NEUTRON IRRADIATION OF BACTERIOPHAGE λ

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ABSTRACT

Double strand breaks (dsb) are the most dangerous lesions in DNA caused by irradiation, but many other lesions, usually called mutations, have not been clearly identified. These lesions, like dsb, can be the source of serious chromosomal damages and finally - cell death. Growing interest in heavy particles for radiotherapy and radioprotection encourages the search of the molecular basis of their action. In this respect, we chose bacteriophage λ1390 as the model system for the study of consequences of neutron irradiation. This derivative of λ phage possesses an unique ability to reversibly reorganize their genome in response to various selective pressures. The phages were irradiated with 13Gy of mixed neutrons (7.5Gy from fast and 5.6Gy from thermal neutrons) and phages genomes were tested to dsb and mutations. Additionally, the stability of *λ* capsid proteins were tested. After all tests, we can conclude that, under our conditions, low flux of neutrons does not induce neither DNA strand break or DNA mutation nor the stability of *λ* capsid proteins.

Key words: DNA, bacteriophage, neutron irradiation

1. INTRODUCTION

Life on the Earth has evolved against a continuous background of ionizing radiation. High-LET particles however originate from man-made sources. This is not natural radiation, especially not from the time living organisms began their evolution on our planet. We may, therefore, assume that contemporary living cells do not possess efficient defense mechanisms against such type of radiation. However, one should bear in mind that it has been shown on viruses, bacteria, yeast and mammalian cells that correction of the damage to the DNA, induced by high-LET particles – acts, definitely *via* repair systems specialized in defending the cell from natural types of ionizing radiation. At the same time, it is not exactly known which one of a few very well characterized DNA repair systems act.

The initial damage caused to the cellular DNA by a highly energetic charged particle is complex and results in a variety of lesions. Many studies point to DNA double strand breaks (dsb) as critical lesions for different types of radiation and suggest that this is due to the lack of repair of at least a part of dsb [1]. The high radiobiological effects of high-LET radiations [2] might be thus due to these radiations inducing more dsb than low-LET ionizing radiation, and that at least part of these numerous dsb remain unrepaired [3] or, as proposed by Taucher-Scholz et al., that dsb produced by high-LET radiation may differ from those induced by low-LET [4]. Actually, there is a wealth of

information about a high-LET irradiation consequence, but their interpretation is not clear and thus far from complete. Although it has been clearly established that double strand breaks are the source of mutations, chromosomal aberration and cell death, the lesions responsible for more recently discovered effects of radiation (gene induction, repair induction, etc.) have not been clearly identified.

Growing interest in heavy particles for radiotherapy and radioprotection encourages the search of the molecular basis of their action. In this respect, we chose bacteriophage λ1390 as the model system for the study of consequences of neutron irradiation. This derivative of λ phage possesses an unique ability to reversibly reorganize their genome in response to various selective pressures.

2. EXPERIMENTAL PROCEDURE

Phage and Host Stocks. The bacteriophage *λ*1390 strain and different *E. coli* bacterial strains used in this study were from Radman's laboratory (M. Radman, France; original source of the phage is from G. Smith, U.S.A.). *λrex173::Tn10* phage exist in two configurations: spi^- and spi^+. *λ*1390 is spi^- configuration. Stock of *λ*1390 bacteriophage was prepared by confluent lysis on a plate [5]. The medium used for plates and for the top agar was Luria broth supplemented with 1M $MgSO_4$ and 20% maltose to final concentration of 10mM and 0.2%, respectively. The plates were used as soon as they harden. For each plate, one single plaque of lambda was transferred to 0.5ml of LB media for 10min at 37°C to allow phage liberation in medium and then add 10µl of fresh overnight *E. coli* P2 bacterial strain. After 15min at 37°C to allow phage adsorption, molten 0.5% soft agar was added and the mixture was poured onto the fresh plate. The plates are incubated right-side-up at 37°C. Lysate is ready for harvesting after 4-6 hours, i.e. when the plaques touch one another. For harvesting of the lysate, the top layer is scraped into a tube containing 0.2ml of $CHCl_3$, the content then mixed well and left for 5min at room temperature. Two cycles of centrifugation (10min at 8000rpm) removed large cellular debris and unlysed bacteria. The lysate is immediately filtered through 0.45µm Millipore filter, 3 times dialyzed against 3l 10mM $MgSO_4$ on cold and λ stock with a few drops of $CHCl_3$ is store at 4°C. Typical titer is $2x10^{11}$ plaque forming units per ml (pfu/ml). The spontaneous inversion frequencies from spi^- to spi^+ configuration in *λ*1390 stock was measured by titration on *recA*$^-$ host (for spi^+ configuration) and on P2 lysogene host - for spi^- configuration, and it was $1x10^{-6}$ (this means that 1 from 10^6 phages in *λ*1390 stock has spi^+ phenotype). *E.coli* P2 lysogene, the host for preparation of phage stock (original source from Nancy Kleckner, U.S.A.), and *recA*$^-$ and *recA*$^-$*uvrA*$^-$ strains were grown according to Arber et al. [5].

Surviving and DNA damage test. *λ*1390 bacteriophage gives transparent plaques on a lawn of *E. coli* plating bacteria. The counting of plaques was performed after incubation at 37^0C for 18h. The relative number of surviving bacteriophage was the ratio of both titers measured for non-irradiated control bacteriophage and neutron irradiated *λ* on a lawn of *E.coli* P2 plating bacteria. DNA breaks were assayed *via* frequency of recombination tested by plating non-irradiated and neutron irradiated *λ* on a lawn of P2 permissive and *recA*$^-$ non-permissive bacteria, respectively. For DNA mutations test, the number of plaques of control and neutron irradiated *λ*, respectively, on a lawn of P2 and *recA*$^-$*uvrA*$^-$ double mutant plate bacteria, were compared.

Capside proteins test. To test the stability of *λ* capsid proteins, samples of non-irradiated and irradiated *λ* were transferred to 37°C or 50°C, and the number of survivors was screened every day for 10 days.

Irradiation and dosimetry. Irradiation of 0.15ml of *λ* stock (1.5×10^9 phage particles) by neutron was performed in RB reactor at the Vinča Institute of Nuclear Sciences, Belgrade, SCG. As a neutron source, the configurations No. 114 of the RB heavy water critical assembly [6], designed to produce fast neutron fields [7], was used. In the central part, this configuration contained an air hole surrounded by the cadmium layer (with thickness of 0.1cm) and the inner neutron converter filled with

2% enriched metal uranium fuel slugs. The driver core for this configuration is the standard core of the RB critical assembly with 40 fuel channels of 2% enriched metal uranium and 100 fuel elements with metal natural uranium. For determination of the neutron flux in this configuration, characterized with the strong neutron absorption and neutron leakage, a combination of numerical and experimental procedures was used. Numerical procedure is based on the SCALE-4.4a code system [8] application with the use of Dancoff factor determined by the VEGA2DAN code [9]. Experimental methodology consists of the irradiated foils activity measurement, and foil averaged neutron absorption cross-section determination via mentioned SCALE-4.4a calculation procedure. The absorbed neutron dose rate is obtained by using measured neutron flux and ANSI standard neutron flux-to-dose-rate factors [8]. The energy-distribution of the neutron flux in the irradiation position of samples is shown in Fig.1. The values of obtained absorbed neutron dose rate in the experimental vertical channel (at half height of thermal core, i.e. at sample position) for fission power of the RB assembly equal to 51W are 7.5Gy from fast (E>0.465eV) and 5.6Gy from thermal neutrons (E≤0.465eV). To reach the total absorbed neutron dose of 13Gy, the RB critical assembly had to operate 60 minutes.

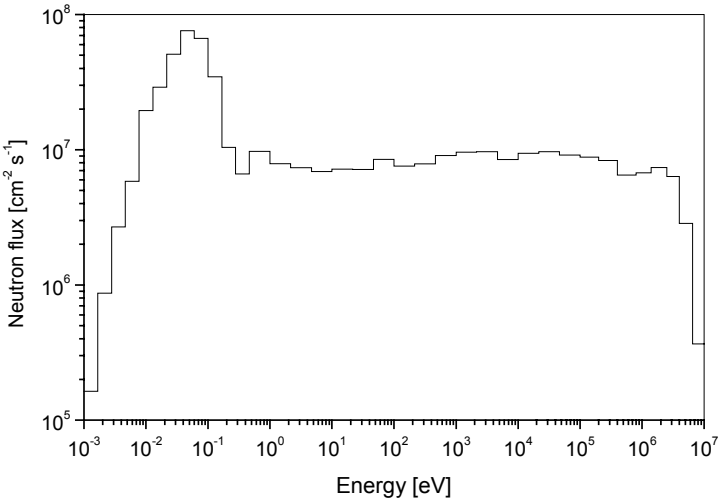

Fig. 1. Energy spectrum of neutron flux in the sample position

3. RESULTS AND DISSCUSION

Transposable elements in prokaryotic organisms mediate directly a variety of different types of DNA rearrangements including deletion, inversions and replicon fusions. They can also contribute to DNA rearrangements in a less direct way, by simply serving as "portable regions of homology" in normal general recombination between homologous elements located at different positions in the chromosome or in different replicons. Transposable elements can clearly affect not only the organization of genetic information, but also its expression. λ phage, which we used as the model system for the study of consequence of neutron irradiation, possesses a Tn10 composite transposable element inserted in the non-essential *rex* gene. Tn10 element allows λ phage the genetic flip-flop *via* recombination event that occurs within one DNA molecule, thus the phage exists in two phenotype forms (spi- and spi^+). This means that two closely linked phage genes (*red* and *gam*) reversibly alternate between expressed (spi^+) and non-expressed (spi-) states. The state of expression of these genes can be tested by plating phages on a pair of special selective *E. coli* hosts: on a $recA^-$ bacterial host, spi^+ phages plate normally, while spi- phages fail to plate; on a P2 bacterial host, the opposite plating behavior is observed; spi^- phages plate at 100% efficiency, but spi^+ phages fail to plate [10]. The frequency of spontaneous flip-flop is about 1% for both directions [11], but changes in response to various selective pressures, for example, in the case of many dsb in phage DNA caused by heavy

particle irradiation. Thus, there is an elegant possibility to measure changes of the frequency of recombination by plating phages on a pair of selective bacterial host, as the absence of any repair processes and of any interaction of DNA with other molecules during the irradiation, actualized this λ strain as model of choice for our studies.

Samples of λ1390 (spi^- phenotype) containing 1.5 x 10^9 pfu (plaque forming units) were irradiated with 13.1 Gy of neutrons (unequal mix of thermal and fast neutrons). The killing effect of irradiation was measured by plating non-irradiated and irradiated phages, respectively, on lawn of P2 bacterial host and count survival phages. The $\lambda_{irr}/\lambda_{contr}$ ratio was close to 1; meaning that almost all lambda phages survived irradiation. Well, it has been shown that efficiency of the heavy ion to the cellular DNA are highly dependent on ionizing density [12]. In our case, the source of radiation was high-LET, high-energy but low flux of particles and, for these experiments, the reactor was near the maximum of its power. For one hour of irradiation, the sample, i.e. $1.5x10^9$ phage particles in 0.15 ml nutrient medium supplemented with maltose and $MgSO_4$, was picking up about $4x10^{11}$ neutrons. The ratio of the number of neutron particles versus the number of atoms of all ingredients in the sample is very unfavourable. What can we do for the next experiment, because we can't exchange the intensity of irradiation? We have prepared the sample in a different way. As it seems, for the irradiation of λ under conditions of indirect action of low fluks of neutrons, it is very important to purify the phages of all possible impurities, because numerous organic substances have a protective effect even at low concentrations. For this purpose, the phage stock should be submitted to CsCl gradient high-speed centrifugation. In this way, the samples are pure phages and we can use 100 times more phages in remarkably smaller volumes of the sample. In this way, we suppose, the ratio of λ vs. neutron particle numbers will be succsessful and probability of collision will be certainly great.

At the same time, non-irradiated control and irradiated phages were plated on lawn of *recA*$^-$ bacterial host to take the number of spi^+ phenotype and speculate about changes of frequency of recombination. Of course, the frequency of recombination was at the natural level: from 1.5 x 10^9 spi^- pfu, 0.9% was spi^+ phenotype. This is in agreement with almost all phages surviving irradiation.

Although it has been established that double strand breaks are the most dangerous lesions in DNA caused by irradiation, many other lesions, called mutations, have not been clearly identified. These lesions can also be the source of dangerous chromosomal changes and cell death. To test irradiated phages for mutatios caused by neutrons, non-irradiated controls and irradiated phages, respectively, were plated on *recA*$^-$*uvrA*$^-$ double mutant bacterial host deficient in dsb repair and repair of major lesions caused by UV rays. We did not note differences between non-irradiated and irradiated phages, i.e. irradiation lacking mutagenic effect.

Finally, to test the stability of λ capsid proteins, samples of non-irradiated and irradiated λ were transferred to increased temperature and the number of survivors was screened for 10 days. Some differences were detected but they were not significant. After all tests, we can conclude that, under our conditions, low flux of neutrons does not induce neither DNA strand break or DNA mutation nor the stability of λ capsid proteins.

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