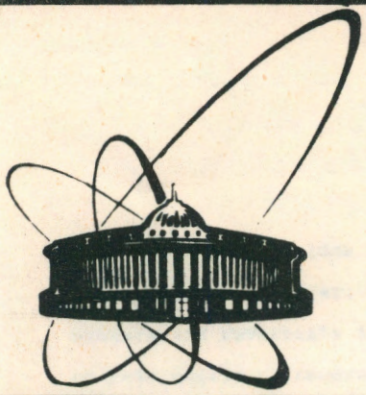

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MUTAGENIC ACTION OF HEAVY IONS ON ESCHERICHIA COLI CELLS

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The investigations of the mutagenic radiation action have been infrequent so far. The induced mutation rate of both direct mutants and revertants in bacterial cells were measured after x- or γ-irradiation (Demerec and Latarjet, 1946; Anderson,1951; Kelner et al., 1955; Bridges et al., 1968; Bridges and Mottershead, 1978; Glickman et al., 1980), The dose response dependences were both linear (Kada et al., 1960; Bridges et al., 1968; Demerec and Sams, 1960) and non-linear with a tendency to a quadratic function (Anderson, 1951, Zelle, 1955; Bridges and Mottershead, 1971; Bridges, Mottershead, 1978; Kato and Nakano, 1981, Glickman et al., 1980). Densely ionizing radiations were used in mutagenic experiments even more rarely. The first investigation with this type of radiation was performed with bacterial cells (Kriviskij, 1938). The efficiency of α-particles from radon to induce mutant cells increased supralinearly with the dose of radiation. Later on, however, in the experiments with heavy ions this observation was not confirmed (Deering, 1963). Linear dependences of the mutation rate on the dose of ionizing radiation were determined for mutations to prototrophy of three auxotrophic strains of E. coli; the effectiveness decreased steadily with increasing LET of protons and α-particles (Munson and Bridges, 1969). On the other hand, the effectiveness of heavy ions to induce mutants in extracellular phages had to be described by a function with a local maximum (Bresler et al., 1975). Owing to these contradictory results we have investigated the regularities of mutagenic action of

different types of ionizing radiation in a sufficiently broad region of LET. Direct mutations in lac operon have been used as a test system. The wild type strain as well as some repair deficient mutants of E. coli have been examined.

Materials and Methods

Bacterial strains.

The main part of experiments has been performed with wild type strain Hfr E. coli K-12 H. Pol A1 repair deficient mutant P3478 with its own wild type strain W3110 have been tested to check the role of DNA-polymerase I in the mutagenic process. For comparison, the lexA strain has been investigated.

Media

The cultures were grown overnight at $37^{o}C$ in a standard liquid medium (Gamaleya Institute of Microbiology, Moscow). The suspension was washed and resuspended in the M9-buffer (Miller, 1972). After irradiation and appropriate dilution the suspension was spread over plates with bottom agar and overlayered with TOP-agar. Standard agar (Gamaleya Institute of Microbiology, Moscow) was supplemented by 50 mg 2,3,5-triphenyltetrazolium chloride and 50 ml of 20% lactose per 1 of the selective medium.

Irradiation by γ-rays and heavy ions.

The ^{137}Cs gamma ray source giving the dose rate of about 23 Gy/min was used. The irradiation by heavy particles was performed at the accelerator of heavy ions U-200, Joint Institute for Nuclear Research. The cells were irradiated on the surface of Dacron filters (pore diameter 0,5 μm) placed on the surface of minimal agar (3% DIFCO agar) poured inside metallic

plates. The plates were set into a round magazine of the "GENOME" apparatus (Cherewatenko, 1986) and irradiated by accelerated ions of desired characteristics. The dose could be monitored by the ionization chamber set at the beam exit window.

Counting of mutant colonies.

Up to 20 000 microcolonies per plate could be checked. Higher concentrations lead to an erroneously decreased mutation rate. In parallel the number of all colonies was determined after the dilution 1:100 of the corresponding sample of the cell suspension which was spread on the surface of Petri plates. Counting of the mutant colonies was done under a slight magnification. The experiments were repeated 3-5 times.

Processing of experimental data.

The mutation rate was measured as the ratio of mutant colonies (N_m) to the number of survived cells (N). The dose dependence $N_m/N(D)$ has been fitted to the following functions:

$$N_m/N = k * D^{*}, \qquad (1a)$$

$$N_m/N = \alpha D + (\beta D)^2, \qquad (1b)$$

where D is the dose of radiation. The parameters $k,*,\alpha,\beta$ were determined by minimizing the sum of squares of the differences between the experimental and theoretical mutation rates calculated acccording to Eqs 1a,b. The squares were divided by standard deviations of the experimental points obtained as square roots of the total number of mutants. The standard deviations of the parameters were calculated as square roots of diagonal elements of an inverse covariance matrix (the matrix of the second derivatives of the sum of squares). The relative genetic effectiveness (RGE) can be determined as the ratio of

the doses inducing the same number of mutations, the dose of γ-rays (D_γ) and the dose of heavy ions (D_i): RGE = D_γ/D_i. In principle RGE can be a dose dependent quantity. However, in our experiments the character of $N_m/N(D)$ functions remained approximately the same for all types of ionizing radiations. The optimal values of RGE could be, therefore, calculated from the best fits of Eqs 1a,b, where the dose is replaced by RGE*D, to the experimental data on heavy ions with the parameters $k,\varkappa,\alpha,\beta$ taken the same as in the case of γ-radiation. The standard deviations of RGE were determined by the same procedure as the deviations of the parameters.

Results

The cell survival (S) and the mutation rate (N_m/N) of E. coli Hfr H cells as a function of the dose of γ-radiation (D) are presented in Fig.1. The survival curve has an exponential shape with the sensitivity (D_0^{-1}) equal to D_0^{-1} = 0,01 Gy^{-1}.

The mutation rate increases sharply with the dose. The linear component of Eq. 1b is much smaller than the quadratic one: $\alpha = (1{,}8 \pm 0{,}6)*10^{-6}$ Gy^{-1} and $\beta = (1{,}6 \pm 0{,}2)*10^{-4}$ Gy^{-1}. The dependence $N_m/N(D)$ is therefore very near to the quadratic one. If this is the case, the exponent $\varkappa$ from Eq. 1a should be close to 2. Actually, its value presented in Table 1., equals 1,92 $\pm$ 0,30.

In the experiments similar to the one in Fig.1. different types of ionizing radiations were used (Fig.2.). The survival curves were exponential in all cases. An increased sensitivity was observed for helium ions. The mutation rates increased with the dose according to the power function (close to the quadratic function). The values of the exponents $\varkappa$ from Eq. 1a are

presented in Table 1. The exponents do not differ from the value $\varkappa$ = 2 within one standard deviation. Therefore, the $N_m/N(D)$ dependences will be analysed as quadratic functions.

The data can be presented as linear functions in the log-log plot where the slope of the dependences equals $\varkappa$ from Eq. 1a. In the case of the quadratic function the slope is equal to $\varkappa$ = 2. The lines for heavy ions are shifted from the line corresponding to γ-radiation by the log value of RGE. Another simple analysis can be done in the $(1/D)*(N_m/N)$ vs D plot where the linear-quadratic function is represented by a straight line with the slope β^2 crossing the y-axis at the distance α. The slopes β^2 are listed in Table 1, α = 0 in all cases. The value of RGE can be calculated as the square root of the ratio of β^2 for heavy ions and γ-rays. The values of RGE calculated according to the optimizing procedure well corresponded to the values determined from the above mentioned plots; they are presented in Table 1.

The mutation rates were also measured for some repair deficient mutants. A quadratic dose-response dependence was observed in the polA strain (Fig.3.). Its mutation rates were higher than that in the wild type cells. The quadratic dependence was observed after irradiation by accelerated heavy ions, too. The most efficient were deuterium ions with LET = 7 KeV/μm. The value of RGE in this case is 3,2, which is more than the value 2,3 for the wild type strain.

In the experiments with the lexA strain irradiated by γ-rays low mutation rates and linear dose-response dependences $N_m/N(D)$ were observed. Similar dependences were obtained by action of heavy ions. The values of RGE were smaller than 1 in all experiments (Fig.4). The dose modifying factors (DMF) for

repair deficient mutants, the values of their sensitivity and RGE are shown in Table 2.

The LET dependences of RGE for the wild type, polA- and lexA-mutants are shown in Fig.5. The maximum RGE values for the wild type strain were obtained at LET = 20 KeV/μm. The same type of RGE(LET) dependence was found in the polA strain. However, the repair deficient lexA strain exhibits decreasing RGE values as LET grows.

Discussion

Dose-response dependences in wild type strains.

The results of our experiments enabled us to conclude that:

1/ The dose-response dependence in the wild type strain is close to the quadratic function without a maximum for doses of γ-radiation or heavy particles decreasing the survival to S = 10^{-5}.

2/ The character of the $N_m/N(D)$ dependence is quadratic for all types of ionizing radiation used in our experiments. This fact allows the evaluation of relative genetic effectiveness independently of the dose.

3/ The value of RGE increases with LET, passes the maximum and falls down again.

4/ The maximum appears at fairly low values of LET (20 KeV/μm).

The conclusive interpretation of the above mentioned facts cannot be done at present time. We shall try to explain them on considering current hypotheses. The quadratic dependence $N_m/N(D)$ was obtained by others authors, too (Bridges and Mottershead, 1971; Bridges, Mottershead, 1978; Kato and Nakano, 1981; Glickman et al., 1980). Generally, the quadratic dose-response dependence can be explained by a two-hit mechanism of the end-effect. The first hit is needed as a target, i.e. the site

in DNA where a change in the nucleotide sequence could arise. Such an injury is usually called a premutational DNA damage. remutational damages can be repaired or converted into mutational changes by mispairing (misreplication) or misrepair (Shinoura 1983). The second hit is required for the fixation of the first one. The fixation can be realized at a short distance as some kind of interaction between the two damages (the first hypothesis) or by means of induction of repair enzymes (the second hypothesis).

The former hypothesis (Bresler S.E., 1976) is supported by the relatively long patches arising in bacterial DNA after repair. The interaction can be realized as a consequence of DNA degradation of one DNA strand if the base injury was produced in the second DNA strand. The interaction distance is given by the length of the exonucleolytic gaps, which is about 10^3 nucleotides.

The induction of repair enzymes by a DNA injury is a well known phenomenon called SOS-induction and the SOS-repair is known as the error-prone repair (Witkin, 1976). If the SOS-repair is responsible for the quadratic dose dependence of the mutation rate, linear dependences $N_m/N(D)$ should be obtained in E. coli mutant strains with a constitutive SOS-response. Such a mutant strain actually does exist (tif-1 at nonpermisive temperature) and its $N_m/N(D)$ dependence after UV-irradiation is linear (Witkin, 1976). On the other hand, it was demonstrated that the mutagenesis induced by γ-rays does not require protein synthesis (Bridges,Mottershead, 1978).

In regard to our data an important difference between the two hypotheses is the density of DNA injuries needed in average for one mutational event. The former hypothesis requires two

hits in DNA within about 10^3 nucleotides, one being inside the investigated operon. The latter hypothesis requires only one hit inside the investigated operon; the second hit inducing error prone repair can occur at any site of DNA. Higher production rates of DNA injuries are linked with smaller energy depositions and, therefore, with decreasing efficiency for greater LET of ionizing radiation (Munson, Bridges, 1973). Lower production rates should lead to increased efficiency at some intermediate LET. Our results support the hypothesis of the induction (or at least activation) of error-prone repair. The values of RGE in the wild type strains are greater than 2 for helium ions with LET = 22 KeV/μm. It is probably connected with the increased production of DNA damages triggering error-prone repair. Our results with the SOS-chromotest support this interpretation (unpublished results).

The dependence RGE(LET) passes the maximum and falls down again. It can be explained on considering specific energy fluctuations in sensitive cell volumes. The cells hit by a high LET particle are killed and do not contribute to the measured mutation rate. On the other hand, the bulk of surviving cells are not damaged at all and, therefore, they also do not contribute to the mutation rate. The greatest part of mutants arise among the cell compartment damaged only by δ-electrons. It also explains the quadratic dependence which remains in the case of heavy ions. δ-electrons have properties very similar to γ-rays. The energy depositions are clustered but the clusters are not concentrated as in the track core of heavy particle (Wideroe, 1977). If δ-electrons are responsible for the mutagenic effect, it can be expected that the dose-response relationship would be of the same shape as in the case of γ-rays.

Dose response dependences in repair deficient mutant strains.

The efficiency of γ-rays in the induction of lac^+ - lac^- mutants in the polA1 strain of E. coli is markedly increased. The simplest explanation is that the number of DNA damages repaired by the error-prone pathway of cellular repair is enhanced owing to the absence of the polA-dependent fast repair process. RGE for helium ions is grater than one, which can be interpreted in a similar way as in the wild type strain, i.e. as a result of increased production of DNA damages triggering error-prone repair for greater values of LET. However, the relative efficiency of heavy ions to induce mutants in the wild type strain is greater than that for the polA1 mutant strain. This difference cannot be connected with different spectra of initial DNA injuries induced by γ-rays and accelerated heavy ions. The shape of the dose-response curves remains the same, irrespective of LET of ionizing radiation.

The rate of mutagenesis is drastically reduced in the repair defficient lexA strain. This evidence strongly supports the idea that the error-prone repair is linked to the SOS-system induction. The dose-response dependences are linear in the lex A strain and RGE falls down with increasing LET of ionizing radiation. These facts are in agreement with the interpretation given above. In the lexA cells the SOS system cannot be induced and linear $N_m/N(D)$ dependences can be expected. The production of premutational DNA injuries and lethal injuries changes with growing LET in such a manner that RGE falls down.

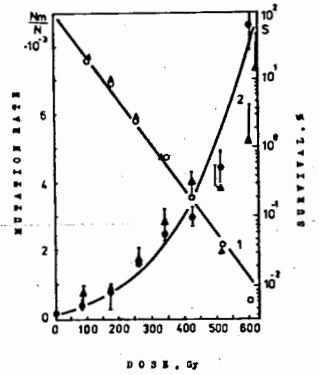

Fig.1: The survival curve and the dependence of Hfr H cells mutation rate on the dose of γ-radiation. Ordinate: survival, % (on the right) and mutation rate (on the left). Abscissa: the dose of γ-radiation, Gy.

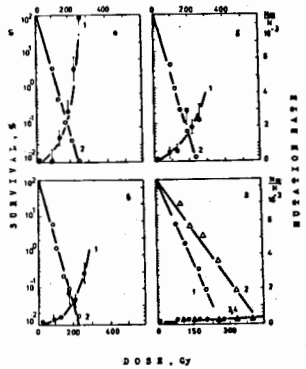

Fig.2: The survival curves and the dependences of Hfr H cells mutation rate on the dose of various accelerated ions: a/ He ions, LET=22 KeV/μm; b/ He ions, LET=54 KeV/μm; c/ He ions, LET=72 KeV/μm; d/ C ions, LET=220 and 330 KeV/μm. Ordinate: survival, % (on the left) and mutation rate (on the right). Abscissa: the dose of radiation, Gy.

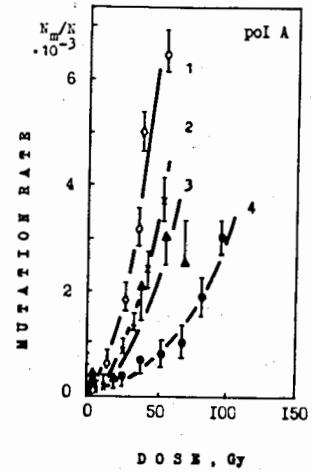

Fig.3: The dependences of mutation rate of polA strain cells on the dose of various kinds of ionizing radiation: 1/D ions, LET=7 KeV/μm; 2/D ions, LET=18,3 KeV/μm; 3/He ions, LET=22 KeV/μm; 4/γ-rays. Ordinate: mutation rate. Abscissa: the dose of radiation, Gy.

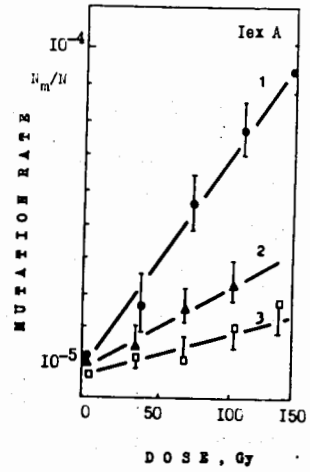

Fig.4: The dependences of mutation rate of lexA strain cells on the dose of different kinds of radiation: 1/γ-rays; 2/He ions, LET=22 KeV/μm; 3/He ions, LET=54 KeV/μm. Ordinate: mutation rate. Abscissa: the dose of radiation, Gy.

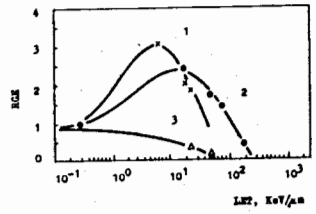

Fig.5: The dependences of RGE on LET for three different E. coli strains: 1/polA mutant, 2/wild type, 3/lexA mutant. Ordinate: RGE; Abscissa: LET, KeV/μm.

Table 1. The values of RGE and the parameters of Eq.1.

Type of radiation	L KeV/μm	E MeV/n	D_0^{-1} $10^{-2}Gy^{-1}$	⁎	β^2 $10^{-9}Gy^{-2}$	RGE
γ-rays	0,3	-	1,40±0,05	1,92±0,30	24±2	1
^4_2He	22	8,0	3,8±0,2	2,9±0,9	130±7	2,3±0,3
^4_2He	54	2,5	3,7±0,2	1,9±0,5	65±5	1,6±0,2
^4_2He	72	1,7	3,5±0,2	2,1±0,5	38±4	1,25±0,20
$^{12}_6C$	220	7,0	3,6±0,2	-	0,6±0,2	0,16±0,05
$^{12}_6C$	330	4,5	2,3±0,1	-	-	-

Table 2. The values of RGE, DMF*, the sensitivities D_0^{-1}, and the values of parameter ⁎ (Eq. 1a) in repair deficient mutants of E. coli cells

Strain	Type of radiation	L KeV/μm	E MeV/n	D_o^{-1} $10^{-2}Gy^{-1}$	⁎	RGE	DMF
	γ-radiation	0,3	-	8,3± 0,3	1,7± 0,3	1	3,5± 0,4
polA1	^2_1D	7,0	5,8	8,3± 0,2	1,5± 0,2	3,2± 0,3	-
	^2_1D	18,3	1,6	8,3± 0,3	1,6± 0,3	2,0± 0,2	-
	^4_2He	22	8,0	8,3± 0,4	1,5± 0,3	1,7± 0,3	2,6± 0,4
	γ-radiation	0,3	-	6,0± 0,2	1,0± 0,2	1	0,30± 0,02
lexA	^4_2He	22,0	8,0	7,3± 0,3	1,0± 0,1	0,4± 0,1	-
	^4_2He	54,0	2,5	6,3± 0,2	1,0± 0,1	0,20± 0,03	-

* Dose modifying factors have been calculated as the ratio of coefficients β (Eq.1b) of the polA1 and wild type strains or as the ratio of coefficients α (Eq.1b) of the lexA and wild type strains.

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