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Rate of double strand breaks of genome-sized DNA in tritiated water: Its dependence on tritium concentration and water temperature

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Abstract. The goal of this study is to establish a simple experimental system to examine the rate of double strand breaks (DSBs) of genome-sized DNA molecules under irradiation of β-rays from tritium under well-controlled conditions for the validation of computer simulation on interactions of biomolecules and ionizing radiation. Irradiation effects were insignificant at tritium concentration of 1300 Bq/cm^3, indicating that the effects of β-rays were far smaller than those of oxidation and/or thermal motion at the low dose rate (4.3 μGy/h). Clear increase in DSB rate was observed at tritium concentrations of 3.0–4.0 MBq/cm^3. The temperature dependence of DSB rate was examined by using the high concentration tritiated water.

Keywords: Tritium, DNA, Double strand break, Single molecule observation method

1. Introduction

Development of nanoscopic computer simulation methods on interactions of biomolecules including DNA with ionizing radiation would provide better understanding of biological effects of radiation [1-3]. In parallel, a relatively simple experimental system easy to be simulated should also be developed for the validation of computer simulation results. Tritium is a radioisotope of hydrogen emitting low energy β-rays (≤18.6 keV) and one of the major radioisotopes released from existing nuclear facilities and future fusion power plants. From these viewpoints,

the authors have developed a molecular dynamics technique for simulating structural change of DNA by the disintegration of tritium replacing to protium in DNA [4,5] and an experimental technique to examine double strand breaks (DSBs) of genome-sized DNA in tritiated water under well-controlled conditions [6,7]. In this experimental technique, the single molecule observation method developed by Yoshikawa et al. [8-11] was employed to measure the length of each DNA molecule and evaluate the rate of DSBs. The single molecule observation method allows quantitative evaluation of DSBs per unit length of genome-sized giant DNA, while such evaluation is rather difficult with other methods including the comet assay and single cell gel electrophoresis assay [11]. Clear irradiation effects of β-rays from tritium on DSB rate was observed in the previous study at tritium concentration of 5.2 MBq/cm^3 and water temperature of 10 °C [6]. However, the dose rate was far higher than the value expected for public exposure. In addition, it was found that DSBs occur even in the non-radioactive sterilized water at a noticeable rate.

In this study, the effects of β-ray irradiation on the rate of DSBs was examined at far lower tritium concentration (1300 Bq/cm^3) at the same water temperature as the previous study. Then, the temperature dependence of the rate of DSBs was examined in tritiated water and non-radioactive water to find optimum conditions to examine irradiation effects at low dose rate.

2. Experimental

Detailed experimental procedures are given elsewhere [6], and only important points are described here. Giant genome-sized DNA molecules of bacteriophage T4 GT7 (Nippon Gene Co., Japan) were used as samples. The nominal length of a DNA molecule is166 kilo base pairs (kbp) or 57 μm. The DNA molecules were immersed and suspended in sterilized tritiated water and non-radioactive sterilized water at 3 °C, 10 °C and 25 °C for 1–35 days. The tritium concentration was adjusted to be 1300 Bq/cm^3 and 3.0–4.0 MBq/cm^3. The corresponding absorbed dose rates were calculated as the product of mean energy of β-rays (5.7 keV) and the number of emitted β-particles by assuming all energy of β-rays was absorbed by water. The values thus evaluated were 4.3 μGy/h (Gy = J/kg) at 1300 Bq/cm^3 and 9.9–13 mGy/h at 3.0–4.0 MBq/cm^3. The concentration of DNA was set to be 17.5 nmol base pairs/cm^3. After mixing with a fluorescent dye, a drop of DNA solution was put on a glass substrate coated with poly-L-lysine, and then the DNA molecules were extended and fixed on the surface of glass substrate. The length of DNA molecules was measured using a fluorescence microscope. The number of DSBs N was evaluated from the average length of DNA L_{AVE} as:

$$N = L_0/L_{AVE} - 1, \quad (1)$$

where L_0 is the initial length of DNA [6-11]. The real value of L_0 was ~39 μm and smaller than

the nominal length due to DSBs occurred during handling of DNA molecules (pipetting, mixing, extension, etc.). If N is proportional to the immersion time t in water, Eq. (1) can be modified as

$$L_{AVE} = L_0/(\alpha t + 1) \quad (2)$$

where α is a constant.

3. Results and discussion

Figure 1 shows the change in L_{AVE} with immersion time in the non-radioactive sterilized water and sterilized tritiated water (1300 Bq/cm^3 and 4.0 MBq/cm^3) at 10 °C. The length of DNA molecules gradually decreased even in the sterilized water due to DSBs induced most probably by thermal motion and/or oxidation. These factors inducing DSBs in the non-radioactive water are hereafter called as the contributing factors to DSBs other than β-rays. It should be noted that L_{AVE} in the 1300 Bq/cm^3 tritiated water was comparable with that in the non-radioactive water. Namely, the effects of β-rays from tritium were not observable at 1300 Bq/cm^3 after the immersion for 35 days at 10 °C. On the other hand, clear increase in the rate of DSBs by β-ray irradiation was observed at 4.0 MBq/cm^3 even after the immersion for 7 days. These

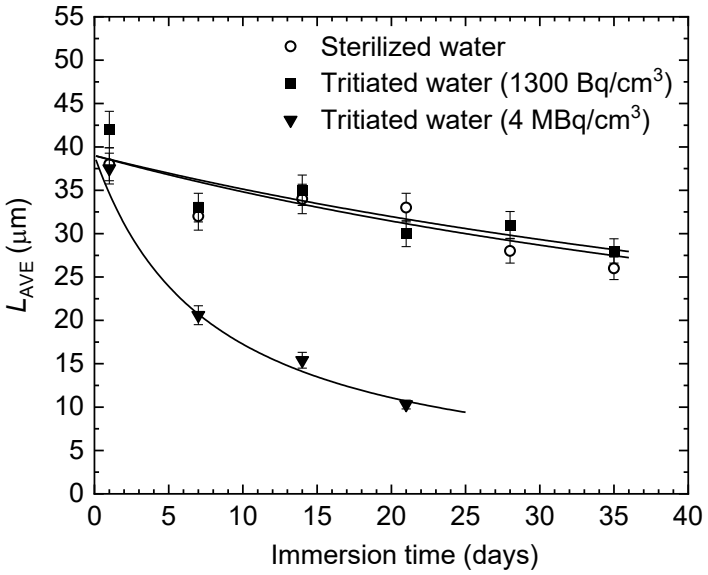

Figure 1: Change in average DNA length with immersion time at 10 °C and different dose rates (0 μGy/h in non-radioactive sterilized water, 4.3 μGy/h at 1300 Bq/cm^3 and 13 mGy/h at 4.0 MBq/cm^3). Lines were obtained by fitting Eq. (2) to experimental data by setting L_0 = 39 μm.

observations indicate that the effects of β-ray irradiation was far smaller than those of the other contributing factors at the low dose rate (4.3 μGy/h).

The change in L_{AVE} in the non-radioactive sterilized water and sterilized tritiated water (3.0 MBq/cm^3) at 3 °C and 25 °C is shown in Fig. 2. The rate of DSBs in the non-radioactive sterilized water at 25 °C was clearly higher than that at 3 °C. This acceleration was ascribed to the enhancement of thermal motion and/or oxidation at the higher temperature. The DNA length in the tritiated water was shorter than that in the sterilized water at both 3 °C and 25 °C. It should be noted that the temperature dependence of DSB rate was observed also in the tritiated water.

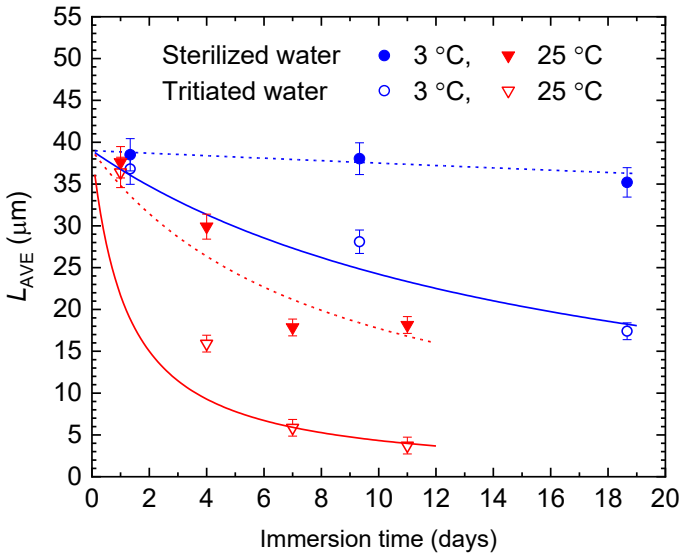

Figure 2: Change in average DNA length with immersion time in non-radioactive sterilized water and sterilized tritiated water (3.0 MBq/cm^3) at different temperatures (3 °C and 25 °C). Lines were obtained by fitting Eq. (2) to experimental data by setting L_0 = 39 μm.

The number of DSBs N was calculated from L_{AVE} given in Figs. 1 and 2. The values of N in the non-radioactive water are plotted as a function of immersion time in Fig. 3 (a), while the correlation between β-ray dose and N is given in Fig. 3 (b). The latter figure shows solely the contributions of β-rays which were evaluated from the difference in L_{AVE} between the non-radioactive water and tritiated water at the same temperature. N was in proportion to the immersion time and β-ray dose under the present conditions, as assumed in the previous section. The rates of DSBs were calculated from the slopes in Figs. 3 (a) and (b) in the unit of DSBs/day/100 kbp for the non-radioactive water and DSBs/Gy/100 kbp in the case of tritiated

water, as summarized in Table 1. It is clear that both the rates of DSBs induced by β-ray irradiation and the other contributing factors increased with increasing temperature. This means irradiation effects and thermal effects are synergistic with each other. Such synergism should be taken into account in computer simulation codes for evaluation of irradiation effects on biomolecules. One of the possible mechanisms underlying this synergism is that a single strand break (SSB) induced by radiation developed to DSB by thermal effects and vice versa. It is also possible that a minor damage induced by radiation developed to a strand break by thermal effects and vice versa. However, further investigation is required for more detailed understanding of the mechanisms underlying the observed synergism. Nevertheless, it is evident that the influence of contributing factors to DSBs other than β-rays is weaker at lower temperature. Hence, an experiment at low temperature (i.e., 3 °C) is more suitable for examination of β-ray irradiation effects at a low dose rate.

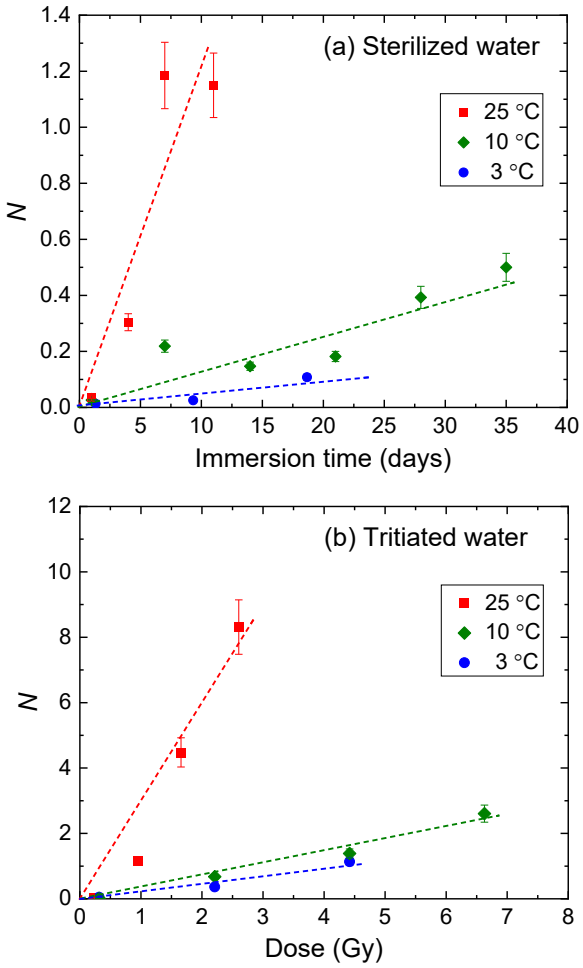

Figure 3: Correlation between N and immersion time in non-radioactive sterilized water (a) and that between N and β-ray dose in tritiated water (3.0–4.0 MBq/cm^3) (b).

In the end, the current status and future directions of computer simulation of DNA strand breaks are mentioned. Recently, reactive molecular dynamics simulations of DNA have begun to reveal the molecular mechanisms of hydrogen atom abstraction from DNA backbone and SSBs [12]. However, DSBs have not been reproduced by molecular simulation. This may be due to the short timescale of the molecular simulation (~1 μs at most). Besides, molecular simulations of DNA are usually performed at 300K or 310K, but simulations should also be done at much lower temperatures to make comparisons with experiments. On the experimental side, on the other hand, it is desirable to construct an efficient method to detect SSBs such as nicks and gaps.

Table 1: Temperature dependence of rate of DSBs caused by β-ray irradiation in tritiated water (3.0–4.0 MBq/cm^3) and those induced by contributing factors other than β-rays.

(a) DSBs caused by β-ray irradiation

Temperature (˚C)	3	10	25
DSB rate (DSBs/Gy/100 kbp)	0.24±0.03	0.36±0.03	2.9±0.3

(b) DSBs induced by contributing factors other than β-rays

Temperature (˚C)	3	10	25
DSB rate (DSBs/day/100 kbp)	0.004±0.001	0.012±0.002	0.12±0.03

4. Summary

The simple experimental system to examine DSB rate of DNA in tritiated water was proposed for validation of computer simulation of radiation effects on biomolecules. The rate of DSBs was examined in non-radioactive sterilized water and tritiated water at different tritium concentrations and water temperatures. No noticeable increase in the DSB rate was observed at 1300 Bq/cm^3 while the irradiation effects of β-rays were evident at higher tritium concentration (> MBq/cm^3). Both the rates of DSBs in the non-radioactive water and the high concentration

tritiated water increased with increasing water temperature. It was concluded that an experiment at low temperature is more suitable to examine the irradiation effects of β-rays at a low dose rate. Issues in both computer simulation and experiment were discussed.

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