Effects of the Mixed Thermal Neutron-Gamma Radiation on Plasmid DNA From IEA-R1 Reactor of IPEN

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Abstract. The experimental results of the mixed thermal neutron-gamma radiation-DNA interaction are presented in this paper. The experiments were conducted at a research Boron Neutron Capture Therapy (BNCT) facility using the IEA-R1 reactor of IPEN-CNEN, Sao Paulo. This paper also includes the method used for neutron and gamma dose calculations. The fractions of supercoiled (undamaged), circular (resulted from single-strand breaks (SSBs)) and linear (resulted from double-strand breaks (DSBs)) molecules of plasmid DNA produced by the mixed thermal neutron-gamma radiation from the IEA-R1 reactor are presented.

1 Introduction

Nowadays, there is a growing interest in neutron interaction of DNA molecule. This is due to the fact that neutrons are used in the radiation treatment of cancer patients.

The interaction of ionizing radiation with living matter involves direct and indirect effects. In the direct effect, the ionizing radiation deposits energy directly in the DNA (ex. heavy particles, protons and neutrons). The indirect effect consists on the interaction of DNA molecules with the products of water radiolysis: hydroxyl radicals (OH), hydrogen atoms and aqueous-electrons (ex. X or gamma rays and

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electrons). The interaction is studied through the observation of single-strand and double-strand breaks of the DNA molecule. Radiation damage to DNA is evaluated by electrophoresis through agarose gels, which shows the conformation of the DNA after irradiation. The method used for neutron and gamma doses measurements is shown.

2 Materials and Methods

2.1 DNA sample specification

Plasmid DNA has been used for studying how radiation can induce breakage of DNA molecules [1-5]. Plasmids are a convenient system, because they are well-defined size and breakage detection is readily accomplished by gel electrophoresis.

DNA plasmid samples containing +2.961 kb of pBsKS were isolated from Escherichia coli using the lise alkali method; wich involves the extraction using organic solvents and separation by centrifuge in Cesium Chlorid. 15 μl-samples were placed in small eppendorf for the irradiation. A support was built to place each eppendorf. Also, a mechanism was introduced to aid the placement and removal of the eppendorf while the reactor was in operation.

The DNA sample at concentration of 50 ng/μl was prepared. One sample was used as a control and the others as irradiated. The DNA solutions were irradiated, in 0.5 ml eppendorf tubes (polypropylene material). The plasmid was purified according to [6]. In this irradiation, the plasmid DNA used was of approximately 10 % supercoiled form. This plasmid was originally 85 % supercoiled and degraded as time went by.

2.2 Neutron irradiation

Neutron irradiation was performed in the BH-3 at the research Boron Neutron Capture Therapy (BNCT) facility at the IEA-R1 reactor of IPEN-CNEN. See figure 1. The optimal filters sets were obtained by means of Monte Carlo simulations in a previous work[7].

The reactor was operated with 3.5 MW. The neutron field is compound for: 48% of the thermal neutrons, 13 % of epithermal neutrons and the 19% of the fast neutrons. For this cause prevail the (n,γ) radiative capture in this irradiation field. The contribution of gamma photons (originating from the reactor) was about 20% of the fluence.

Gold activations foils were used to the thermal and epithermal neutron fluxes measurements, while applying the Cadmium rate technique in each mixed-field radiation. The LiF Thermoluminescent dosimeter was used to estimate the gamma exposure of the DNA, which constitutes itself an unavoidable contamination of the

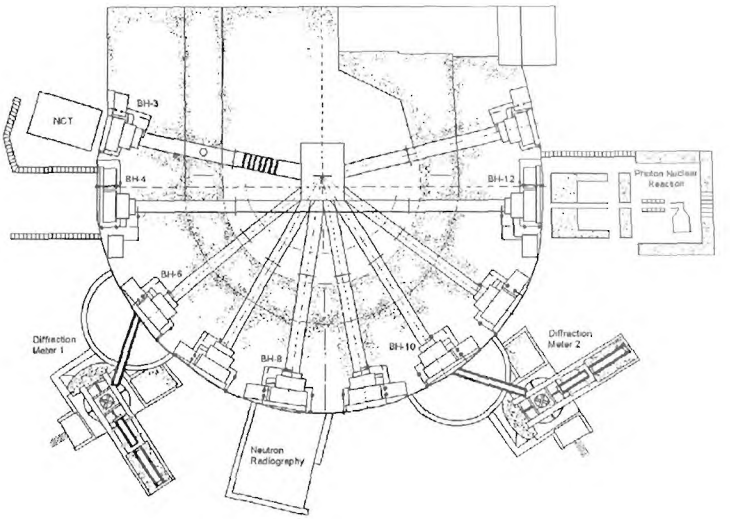

Figure 1 Structural layout of the IEA-R1 reactor at IPEN-CNEN.

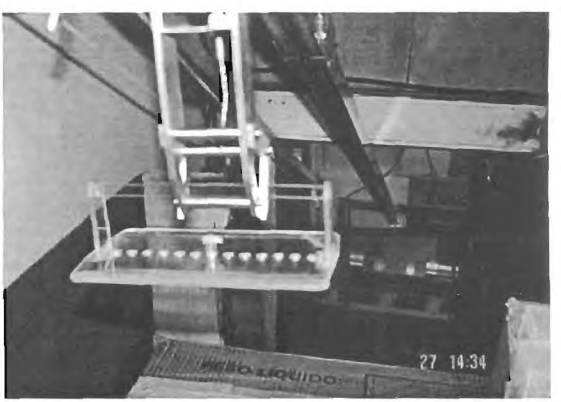

Figure 2 Mechanism to place and remove the sample in the irradiation beam-hole

thermal neutron field. The count rate was measured with a High Pure Germanium (HI'Ge) Detector. The neutron doses rate was of 2.8 Gy/h.

To place and remove the sample in the irradiation beam hole with the reactor in operation, a mechanism was construed. This mechanism is represented in fig. 2.

The system is formed by an Aluminum rail 10 m long, over which runs a 4x4 cm^2 wagon. In the experimental installation, the rail has a declined of 25 degrees and the wagon is manually moved through a flexible cable. Another similar cable is

used for moving a mechanical hand which is used to grasp the sample holders. The construction was carried out in the facilities of IF–USP (Brazil).

2.3 Electrophoresis and DNA quantification

Supercoiled DNA (form I) consists of the undamaged plasmid, circular (form II) resulting from single- strand breaks (SSBs), and linear (form III) resulting from double-strand breaks (DSBs).

The agarose gel was prepared with 0.7% of agarose, and 5 μL de ethidium bromide. 10 μl samples were placed in each well. The run time lasted 2 h under 75 volts potencial.

The gel images were obtained in the EAGLE EYE II transilluminator, using a CCD camera connected to a computer. This way, the images were stored in BMP format with a 640x480 pixel resolution. Agarose gel electrophoresis (figure 3) show a decrease in the amount of the supercoiled form, an increase in that of the circular form, and the appearance and increase in the amount of the linear form upon irradiation. The damage quantification of the DNA bands was obtained with the program Gel Analysis[8]. We used pictures with three integrations, wich presented a low background. Also, the mass ladder was used in the mass calibrations of the samples. Furthermore, a factor of 1.7 was applied to the adherence correction of the Ethidium Bromide in the supercoiled DNA.

2.4 Neutron and gamma doses measurements

The characteristics of the mixed radiation field were determined with gold activation foils and TLD-100 (7.5% 6Li) at a reactor power of 3.5MW.

The photon and thermal neutron doses must be determined separately:

$$D = D_n + D_\gamma$$

Neutron measurements were performed using an Au-197 activation foils. After neutron irradiation, the Au-197 foil is activated. The neutron flux is obtained after activation rate calculations. Gold foils were irradiated both bare and covered by 0.6 cm thick of Cadmium (Cd) foils. In order to measure the epithermal neutron flux the Au foils is covered with Cd. With the Cd foil in place, all neutron contributions with energies less than 0.4 eV are neglected. Neutron flux to kerma conversion factors were used to determine the thermal and/or epithermal (with Cd) neutron dose.

The gamma doses were carried out with TLD-100 dosimeters. It is more attractive for gamma measurements. The Li natural (TLD-100) thermoluminescent detectors are most used in mixed neutron and gamma fields, due to the high thermal neutron capture cross section (941 barns).

3 Results and Discussions

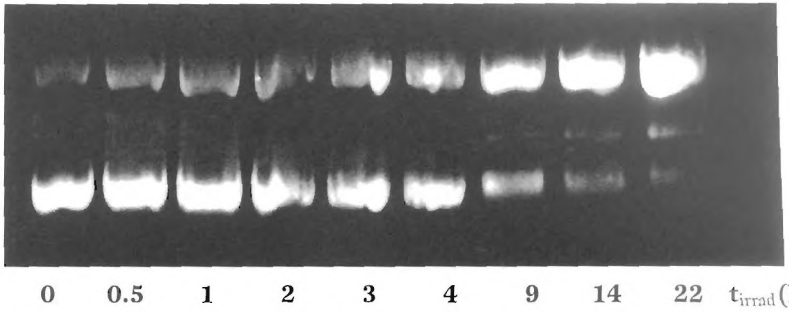

Figure 3 Ethidium fluorescence for samples with 50 ng/μl irradiated at the BH-3

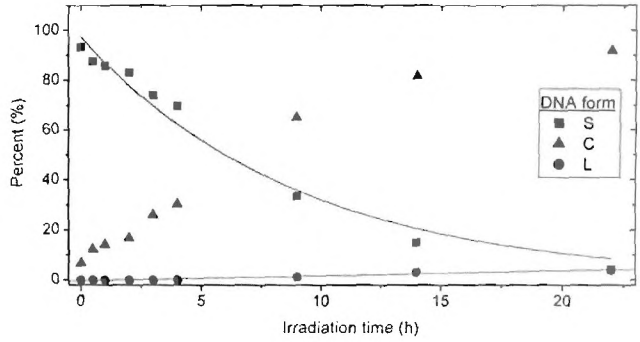

Figure 4 Percent of the forms of plasmid: supercoiled(S), circular(C) and linear (L) for thermal neutron-gamma mixed radiation as a function of irradiation time.

We fit an exponential curve to the data of the supercoiled conformations. The fraction of supercoiled form(S) is the decrease decay function of the irradiation time and can be well fitted by the following equation: $S = A^{*}\exp(B^{*}t)$, where t is the irradiation time:

Parameter	Value	Error
A	97.4	3.1
B	-0.111	0.011

The linear conformations were fit to a linear equation, $L = A * t$,

Parameter	Value	Error
A	0.180	0.016

The three sets of data show the expected behavior. During irradiation, the amount of supercoiled form decreases, while the circular- and linear forms increase, due to the the production of SSB and DSB.

4 Conclusions

1. A better understanding of the neutron radiation effects in the DNA molecule is reached.

2. When the DNA molecules are irradiated in a mixed thermal neutrons and photons field the damage is produced due to both the neutrons and the gamma rays.

3. It is illustrated the constructed mechanism for positioning and removing of the sample in the irradiation beam hole with the reactor in operation.

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