объединенный
институт
ядерных
исследований
дубна

К 81

E19-88-197

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THE ROLE OF DNA REPAIR PROCESSES IN THE BIOLOGICAL EFFICIENCY OF HEAVY IONS

Submitted to "Studia Biophysica"

1988

INTRODUCTION

The explanation of the relative biological efficiency (RBE) of heavy ions is of crucial importance in radiobiology owing to lack of our knowledge on the mechanisms governing the biological effects of radiation. The attempts to explain the values of RBE have been made mostly on considering differences in physical characteristics of ionizing radiation. However, there was evidence that RBE could also be influenced by the properties of cells, probably by the state of their repair systems[1]. In order to investigate the role of DNA repair processes in RBE(L) dependence, we have chosen bacterial cells Escherichia coli. The structural organisation of the E.coli genome and DNA repair processes have been thoroughly investigated earlier. Several repair systems could be distinguished and repair deficient mutants were derived. In our investigation we have used wild type cells, isogenic rec A, and superresistant mutants of E.coli. The rec A mutant is known to back the inducible repair of DNA[2], which leads to the formation of extensive gaps in DNA strands, being lethal events for this mutant[3-5]. Superresistant mutant is highly resistant to γ-radiation. Its higher resistance could be due to smaller induction rate, in comparison with wild type cells, of enzymatic DNA double strand breaks owing to the fact that the processes of DNA degradation and resynthesis are better coordinated[6]. Theoretical analysis of a possible role of DNA repair in the efficiency of high-LET radiation has been done[4,5]. The dependence of cell sensitivity (D_o^{-1}) on the linear energy transfer (L) of radiation could be interpeted in terms of both physical characteristics of ionizing radiation and biological factors such as DNA repair capability. This experimental work has confirmed our assumptions.

MATERIALS AND METHODS

Isogenic strains of E.coli K-12 were used: wild type - AB 1157, sensitive mutant rec A13 - AB 2463, and superresistant mutant Gam^r 444. Superresistant mutant Gam^r 444 was derived by V.L.Kalinin et al. at the Leningrad Institute of Nuclear Research of the Academy of Sciences of the USSR. The bac-

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terial cells were cultivated to the stationary phase of growth (concentration 2-3·10^8 cells per ml) in YEP medium (pepton - 10 g, yeast extract - 10 g, NaCl - 10 g per liter). After cultivation the cells were washed out by centrifugation and resuspended in M9 buffer. Then they were irradiated on the surface of agar. In comparative experiments the cells were irradiated both on the surface of agar and in flasks (in volumes of about 10 ml). There were no differences in cell sensitivities.

We have used electromagnetic types of radiation from two sources ^{137}Cs with a dose rate 0.58 Gy/s and unfiltered X-ray (apparatus RUP-200, voltage 200 kV, current 14 mA) with the same dose rate. The irradiation by heavy particles was performed on the accelerator of heavy ions U-200 at the Joint Institute for Nuclear Research on a specially designed apparatus controlled by electronics. Beam monitoring and dosimetry was performed by means of a calibrated air ionizing chamber. Physical characteristics used in our experiments are shown in Table 1.

Table 1. Physical characteristics of the radiation used

Type of radiation	Energy MeV/nucleon	LET keV/μm	Dose rate Gy/s	Source
γ-rays	-	0.3	0.58	^{137}Cs
x-rays	-	2.5	0.58	RUP-200
α-particles	-	110	0.35	^{139}Pu
^{4}He	8.0	22	1.5	U-200
^{4}He	3.5	42	1.5	U-200
^{4}He	2.0	63	1.5	U-200
^{12}C	7.5	205	1.5	U-200
^{12}C	4.0	333	1.5	U-200
^{12}C	3.0	410	1.5	U-200

The results of our experiments were statistically analysed. In order to obtain the cell sensitivity, a multitarget-single hit formula was fitted to the experimental data weighted according to their possible statistical and dilution errors. The sum of squares was minimized by computer procedure.

RESULTS AND DISCUSSION

Servival curves of E.coli wild type, rec A , and Gam^r 444 strains for different types of radiation are shown in Fig.1b, a, and c, respectively. The sensitivities calculated from the final slopes of the survival curves, the values of RBE, and

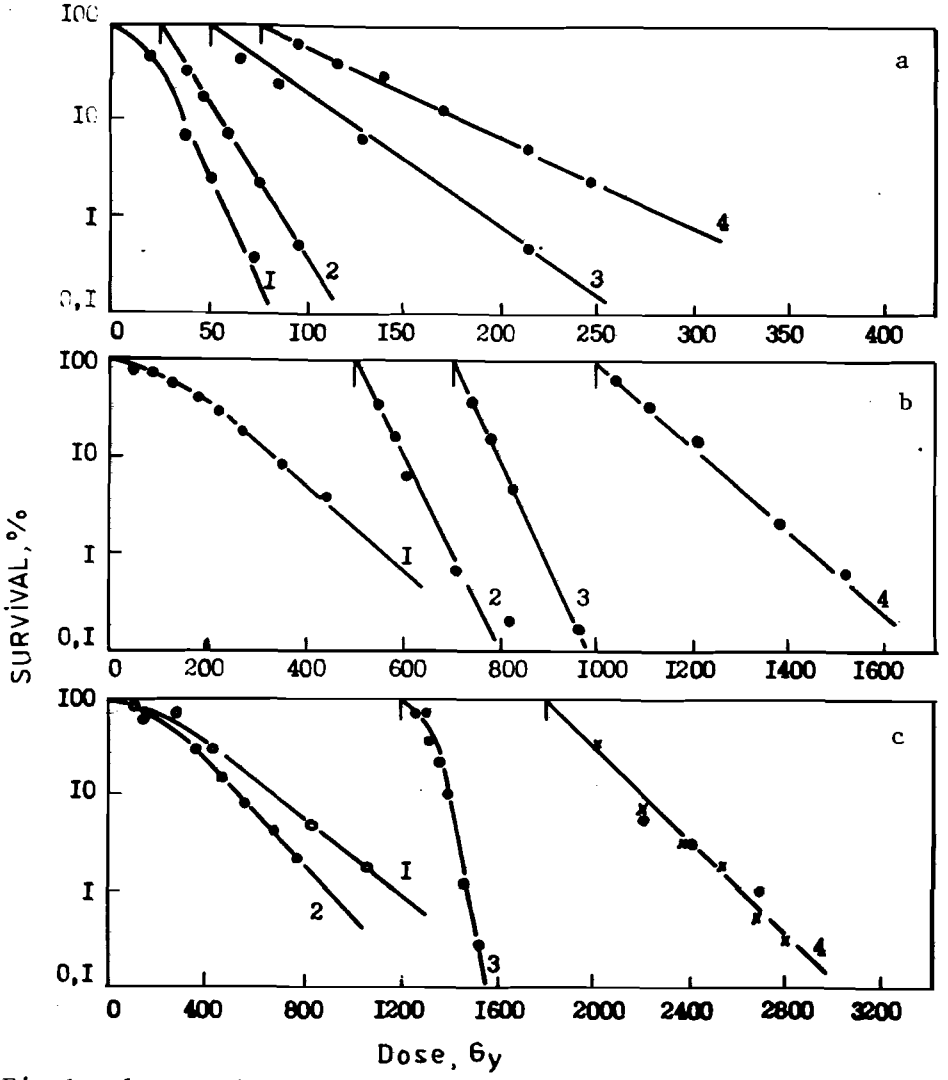

Fig.1. The survival curves of wild type (b), rec A mutant (a), and Gam^r 444 mutant (c), for various types of radiation: (1) γ-radiation, (2) ^{4}He-ions, (3) α-particles, (4) ^{12}C-ions. (Ordinate: cell survival (%), Abscissa: dose (Gy).

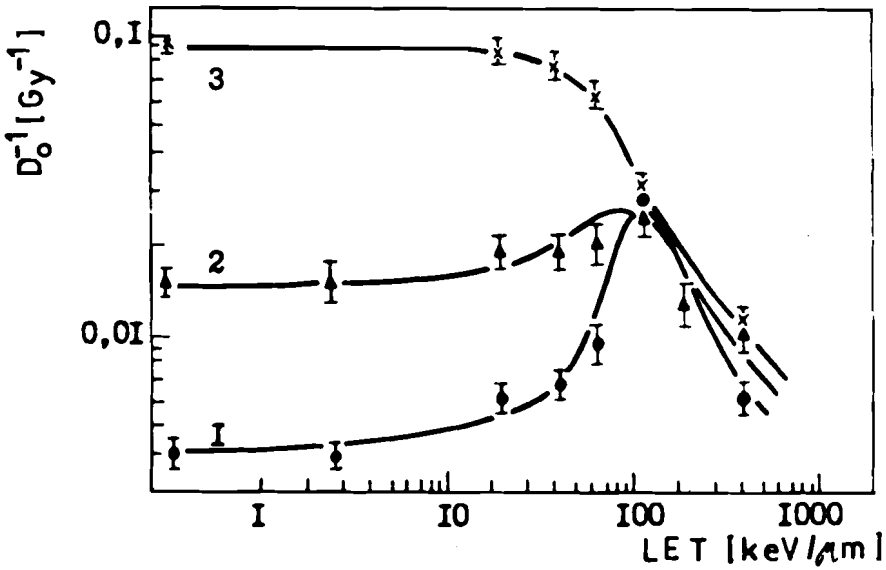

Fig.2. The dependence of radiosensitivities of rec A mutant (1) wild type (2), and Gam^r 444 mutant (3) on LET. Ordinate: radiosensitivity (Gy^{-1}), abscissa: LET (keV/μm).

dose-modifying factors (DMF) are summarized in Table 2. The dependences of cell sensitivities on LET for the three E.coli strains are shown in Fig.2.

The sensitivity to γ-radiation markedly differs in various E.coli strains. Rec A mutant is 10 times more sensitive than wild type cells and 20 times more sensitive than superresistant mutant. According to our interpretation[4,5], these differences are caused by repair peculiarities of the strains. In the case of rec A mutant there exists only pol A-dependent repair[7]. It repairs a great part of single strand breaks (SSB) of DNA induced directly by radiation or arisen in the process of endo-nuclease incision from ferment labile sites. Some part of DNA SSB is affected by exonucleases (which is connected with degradation not restricted by Rec A protein[8]). Therefore, the degradation leads to disappearance of DNA being the lethal event, of course.

Increasing LET influences the sensitivity of rec A mutant in two ways. Firstly, the number of SSB induced decreases with increasing LET owing to more localized energy depositions in tracks of heavy particles. In DNA strands are more energy departed than needed for the induction of SSB. E.g. if L = = 100 keV/μm = 100 eV/nm, then the energy departed per one SSB

Table 2. The sensitivities of different E.coli strains to various types of radiation

Strains	Type of radiation	Energy MeV/nucl.	LET keV/μm	D_0^{-1} 10^{-2}, Gy^{-1}	DMF*	RBE
AB 2463	γ-rays	-	0.3	9.97±0.57	0.17±0.08	1
	^{4}He	8.0	22	8.81±0.14	0.22±0.09	0.88±0.04
	^{4}He	3.5	42	7.87±0.19	0.25±0.01	0.79±0.04
	^{4}He	2.0	63	6.17±0.60	0.32±0.02	0.62±0.06
	α-particles	-	110	3.20±0.08	0.78±0.04	0.32±0.02
	^{12}C	3.0	410	1.17±0.11	0.99±0.10	0.12±0.01
AB 1157	γ-rays	-	0.3	1.71±0.04	1	1
	X-rays	-	2.5	1.69±0.13	1	1
	^{4}He	8.0	22	1.98±0.07	1	1.16±0.04
	^{4}He	3.5	42	1.97±0.06	1	1.15±0.03
	^{4}He	2.0	63	2.00±0.06	1	1.17±0.04
	α-particles	-	110	2.50±0.06	1	1.46±0.04
	^{12}C	6.5	215	1.29±0.03	1	0.75±0.02
	^{12}C	3.0	410	1.16±0.09	1	0.68±0.06
Gam^r 444	γ-rays	-	0.3	0.40±0.03	4.27±0.32	1
	X-rays	-	2.5	0.37±0.05	4.56±0.59	0.93 0.12
	^{4}He	8.0	22	0.57±0.01	3.47±0.18	1.42 0.10
	^{4}He	3.5	42	0.63±0.01	3.13±0.19	1.57 0.12
	^{4}He	2.0	63	0.83±0.02	2.06±0.10	2.08 0.15
	α-particles	-	110	2.70±0.27	0.93±0.06	6.75 0.51
	^{12}C	3.0	410	0.59±0.09	1.96±0.29	1.47 0.11

*$DMF = D_0^{-1}$ (wild type)/D_0^{-1} (mutant)

is approximately 2 time greater than in the case of γ-radiation. In fact, this effect appears already for smaller values of LET.

Secondly, the energy departed in the cell sensitive structure by one particle becomes with increasing LET greater than in the mean lethal energy in the case of γ-radiation.On the other hand,there is no energy deposited in other cells.These energy fluctuations are well known as the "overkill effect" or "saturation effect" as the cross-section for cell inactivation calculated from cell sensitivity approaches geometrical dimensions of the cell nucleoid.

Both effects lead to continually decreasing sensitivity of rec A mutant with increasing LET. The induction of double strand breaks also influences somewhat the sensitivity of rec A mutant. The number of DSB induced by particles with LET in the region 100 keV/μm is according to our model[4,5] comparable with the number of SSB irrepairable by pol A-dependent repair - the main lethal injuries of DNA for rec A mutant. In this region of LET, however, one particle departs in a cell nucleoid energy sufficient for cell killing. The surviving cells are not hit; therefore, the spectrum of DNA injuries is not so important. Both above-mentioned effects are of physical nature - they can be explained as energy fluctuations at nanometric or micrometric scales. We can conclude that the dependence $D_o^{-1}(L)$ in the case of rec A mutant, being continuously decreasing, is determined by the physics of ionizing radiation and target geometry.

The dependence $D_o^{-1}(L)$ in the case of wild type and superresistant mutant cells in comparance to rec A mutant has a local maximum for LET ~ 100 keV/μm.

The phenomena described above are also included in the formation of the dependences $D_o^{-1}(L)$ of the second type typical of wild type cells and superresistant mutant cells. To the right of the local maximum all sensitivities are equal independently of the state of repair system.This can be understood because the geometry of cell targets is equal for the three used strains. The initial parts of the dependences $D_o^{-1}(L)$ are the lower, the greater is the capability of cells to repair DNA injuries. As can be seen in Fig.2 and Table 2 the sensitivities of wild type cells and Gam^r 444 mutant cells to γ-irradiation are $D_o^{-1} = 1.71 \cdot 10^{-2}$ Gy^{-1} and $D_o^{-1} = 0.40 \cdot 10^{-2}$ Gy^{-1}, respectively. In the case of E.coli cells DSB cannot be repaired and so one DSB of DNA appears to be a lethal event[9]. It was suggested that DSB induced by γ-radiation are mostly of enzymatic origin[9,10,5]. As mentioned above, the increased resistance of superresistant mutant has been explained by reduced induction rate of these enzymatic DNA DSB.

It was shown that enzymatic DSB of DNA can arise from SSB. Consequently, the production of this type of DNA injuries decreases with increasing LET of radiation. On the other hand, the production of DSB induced directly by ionizing radiation (direct DSB) is increased.

CONCLUSIONS

The dependence of RBE on LET cannot be interpreted in terms of physics only. The character of this dependence is determined by the efficiency of cell DNA repair systems influencing the induction rate of different DNA injuries leading to lethal events. The sensitivity of cells to γ-radiation can be modified by the genetic block of some repair or, on the other hand, by increasing their efficiency. The sensitivity to high-LET radiation is determined by physical characteristics of ionizing radiation, only.

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Received by Publishing Department
on March 24, 1988.