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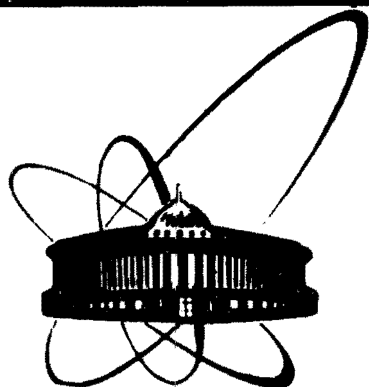

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THE INDUCTION OF SOS-RESPONSE IN ESCHERICHIA COLI BY HEAVY IONS

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The SOS-response affects a number of properties in bacterial cells such as prophage induction (Lwoff et al., 1950), ultraviolet light reactivation and mutagenesis (Weigle,1953, Defais et al.,1971), error-prone mutagenesis (Witkin,1974), radioresistance (Pollard and Achey,1975), etc. The induction of the SOS-system which produces these phenomena can be achieved by a variety of agents or treatments: uv, mitomycin C, thymine starvation, nalidixic acid, as well as by ionizing radiation. No information, however, has been obtained on the induction of the SOS-system by heavy ions although this information would be of great value for the investigation of mechanisms of the phenomena mentioned above, particularly, in the case of mutagenesis.

Heavy charged particles are known to induce different spectra of DNA injuries (Christensen et al.,1972; Munson and Bridges, 1973; Kozubek and Krasavin,1984). The increasing linear energy transfer (LET) of the ionizing radiation leads to decreasing production of DNA single strand breaks (SSB) and base damages. On the other hand, the production of DNA double strand breaks (DSB) and multiply damaged sites is increased. It can be expected that the signal for the SOS-induction in the case of ionizing radiation is also some type of DNA damages. The efficiency of heavy ions to induce the SOS-system can give us the information on the nature of these injuries and their genesis.

The efficiency of the SOS-system induction has been assessed according to the result of the SOS - chromotest - a new quantitative bacterial colorimetric assay for genotoxins (Quillardet et al.,1982). The strain with an operon fusion

placing lacZ, the structural gene for β-galactosidase under control of the sulA gene which is itself SOS-controlled, has been used. The level of β-galactosidase activity in cell suspension after irradiation has reflected the intensity of SOS-response.

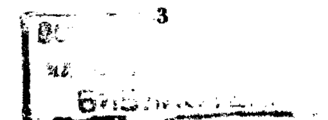
Materials and methods

Bacterial strain

Escherichia coli PQ37 (Quillardet et al.,1982) was kindly supplied by Dr.M.Hofnung, Institut Pasteur. This strain carries a sulA::lacZ fusion and has a deletion for the normal lac region so that β-galactosidase activity is strictly dependent on sulA expression. The strain is constitutive in alkaline phosphatase synthesis, which can be used for monitoring general protein synthesis. The strain has been described in detail (Quillardet et al.,1982) as well as the procedures of its construction.

Media

Cultures were grown overnight at 37°C in L-medium (Bacto tryptone 10g, Bacto yeast extract 5g, NaCl 10g/l of distilled water) supplemented with 20 μg/ml ampicilin. The suspension was diluted 1:20 into a fresh medium and incubated at 37°C with shaking up to 2×10^8 bacteria/ml. The suspension was distributed onto pieces of peptone agar (Gamaleya Institute of Microbiology, Moscow), irradiated by heavy ions, washed at 1ml of L-medium and incubated with shaking for 2 hours at 37°C. After incubation the suspension was assayed for β-galactosidase and alkaline phosphatase. The procedure is described in (Quillardet et al.,1982). Briefly, β-galactosidase assay: 0.9ml of Z-buffer (Na_2HPO_4 16.1g, $NaH_2PO_4.H_2O$ 5.5g, KCl 0.75g, $MgSO_4.7H_2O$ 0.25g, sodium dodecylsulfat 1g, β-mercaptoethanol 2.7ml/l distilled water, adjusted to pH7), 0.2ml of ONPG solution 4mg/ml of phosphate buffer (61ml Na_2HPO_4. $7H_2O$ 0.1M and 39ml NaH_2PO_4.



H_2O 0.1M), and 0.1ml cell suspension was the reaction mixture; the reaction was terminated after 30-40 min by adding 0.65ml 1M Na_2CO_3 solution; alkaline phosphatase assay: 0.9ml of T-buffer (tris hydroxymethylaminomethane 121g, sodium dodecylsulfat 1g/l distilled water, adjusted to pH8.8 with HCl), 0.2ml PNPP solution 4mg/ml of T-buffer, and 0.1ml cell suspension; the reaction was terminated by adding 0.4ml 2N HCl and in about 5min the pH was changed again by adding 0.4ml 2N tris.

Irradiation by γ-rays and heavy ions

The ^{60}Co and ^{137}Cs gamma ray sources giving dose rates of about 3 Gy/min and 22Gy/min, respectively, were used. The irradiation by heavy particles was performed at the accelerator of heavy ions U-200, Joint Institute for Nuclear Research. Agar pieces with cell suspension on the surface were placed on sterile metal plates, inserted in a round magazine, and transported by remote control to the beam exit window. The exposure of various biological samples was monitored by an ionization chamber set between the exit window and the biological sample (Cherevatenko,1986). Physical characteristics of the particles used in our experiments are shown in Table 1.

Table 1. Physical characteristics of the radiation beams

Type of radiation	Energy MeV/nucl.	LET keV/μm	Dose rate Gy/min	Source
γ-rays	-	0.2	3	^{60}Co
	-	0.3	22	^{137}Cs
$^{4}_{2}He$	8.0	22	20	U-200
	3.9	40	-"-	-"-
	2.2	60	-"-	-"-
$^{9}_{4}Be$	6.5	117	-"-	-"-
	3.2	200	-"-	-"-
$^{12}_{6}C$	7.5	210	-"-	-"-
	4.0	330	-"-	-"-

SOS-induction potency

The extinction was measured for β-galactosidase (β) and alkaline phosphatase in the samples irradiated with various doses (D). The measurements were done against blank sample which was neither irradiated nor incubated (it was kept on ice). The quantity which reflects the efficiency of SOS-system induction is SOSIP (SOS-induction potency) defined as (Quillardet et al.,1982):

$$SOSIP = \frac{d\ IF(D)}{d\ D}\bigg|_{D=0} ,$$

where $IF=K_\beta/K_p$; $K_\beta=\beta(D)/\beta(0)$ and $K_p=p(D)/p(0)$. IF is the induction factor and SOSIP represents the initial slope of the IF(D) dependence.

Results

β(D) and p(D) dependences for γ-rays, helium ions (60 keV/μm), and carbon ions (330 keV/μm) are shown in Fig.1. Other ions displayed the same character of β(D) and p(D) dependences. The activity of β-galactosidase increased with the dose to some maximum value, similar for different types of ionizing particles. The slope of the initial linear region somewhat increases for greater LET, passes a maximum and falls down again. On the other hand, p(D) dependences are continuously falling to approximately one third of its initial value. The initial slope is negative and steeper for greater LET.

The initial regions of IF(D) dependences are linear (Fig.2). The greatest slope was obtained for helium and berylium ions, the smallest for carbon ions with LET=330 keV/μm. The γ-irradiation was performed both on the surface of agar and in suspension. The efficiency is somewhat greater when the samples were irradiated on the surface of agar. Fig.3 shows the dependence of SOSIP on LET. Maximum SOSIP values were obtained

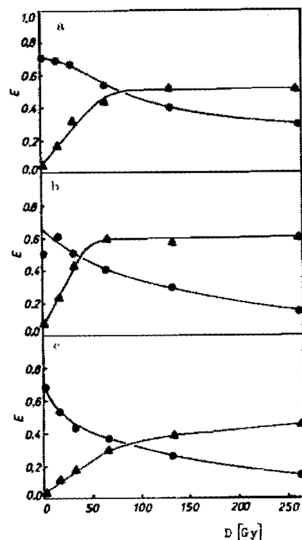


Fig.1. Examples of the dose-response curves for the β-galactosidase activity, $E=\beta(D)$, (▲) and alkaline phosphatase activity, $E=p(D)$, (●) measured in the cell suspension of E.coli PQ37 irradiated by γ-rays (a), helium ions with LET=60 keV/μm (b) and carbon ions with LET=330 =330 keV/μm.

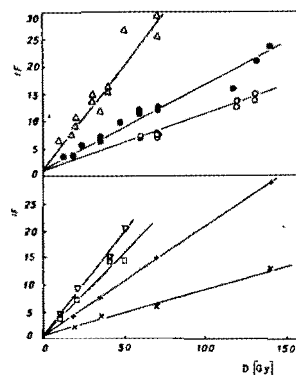


Fig.2. The dose dependence of the induction factor (IF). (o) - γ-rays, irradiation in suspension; (●) - γ-rays, irradiation on the surface of agar; (Δ) - helium ions, LET=22, 40, and 60 keV/μm; (∇) - beryllium ions, LET = 117 keV/μm; (□) - beryllium ions LET = 200 keV/μm; (+) - carbon ions, LET = 210 keV/μm; (x) - carbon ions, LET = 330 keV/μm.

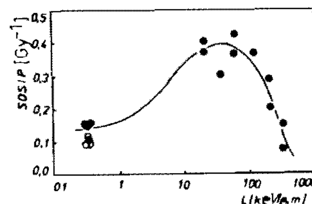


Fig.3. The LET dependence of SOS induction potency (SOSIP), (o) - irradiation in suspension; (●) - irradiation on the surface of agar.

at LET=40-60 keV/μm. Greater LET led to lower efficiency of ionizing radiation to induce SOS-system. The maximum value is SOSIP=0.4 Gy^{-1}, which is approximately 2.5 times greater than the value obtained for γ-radiation under equal conditions.

Discussion

The values of SOSIP obtained according to the standard protocol described by (Quillardet et al.,1982) are markedly greater for intermediate LET values (40-60 keV/μm) than for γ-radiation. This is the result of two independent effects: a) the initial slopes of $\beta(D)$ dependences are increasing in this region of LET, b) the initial slopes of $p(D)$ dependences are decreasing (IF is inversely proportional to $p(D)$).

The assay of alkaline phosphatase activity was proposed to estimate general protein synthesis during the incubation period (Quillardet et al.,1982). However, our kinetic studies (data not shown) showed that the activity of alkaline phosphatase in cell suspension increases exponentially with time of incubation (up to 3 hours) with doubling time corresponding to the growth of the cell population. Irradiation inhibits the growth of the cell population and leads, therefore, to lower values of alkaline phosphatase activity. The dependence $p(D)$ can be approxieately described on the assumption that each cell can produce a constant amount of alkaline phosphatase per time unit, however, surviving cells can pass several divisions during the incubation period meanwhile "killed" cells do not divide.

During the standard incubation period the number of intact cells in the L-medium increases approxieately 8-times. The area under the growth curve which is proportional to the alkaline phosphatase activity decreases 3-times with increasing dose. This explaines the $p(D)$ dependence that reflects to some extent the survival curve $S(D)$. The hypothesis mentioned above leads to the following relation between the two functions:

$$p(D) \approx \left([1-S(D)] + S(D).\frac{\exp(\beta.t) - 1}{\beta.t} \right), \qquad (1)$$

there t is the time of incubation, β is the growth rate. Our experimental data (to be published) on cell survival actually well correspond to the Eq.1.

For this reason the values of SOSIP do not properly reflect SOS-induction. The dependence SOSIP(LET) includes the changes of the cell survival curve with increasing LET.

On the other hand, the induction of β-galactosidase itself is also more efficient for intermediate LET values. The relative biological effectiveness calculated from the initial slopes of $\beta(D)/\beta(0)$ dependences amounts to 1.6 for helium and berylium ions. The enhancement of the efficiency of heavy ions to induce β-galactosidase synthesis in PQ37 cells can be explained as the consequence of the increased production of DNA damages serving as a signal for the SOS-system induction. It means that more cells are induced at the given dose by heavy ions (with LET=40-60 keV/μm) in comparison with γ-rays. Alternative explanation is that the extent of SOS-induction is the same in all cells but increasing dose or LET leads to greater intensity (activity of induced proteins) of SOS-response. In this case, however, the response at greater doses $\beta(D \to \infty)$ should grow gradually with increasing LET even for carbon ions. Experimental results showed no marked change of the final level of β-galactosidase activity at greater doses.

Therefore, the results cannot be interpreted like that there is more repair in cells irradiated by heavy ions. The extent of SOS-response is probably the same in each individual cell independently of the type of ionizing radiation, only the number of induced cells differ.

The production of SOS-signal damages of DNA by γ-radiation is 0.02 $Gy^{-1}.genome^{-1}$ and increases to 0.03 for helium ions. The production of these damages by heavy ions with greater LET should be calculated on considering specific energy fluctuations (some part of cell population is not hit by a particle).

The dose dependence of any measured quantity is modified as a result of the fluctuations of specific energy absorbed in a cell nucleus. Such a transformation of a function $G(D)=1-\exp(-0.01*D)$ with increasing LET is shown in Fig.4. Changes of the initial slope of the above mentioned function depend on the diameter of the sensitive volume. The diameter equals 0.7 μm in Fig.4 and corresponds to our bacterial cell diameter. Taking into account the energy fluctuations we can estimate the production of SOS-signal damages of DNA by carbon ions (220 keV/μm) to 0.3 Gy^{-1}.

Greater production of SOS-inducing DNA damages by heavy ions suggests that they are formed after deposition of a substantial portion of energy inside DNA or near it. The possible candidates could be "complex" DNA injuries proposed by Bridges and Mottershead (Bridges and Mottershead, 1972).

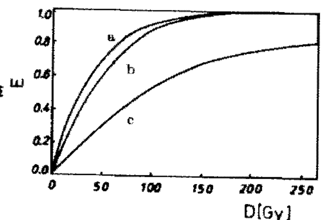


Fig.4. The influence of the fluctuations of specific energy absorbed in the cell sensitive volume on the dose response curve $E(D) = 1-\exp(0.01 \times D)$. The dependence is shown for γ-rays (a), for helium ions, LET = 60 keV/μm (b), and for carbon ions, LET = 330 keV/μm (c).

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Козубек С. и др. E19-88-867
Индукция SOS-системы у клеток
Escherichia coli тяжелыми ионами

Эффективность тяжелых ионов в индукции SOS-системы у клеток E.coli определяется методом SOS-хромотеста. Способность ионизирующих излучений к индукции SOS-системы с ростом ЛПЭ возрастает до значений ЛПЭ=40-60 кэВ/мкм, проходит через максимум и падает с дальнейшим ростом ЛПЭ. Можно сделать вывод о том, что индуцируемые радиацией повреждения, запускающие SOS-систему, являются повреждениями ДНК "комплексного типа".

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна 1988

Kozubek S. et al. E19-88-867
The Induction of SOS-Response
in Escherichia coli by Heavy Ions

The effectiveness of heavy ions to induce SOS-system of E.coli cells has been measured by means of SOS-chromotest. The SOS-induction potency of ionizing radiation increases as LET grows up to 40-60 keV/μm, passes maximum and falls down for greater LET values. The conclusion has been drawn that the SOS-inducing damages in the case of ionizing radiation could be DNA injuries of "complex" type.

The investigation has been performed at the Laboratory of Nuclear Problems, JINR.

Preprint of the Joint Institute for Nuclear Research. Dubna 1988