population and the maintenance of its relative geno- and phenotypic homogeneity. A prerequisite for the AHB action in the "passive" protection system is an additional rise in AHB concentration upon increasing the deleterious factor's dose to the lethal level. This is due to the extrusion of AHBs from the dying cells of the population, which was simulated in this work by increasing the AHB concentration added to the test culture. The resulting rise in the AHB level brings about a pronounced inhibition of metabolic and biochemical processes including the SOS response, causing the development of the hypometabolic and, subsequently, of the anabiotic state [18]. The extreme manifestation of this effect is a loss of bacterial cells' capacity to form colonies on solid media, i.e. the development of the nonculturable state [25]. However, this is accompanied by the processes resulting in AHB-dependent stabilization of cell biopolymers including the DNA, which become highly resistant to a wide variety of stress factors, including UV irradiation. The cells that acquire a metabolically inactive state with the SOS system repressed because of the AHB pretreatment are characterized, therefore, by a considerably higher viability upon lethal UV irradiation. This manifests itself in an increase in both relative and absolute numbers of the colony formation-capable cells that exhibit an enhanced capacity for phase variation. Importantly, there is a direct relationship between protection efficiency and the chemical structure of the AHB and its concentration, which coincide with those required for blocking the metabolism of a bacterial cell [2–6, 18, 19].

Taken together, the results obtained enable us to construe the AHB-controlled mechanisms of general stress resistance of bacteria and SOS repair of the DNA as the alternative "passive" and "active" systems that are both aimed at securing the survival of bacterial populations at various UV irradiation intensities. An additional AHB-dependent protective effect is an increase in the subsequent phase variation of the surviving part of the bacterial population. As a result, the species potentially has a large number of ecological options after surviving extremely intense UV irradiation.

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