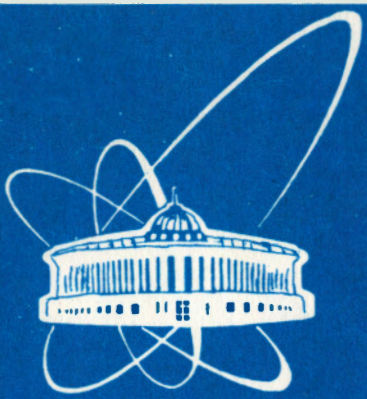

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# OXYGEN EFFECT AND INFLUENCE OF THE ANOXIC RADIOSENSITIZING AGENT TAN ON THE INDUCTION OF λ-PROPHAGE IN polA AND WILD TYPE *E.coli* STRAINS AFTER GAMMA IRRADIATION

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## Introduction

It is well known that the oxygen as well as the organic nitroxyl-free radicals are able to sensitise irradiated cells to the lethal action of ionising radiation. The mechanism of sensitisation by oxygen and N-oxyls consists in interaction of these compounds with radiation-induced DNA radicals (Johansen, 1974; Nakken and Brustad, 1974). The interaction of N-oxyls (for example, TAN) with DNA-radicals generates TAN-DNA adducts, that are difficult to repair (Hohman et al., 1976).

The important role of the "complex" DNA damages in mutagenesis induced by high LET radiation have been shown by study of the effect of the anoxic radiosensitizing agent TAN on the induction of revertants by γ-rays and helium ions in Salmonella tester strains (Basha et al., 1992). In our research on the λ-prophage induction by γ-rays and helium ions we have established that the "complex" single-stranded DNA breaks (ssb), which require a substantial level of energy deposition and which cannot be repaired by fast repair processes, enhance λ-induction potency (λiP) (Bonev et al., 1990). On the other hand, an increase in SOS-response in TAN-sensitised *E.coli* cells under anoxia was shown after γ-irradiation (Komova et al., 1990). These data indicate that

binding of a sensitiser (TAN or oxygen) to DNA radicals leads to formation of SOS-inducing damages. Owing to λ-repressor cleavage, which is under recA protein control, the induction of λ-prophage is a good pointer of the DNA lesions, which are repairable only by the SOS system. The efficiency of these lesions could be studied further by induction of λ-prophage under different conditions (oxygen, anoxia, and anoxic radiosensitizing agent TAN), wich is the aim of the present work.

**Materials and Methods**

The *E.coli* strains used in our experiments are: W3110(B1,thy-36,deoC, λ), P3478(polA1,B1,thy,λ), and C600(thr1,leu6,thi1,supE44,lacY1,tonA21,$F^-$,$Str^r$). The last strain C600 has been used as an indicator of plaque-forming unit (PFU). All strains were obtained from the Institute of Biophysics, Ministry of Health of Russia.

.2,2,6,6-Tetramethyl-4-piperidine-1-oxyl (TAN) was synthesised in the Institute of Chemical Physics of the Academy of Sciences of Russia. TAN was dissolved in destilled water and added to the bacterial suspension in order to obtain a final concentration of $10^{-3}$M immediately before degassing performed by flowing argon for at least 30 min before irradiation.

Bacteria were grown overnight in liquid nutrient broth, diluted 1:20, and incubated at $30^0$C to approximately $2x10^8$ cells/ml. The suspension was centrifuged at 8000g for 15min and resuspended twice in 0.01mol/l $MgSO_4$.

In order to determine the production of infective centers, 0.1 ml of appropriately diluted suspension was added to 3ml of TOP agar (7g/l agar, 1g/l yeast extract, 8g/l NaCl, and 3nmol/l $MgSO_4$) together with 0.1ml of a suspension of indicator bacteria ($4x10^8$ cells/ml; C600 strain) and poured on to plates containing nutrient agar. The fraction of spontaneously induced cells that



may occur on the plates during overnight incubation was one or two orders of magnitude lower than in the irradiated samples.

The surviving fraction (S) was calculated as the ratio $S=N_s/N_0$, where $N_s$ is the number of surviving bacteria after irradiation, and $N_0$ is the number of non-irradiated cells. The number of cells was determined by a standard method, i.e. by counting the number of macrocolonies on the nutrient agar.

The fraction of induced cells (I) was determined as the ratio of the number of observed PFU ($N_i$) and the number of the non-irradiated bacteria: $I=N_i/N_0$. The dose-dependence

$$I(D)=\alpha D\exp(-\beta D)(1-\exp(-D.D_0^{-1})),$$

where $D_0^{-1}$ is the radiosensitivity of the cells (Bonev et al., 1990). The parameters α and β were determined for each I(D) dose dependence by an optimising procedure (Velev et al., 1989).The parameter α will be called λiP further on.

**Results**

The spontaneous λ-prophage induction in non-irradiated cells under anoxia, anoxia in the presence of TAN, and in the presence of oxygen during of period of 3 hours at $0^o$C appears to be the same.

The survival curves of the wild type and $polA^-$ mutant strains under different anoxic conditions after γ-irradiation are shown in figures 1 and 2. Bacteria of both wild type and $polA^-$ strains seem to be most sensitive in the presence of oxygen, less in the presence of anoxia + TAN, and least sensitive under anoxia. The values of the radiosensitivities and λiP with their standard deviations are summarised in the table. The oxygen enhancement ratio (OER) on the cell sensitivity was determined as ratio $D_o^{-1}$(oxygen) / $D_o^{-1}$(anoxia). The effect of TAN + anoxia (TAN enhancement ratio - TER) was presented as $D_o^{-1}$(TAN) / $D_o^{-1}$(anoxia). The effect of oxygen and TAN on the λ-induction (OIR and TIR)

was described as the ratios: λiP(oxygen)/λiP(anoxia) and λiP(TAN)/λiP(anoxia) respectively. The OER for the wild type stain is equal to 5.8, and for polA⁻ is equal to 12.9. The relevant TER ratios are equal to 2.0, and 2.3. The λ-induction curves are presented in figures 3 and 4. For the wild type and for polA⁻ strains the values of λiP are greatest in the case of irradiation in the presence of oxygen. The smallest value of λiP is in the case of irradiation under anoxic conditions. The OIR for the wild type strain is equal to 6.5 and for polA⁻ is equal to 118.5. The relevant TIR values are equal to 1.7 and 2.5.

**Discussion**

We have investigated the effect of the anoxic radiosensitizing agent TAN on cell survival and λ-prophage induction in wild type and polA mutant strains after irradiation with γ-rays. The results of our experiments show that the influence of anoxia on the cell survival and λ-induction after irradiation are similar (a diminution of the both sensitivity to γ-rays and λiP).

The radiosensitivities of the two strains under anoxia decrease. The modification effect of TAN on the wild type strain is significantly smaller than in the presence of oxygen (TER=1.50, and OER=5.80). The similar line of behaviour is observed in the case of polA⁻ mutant cells irradiation. The bigger efficiency of oxygen as compared to TAN suggests that the lesions created by an interaction of oxygen and DNA-radicals after γ-irradiation are more difficult to repair than those created by an interaction of TAN and DNA-radicals.

As it can be seen from the figures 3 and 4, the initial slope of induction curves under anoxia decreases for the two strains. λiP characterises the efficiency of ionizing radiation in inducing λ-prophage (it represents the number of induced cells per inactivated cell and per unit dose). On the other hand, λiP expresses SOS-response, as the λ-repressor cleavage is under recA protein-control. In addition, the increase of λiP in the presence of oxygen reflects the increase of the DNA damages which are repairable by recA-lexA-



dependent repair system. The effect of TAN on the wild type strain is smaller than in the presence of oxygen (TIR=1.7, and OIR=6.5). The similar effects of TAN and oxygen on the polA⁻ strain (TIR=2.5, and OIR=118.5) we can observe too.

The biochemical steps of λ as well as SOS induction are initiated by the activation of RecA protein following DNA damage. This process, which is not understood in details, begins when bacterial DNA is damaged in any of a variety of ways. The final event, activation of RecA protein to bind and promote cleavage of λ and LexA repressors, has been studied mostly in biochemical experiments (Craig and Roberts, 1980); Little,1983). Cleavage-promoting activity of RecA protein depends on the presence of single-stranded DNA (Craig and Roberts, 1980). The results of our experiments show that the values of λip in the presence of oxygen are bigger in comparison to anoxia in the presence of TAN. This means that the amount of single-stranded DNA following the repair process in the presence of oxygen is larger than that under anoxia in the presence of TAN.

The influence of oxygen on the survival and λ-induction increases in polA⁻ strain as compared to the wild type. The sensitivity to γ-irradiation of the polA⁻ strain is approximately 3 times higher than that of the wild type. The ratio λiP(polA⁻)/ λiP(wild type) is equal to 9. The effect of anoxia in the presence of TAN on the survival and λ induction is similar to that in the presence of oxygen, but more slightly. The radiosensitivity of the polA⁻ strain is approximately 2 times higher compared to that of the wild type, and the ratio λiP(polA⁻)/ λiP(wild type) is equal to 1.3. The radiosensitivities and λiP strengthening in polA⁻ strain are due to the absence of polymerase I in the mutant strain (Town et al., 1973). The absence of polA-dependent repair leads to an enhancement in the number of the DNA gaps. This can cause an augmentation of DNA-bound RecA protein molecules and as a consequence an

Table. Values of the radiosensitivities and λiP

| | $D_0^{-1}(10^{-3}\,Gy^{-1})$ | | | $\lambda ip(10^{-3}\,Gy^{-1})$ | | |
|---|---|---|---|---|---|---|
| Strain | anoxia | TAN | oxygen | anoxia | TAN | oxygen |
| W3110 | 4.3±0.2 | 8.5±0.2 | 25.0±0.3 | 0.4±0.01 | 0.7±0.01 | 2.6±0.01 |
| P3478 | 6.3±0.2 | 14.5±0.2 | 81.0±0.3 | 0.2±0.01 | 0.5±0.01 | 23.7±0.02 |






Fig.1. Survival of wild-type strain W3110(λ) in the presence of oxygen (circles), under anoxia in the presence of TAN (triangles), and under anoxia (squares)

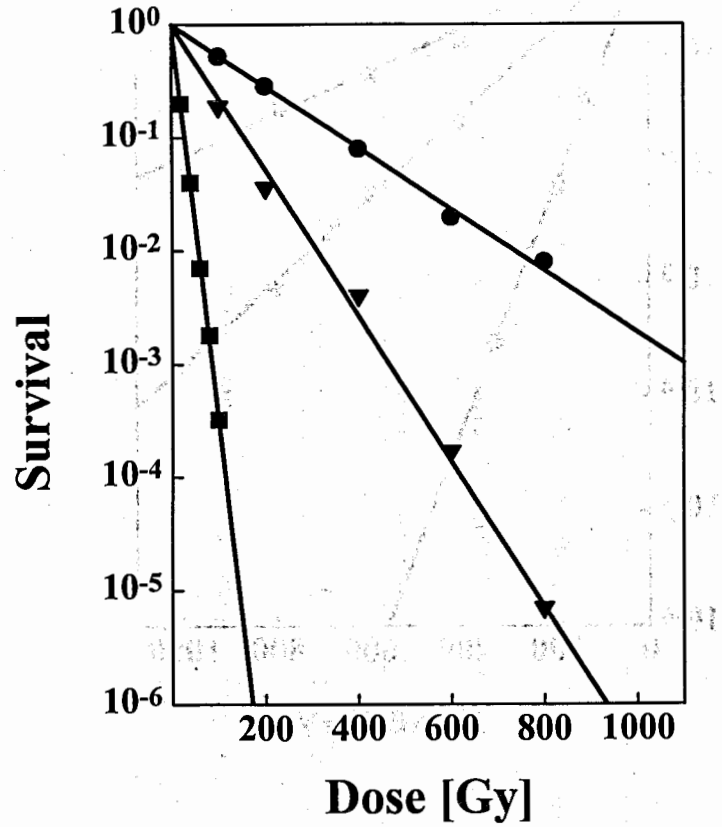


Fig.2. Survival of polA⁻ strain P3478(λ) in the prsence of oxygen (squares), under anoxia in the presence of TAN (triangles), and under anoxia (circles)



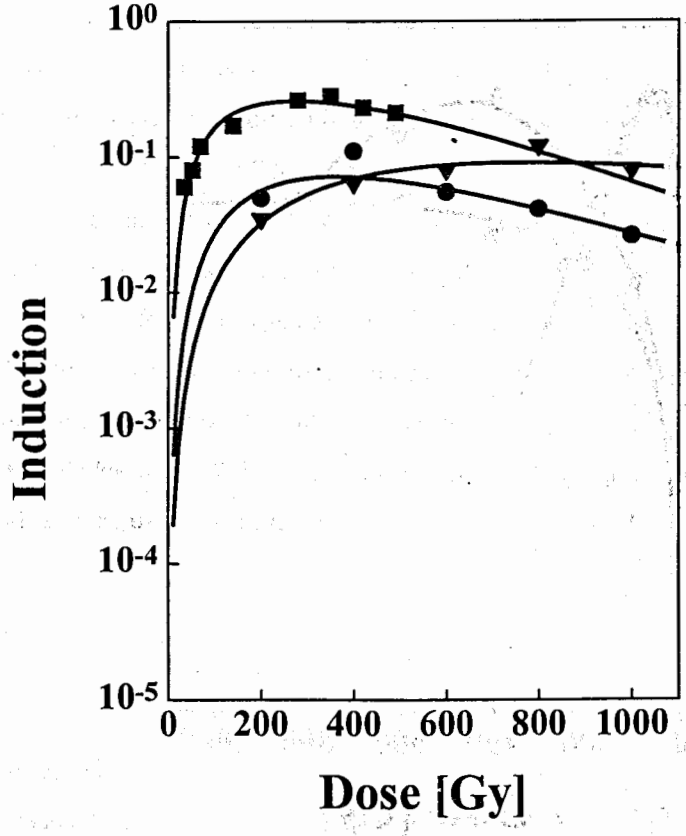


Fig.3. λ-induction of the wild type strain W3110(λ) in the presence of oxygen (squares), under anoxia in the presence of TAN (circles), and under anoxia (triangles)

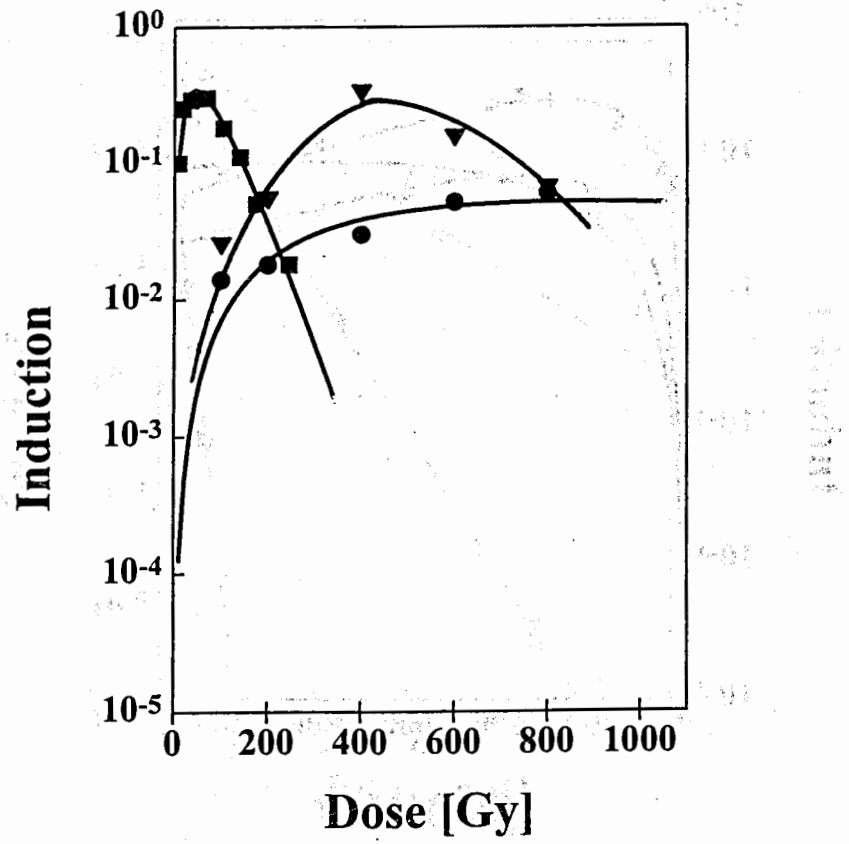


Fig.4. λ-induction of polA⁻ strain P3478(λ) in the presence of oxygen (squares), under anoxia in the presence of TAN (triangles), and under anoxia (circles)



enhancement of the RecA proteolytic activity. Therefore, the expression of λ-prophage induction is greater in the polA⁻ strain than that in the wild type.

In conclusion, our results show that:

(I) the lesions created by an interaction of oxygen and DNA-radicals after γ-irradiation are more difficult to repair as compared to those created by an interaction of TAN and DNA-radicals,

(ii) the amount of single-stranded DNA under oxygen conditions is bigger than that under anoxia in the presence of TAN, and

(iii) the effect of oxygen and anoxia in the presence of TAN is increased in polA⁻ strain, which is due to an enhancement of the RecA proteolytic activity following the absence of DNA-polymerase I.

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