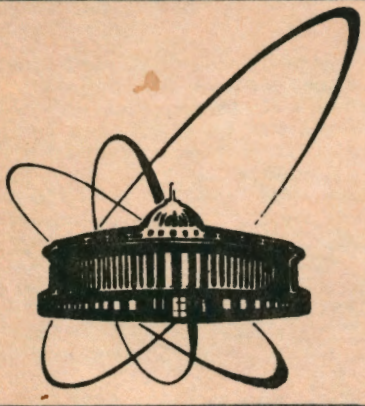

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RADIOPROTECTIVE ACTION OF GLYCEROL AND CYSTEAMIN ON INACTIVATION AND MUTAGENESIS IN SALMONELLA TESTER STRAINS AFTER GAMMA AND HEAVY ION IRRADIATION

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In our previous investigations mutagenic effects of ionizing radiation in E.coli cells (Tokarova et all., 1989), in Salmonella typhimurium after low doses of radiation (Kozubek et al., 1989b), and induction of mutagenic repair in PQ37 cells (Kozubek et al., 1989a,1990) have been studied. It has been shown that heavy ions are mostly more effective in mutation induction as well as in the induction of the SOS mediated repair. The greater production of corresponding injuries 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 the "complex" DNA injuries proposed by Bridges and Mottershead (1972). The nature of these injuries can be investigated at molecular level or somewhat deliminated by chemical modification of the radiation effects. The following results should contribute to the latter approach.

The effect of glycerol on the bacterial cell radiosensitivity has been well established (Amirtaev et al., 1985). It has been shown that the efficiency of glycerol is diminished, however remains significant in repair deficient mutants. This finding was in agreement with generally accepted opinion that glycerol protects DNA against radicals. The presented results show that glycerol protects cells also against heavy ions even when mutation induction is the observed effect. The universal character of glycerol action is confirmed by the fact that the effect was observed in two different strains (TA98 and TA100).

On the other hand the action of cysteamin is less established. Absence of the radioprotective effects in repair deficient mutants of E.coli (polA and recA) when wild type cells are protected (our observation) seems to be very strong argument for the hypothesis according to which cysteamin influences cellular repair. For comparison with glycerol we have investigated also the effect of cysteamin on Salmonella typhimurium cells.

MATERIAL AND METHODS

Sample preparation

Salmonella tester strains TA98, TA100, and TA102 have been used in our experiments. The overnight cultures as well as the plates for mutagenicity assay were prepared according to Maron and Ames, 1983. Glycerol was added to washed cell suspension in standard M9 buffer. The suspension has been incubated for 30 min before irradiation in the presence of 1 M glycerol or 0.02 M cysteamin.

Irradiation by γ-rays and heavy ions

A ^{137}Cs γ-ray soure with the dose rate of about 23 Gy/min was used. The cell suspension after incubation in the presence of glycerol has been irradiated, appropriately diluted and spread on plates.

Irradiation by heavy ions was performed at the U-200 accelerator, Joint Institute for Nuclear Research, Dubna. Beam monitoring and dosimetry were performed by means of a calibrated air ionization chamber set between the exit window and the biological sample. For

irradiation 10^8 cells in droplets were placed on the surface of dacron filters in metalic plates used for accelerator experiments filled with agar containing 1 M glycerol. Three kinds of heavy particles have been used: deuterons, helium and carbon ions. The range of LET values and energies is shown in Tab.2.

Assay

Irradiated samples were washed in 1 ml M9. One part was appropriately diluted for survival experiments, the second part was left in high density. Both sets were immediately spread in TOP agar a little supplemented with histidin on the surface of minimal plates (according to Maron and Ames, 1983). High density population formed barely visible lawn with revertans; low density population could grow to some extend and form visible microcolonies. The number of surviving cells and revertant colonies were counted under slight magnification after 48 h of incubation at 37°C.

Numerical analysis

The surviving fraction (S) was calculated as the ration $S=N_S/N_O$, where N_S is the number of surviving cells after irradiation and No is the number of nonirradiated bacteria. Survival curves were constructed by plotting the logarithm of cell survival versus dose and fitted to the formula:

$$S = \exp(-D/D_o). \qquad (1)$$

The number of induced revertants (N_m) can be calculated from the whole number of mutants detected on the plates (M) by subtracting the spontaneous mutations fraction. Spontaneous mutations present in cell suspension (M_{s1})at the moment of irradiation will be also killed to some extent. Owing to the fact that their survival curves do not differ from those observed for auxotrophic cells, we can simply multiply the number of these revertants by survival fraction and subtract from M. The number of spontaneous mutations arising at the plates (M_{s2}) do not depend on the dose in a broad range and it should be simply subtracted from M. The final formula is therefore:

$$N_m/N(D) = [(M-M_{s1}*S-M_{s2})/S*N], \qquad (2)$$

where N is the number of originally irradiated bacteria.

Dose dependence $N_m/N(D)$ was fitted to the following function:

$$N_m/N = k.D^{\delta}, \qquad (3)$$

where D is the radiation dose. The parameters k and δ were determined for each $N_m/N(D)$ dependence by an optimizing procedure (Tokarova et al., 1989). The dose modifying factor (DMF) with respect to mutagenesis was determined as the ratio of the doses inducing the same number of mutations under normal conditions and in the presence of glycerol. Simultaneous fit has been performed for this purpose for both sets of the data with one common free parameter δ.

The number of lesions participating in mutagenesis induced per unit dose can be formally calculated as $(k)^{1/\delta}$. The ratio $(k)^{1/\delta}/D_o^{-1}$ represents mutagenic efficiency per lethal event (Baltschukat, Horneck, 1990).

In the region of LET values investigated the biological effects depend mainly on LET; the energie of particles is less important (e.g. Kraft, 1987). Therefore, the results will be plotted as LET dependences.

RESULTS

Inactivation and mutation induction following γ-irradiation

Cell survival curves together with mutation frequencies for all three strains in the presence and absence of glycerol are shown in Fig.1. The three strains have on minimal medium identical survival curves. We can see radioprotective effect on cell survival as it is usually observed. The dose modifying factor (DMF) is 2.05±0.20. In the case of cysteamin some protection have been also observed at the level of cell survival with DMF=1.50±0.17.

The DMF factors for mutagenesis are shown in Tab.1. The greatest effect of cysteamin was observed for TA98 strain, the greatest effect of glycerol for TA100 and TA102 strains.

Table 1. The average values of the slopes of power functions describing mutation frequency in dependence on the radiation dose and dose modifying factors (DMF) for glycerol and cysteamin in three Salmonella typhimurium cells after γ-irradiation.

Strains		Glycerol	Cysteamin
	δ	DMF	DMF
TA98	2.0±0.1	1.52±0.15	1.40±0.07
TA100	1.9±0.1	1.83±0.07	1.15±0.15
TA102	2.6±0.3	2.24±0.04	1.34±0.06

$DMF=(k_1/k_1)^{1/\delta}$, where k_1, k_2 and $\delta=(\delta_1+\delta_2)/2$ are the average parameters of Eq.(3) in the absence and presence of protectors.

Inactivation after heavy ion tratment in the presence of glycerol

Inactivation and mutation induction in Salmonella tester strains (normal conditions) have been investigated earlier in low dose region (Kozubek et al.,1989b) and high dose region (Kozubek at al.,submitted for publication). The radiosensitivity slightly

increased with LET in all three above mentioned strains. The presented experiments confirme our earlier observation. All survival curves of all strains are exponential. The radiosensitivities, RBE, and DMF factors for cell survival are given in Tab.2 and Tab.3. The dependence of the radiosensitivity D_o^{-1} on LET is presented in Fig.2. As can be seen the protective effect of glycerol gradually diminishes from 2 to 1.2 for carbon ions. Both curves form local maximum which is however shifted to the right in the presence of glycerol.

Table 2. Radiosensitivity and the values of RBE for Salmonella tester strains (TA98 and TA100) irradiated in the ABSENCE of glycerol. The radiation beam parameters (LET and energy per nucleon) are also shown.

Radiation type	LET, keV/μm	E MeV/n	D_o^{-1} 10^{-2},Gy^{-1}	RBE
^{137}Cs	0.3	-	2.0±0.1	1
^{2}D	5	8.7	2.4±0.2	1.2±0.2
^{2}D	8	5.9	2.7±0.2	1.3±0.2
^{4}He	20	8.4	-	-
^{4}He	40	4.2	-	-
^{4}He	50	3.8	2.8±0.3	1.4±0.3
^{4}He	80	2.1	2.7±0.3	1.3±0.2
^{12}C	220	7.6	2.1±0.1	1.1±0.1

Table 3. Radiosensitivity, the values of RBE and DMF for Salmonella tester strains (TA98 and TA100) irradiated in the PRESENCE of glycerol.

Radiation type	LET, keV/μm	D_o^{-1} 10^{-2},Gy^{-1}	RBE	DMF
^{137}Cs	0.3	1.1±0.1	1	1.8±0.2
^{2}D	5	1.1±0.1	1.1±0.2	2.1±0.2
^{2}D	8	1.2±0.2	1.1±0.2	2.2±0.2
^{4}He	20	1.1±0.2	1.0±0.2	-
^{4}He	40	1.2±0.2	1.1±0.2	-
^{4}He	50	1.5±0.2	1.4±0.2	1.8±0.3
^{4}He	80	2.0±0.2	1.8±0.2	1.4±0.3
^{12}C	220	1.7±0.2	1.5±0.2	1.2±0.2

Induction of mutations after heavy ion irradiation in the presence of glycerol

In our earlier experiments (Kozubek et al., submitted for publication) we have observed increased mutation efficiency for deuteron and helium ions in TA100 and TA102 strains. In the case of TA98 strain this efficiency was continuously decreasing. The results presented here show that the mutagenic effeciency of heavy ions is actually less in TA98 strain in comparison to TA100 strain. The values of RBE for deuteron ions are, however, slightly higher then one.

All radiobiological parameters calculated according to the procedures described above are shown in Tab.3 (absence of glycerol) and Tab.5 (in the presence of glycerol).

Table 4. The slopes of $N_m/N(D)$ dependence and the values of RGE (relative genetic effectiveness) for TA98 and TA100 strains irradiated by heavy ions in the ABSENCE of glycerol

Radiation type	LET, keV/μm	TA98 δ	TA98 RGE	TA100 δ	TA100 RGE
^{137}Cs	0.3	2.0±0.1	1	1.9±0.3	1
^{2}D	5	2.1±0.1	1.2±0.1	2.0±0.5	1.3±0.1
^{2}D	8	1.7±0.2	1.3±0.2	2.2±0.5	2.0±0.2
^{4}He	50	1.5±0.2	0.9±0.1	1.7±0.4	0.8±0.1
^{12}C	220	1.6±0.2	0.5±0.05	1.5±0.5	0.45±0.08

RGE was calculated under assumption that δ is equal for all types of ionizing radiation (δ=2).

Table 5. The slopes of $N_m/N(D)$ dependence, the values of RGE (relative genetic effectiveness) and DMF_m (for mutation induction) for TA98 and TA100 strains irradiated by heavy ions in the PRESENCE of glycerol

Radiation type(LET)	TA98 δ	TA98 RGE	TA98 DMF_m	TA100 δ	TA100 RGE	TA100 DMF_m
^{137}Cs	1.9±0.5	1	1.5±0.1	1.8±0.4	1	1.8±0.4
^{2}D(5)	2.0±0.5	1.3±0.2	1.4±0.2	2.0±0.5	1.8±0.2	1.4±0.3
^{2}D(8)	1.8±0.5	1.3±0.2	1.5±0.2	2.1±052	1.6±0.2	2.1±0.3
^{4}He(50)	1.6±0.5	1.0±0.1	1.3±0.1	1.8±0.4	1.1±0.1	1.5±0.2
^{12}C(220)	1.7±0.5	0.6±0.1	1.1±0.1	1.7±0.5	0.7±0.1	1.2±0.1

The values of δ are average values obtained in the presence and absence of glycerol. RGE was calculated under assumption that δ is equal for all types of ionizing radiation (δ=2).

As can be seen in Tab.4-5 all dependences of mutation frequency on the radiation dose for both strains were non-linear. The values of the parameter δ, however, somewhat decrease with LET. The dependence of RGE on LET for the two strains used in the absence of glycerol is shown in Fig.3. The same dependences for TA100 strain in the presence and absence of glycerol are shown in Fig.4. As can be seen both dependences form local maxima. The maximum in the presence of glycerol is slightly shifted to the left. Fig.5 shows DMF_m(LET) dependences for TA98 and TA100 strains. The decreased efficiency of ionizing radiation to induce mutations for γ-radiation is relatively increased again for heavy ions.

DISCUSION

We have continued the investigation of the mutagenic effects of ionizing radiation on bacterial cells. The effects of radio-protectors was investigated in Salmonella tester strains. The strains are isogenic, except for sequence of several nucleotides in some genes encoding histidine synthesis. The strains are constructed such that they can detect different mutagenic agents. It is connected with different mutation events: in TA98 it is deletion and in TA100 substitution in ..CCC..triplet and in TA102 it is substitution in nonsense codon TAA. The strains can be reverted specifically by some mutagens: for example TA98 by ICR-191, TA100 by daunomycin (natrium azid), TA102 by MMC(oxydative agents). The differences between the strains reflect different production of some kind of DNA lesions. Changes in these differences with LET or in different conditions of irradiation reflect changes in the spectrum of induced premutational lesions.

Glycerol is believed to reduce substantially indirect action of radiation and mostly acts as a dose reducing factor (Amirtaev et al., 1985). The protection is, however, partially determined by cell genotyp. The values of DMF for γ-radiation increased in our experiments from 2.0 for $recA^-$ strain to 2.5 for wild type cells and 2.8 for $polA^-$ isogenic strain. This genetic determination provides evidence that glycerol protects more against repairable lesions and that less repairable lesions are also less modified.As it was mentioned above, cysteamin in the concentration used in our experiments influences probably cellular repair.

Both protectors were found to decrease substantially cell lethality after γ-irradiation with very similar ratios for Salmonella typhimurium strains. Glycerol was a little more efficient. The decrease of the mutagenic efficiency was, however, different for various cell strains. In general the effect was smaller in comparison to the influence on the cell lethality. The greatest effect of glycerol have been observed in TA100 and TA102 strains. Cysteamin was found to be non-efficient in the case of TA100 strain. In all cases the effect of cysteamin was fairly small. Therefore, it was not used for experiments with heavy ions.

From our experiments and previous evidence it can be conluded that in Salmonella tester strains indirect action of ionizing radiation is a substantial source of premutational lesions in all three strains. The spectrum of lesions producet by ionizing radiatiion is very broad and provides lesions for all kinds of mutations.

Some part of these lesions is formed as a result of indirect radiation effects. Therefore, both types of radiation action - direct and indirect provide broad spectrum of DNA lesions.

The protective effect of cysteamin in Salmonella tester strains was generally small, which can be connected with cultivation on minimal medium where the repair processes are restricted and might be difficult to influence.

Heavy ion experiments provide evidence that glycerol protects effectively against ionizing radiation with different LET up to approximately 100 keV/μm in the case of both cell lethality and mutagenesis. The dependences of RBE or RGE on LET form local maxima. The maximum is in the region of about 50-100 keV/μm for cell lethality. This finding is in agreement with our earlier observations (Krasavin et al., 1989). The maximum obtained after irradiation in the presence of glycerol is shifter to higher LET values. It can be explained by the fact that a part of the radiation effect (indirect effect) is cut and therefore in average more energy is needed to form injury leading to lethal event. The greatest is the energy needed to form the given injury, the higher should be the maximum on the LET dependence of the production efficiendy and it should be shifted more to the right. On the other hand the bulk of single strand breaks and base damagese should be produced with greatest efficiency after γ-irradiation (Kozubek et al., 1988).

Mutagenic efficincy of heavy ions is greatest in the region of about 10-20 keV/μm (Fig.3,4). This observation confirms our earlier results (Tokarova et al., 1989, Kozubek et al., submitted for publication). Protective effect of glycerol gradually diminishes with LET in both TA98 and TA100 strains. It remains less for TA98 strain in the whole region of LET values. It supports the conclusion about universal mechanisms of glycerol action. If glycerol cuts indirect radiation effects then our experiments provide evidence that both direct and indirect radiation action are very universal and provide broad spectrum of radiation lesions in cellular DNA. Only in the region of higher LET values (LET>100keV/μm), where nearly every particle passing cell nucleoid produces enough lesions to kill the cell, the effect of glycerol diminishes.

As it can be expected in this region of LET the production of mutations also decreases (Fig.3,4 Tab.4,5) as well as the slope of the dose-response dependences. The mutations which arise can not be protected by glycerol. The lesions that lead to mutation formation in cells passed by higher LET particle are probably very complex, difficult to repair and difficults to modify even at the level of chemical processes. Some part of mutations after heavy ion irradiation arise in non-directly-hit cells population. These cells can be hit only by δ-electrons with physical properties similar to γ-radiation. However, decreased values of the dose dependences $N_m/N(D)$ and decreased protective effect of glycerol suggest that with increasing LET the mechanism of mutation induction changes. For even higher LET linear dose-response dependences can be expected.

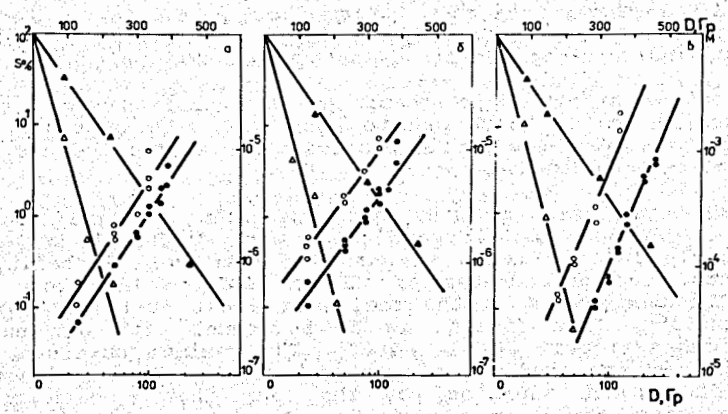

Fig.1. The effect of γ-radiation on cell survival (triangels) and mutagenesis (circles) in Salmonalla typhimurium strains TA98 (a), TA100 (b), and TA102 (c) in the absence (closed symbols) and presence (open symbols) of glycerol.

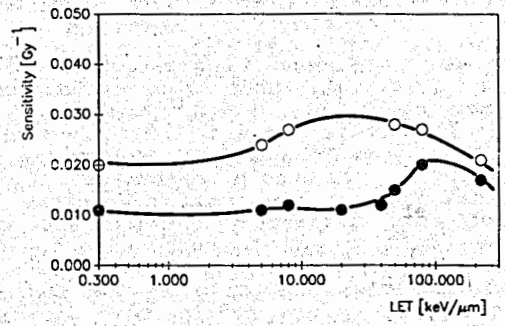

Fig.2. The dependences of the radiosensitivy in the absence (open circles) and presence (closed circles) of glycerol on LET.

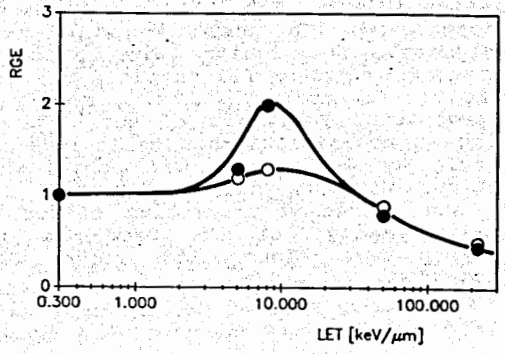

Fig.3. The dependences of RGE (relative genetic effectiveness) on LET for TA98 (open circles) and TA100 (closed circles) strains.

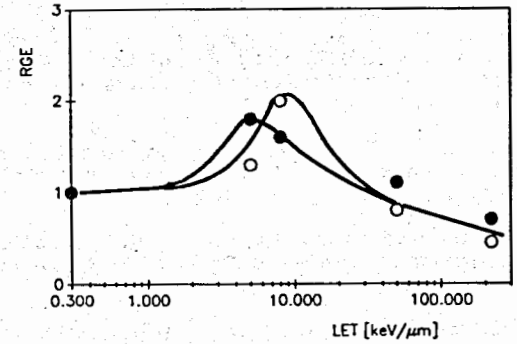

Fig.4. The dependences of RGE on LET for TA100 strain in the absence (open circles) and presence (closed circles) of glycerol.

CONCLUSIONS

1) The radioprotective effect of both glycerol and cysteamin in Salmonella typhimurium strains is less for mutagenesis in comparison to cell lethality. The radioprotection of glycerol is greates in TA100 and TA102 strains.

2) The radiosensitivity in the presence of glycerol increased with LET more rapidly in comparison with normal conditions; both sensitivities become roughly equal for LET>50 keV/μm.

3) The protection of glycerol gradually diminishes with LET for both TA98 and TA100 strains from: 1.5 to 1.1 for TA98 and from 1.8 to 1.2 for TA100.

4) The slopes of the dose dependences of the mutation frequency $N_m/N(D)$ decrease with LET from 2.0 for deuterium ions to 1.5-1.7 for carbon ions for both strains used in the presence and absence of glycerol.

5) The mutagenic efficiency of heavy ions in the both absence and presence of glycerol is greater in comparison to γ-rays for TA100 strain; the same dependence for TA98 strain is continously decreasing (only very slight maximum is not statistically significant). For LET>50 keV/μm the mutagenic efficiency falls down.

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