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Detection of DNA double-strand breaks in boron neutron capture reaction

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HIGHLIGHTS

- The number of double-strand breaks increased with the neutron dose.
- The single-strand breaks increased with the neutron dose and the ^{10}B concentration.
- Our model can quantify the number of DNA breaks regardless of the repair mechanism.

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ABSTRACT

We evaluated DNA double-strand breaks (DSBs) induced by boron neutron capture reaction (BNCR) using plasmid DNA, boron solution, and gel electrophoresis. The amount of the linear form of DNA produced by DSBs increased with the neutron-beam irradiation dose. The amount of the open-circular form of DNA produced by single-strand breaks (SSBs) increased with the neutron-beam irradiation dose and the ^{10}B concentration. The model facilitated quantification of BNCR-induced DSBs and SSBs, irrespective of the DNA repair mechanism.

1. Introduction

Boron Neutron Capture Therapy (BNCT) is a tumor cell-targeted radiotherapy based on the ^{10}B (n,α) ^{7}Li reaction, which results in the release of high linear energy transfer (LET) α(^{4}He) and ^{7}Li particles. These high LET particles cause DNA double-strand breaks (DSBs) and produce strong biological effects. Because the path lengths of α(^{4}He) and ^{7}Li particles are almost equal to the diameter of a single tumor cell, ^{10}B-containing tumor cells are selectively destroyed in theory, minimizing radiation injury to normal tissue.

DSBs induced by boron neutron capture reaction (BNCR) have been evaluated by immunochemical staining for detecting the phosphorylation of core histone variant H2AX (gammma-H2AX) foci (Kinashi et al., 2011; Masutani et al., 2014) and p53-binding protein 1 (53BP1) foci (Kinashi et al., 2011, 2014; Okumura et al., 2013). Gamma-H2AX molecules appear in discrete nuclear foci at the sites of DSBs immediately after irradiation (Rogakou et al., 1999) and colocalize with DNA repair proteins, such as 53BP1 (Rappold et al., 2001). Since gammma-H2AX and 53BP1 foci are markers of DNA repair as well as markers of DSBs, these foci are unlikely to detect DSBs selectively. Strand break assays with plasmid DNA and gel electrophoresis are conventionally used for analyzing radiation-induced DSBs regardless of the DNA repair mechanism (Hempel and Mildenberger, 1987; van Touw et al., 1985). The plasmid DNA is originally in the supercoiled form (SC-DNA) and changes to the linear form (Lin-DNA) following introduction of DSB or the open-circular form (OC-DNA) by introduction of single-strand break (SSB) (Fig. 1A). These different forms of plasmid DNA can be separated by gel electrophoresis (Hempel and Mildenberger, 1987; Roots et al., 1985; van Touw et al., 1985).

Quantification of DSBs induced by BNCR would help to elucidate the mechanism of tumor cell death and to identify boron compounds that could effectively induce DSBs. The strand break assay has been used in fast neutron radiotherapy for measuring additional SSBs and DSBs generated by BNCR as a method for boron neutron capture enhancement (Sèche et al., 2002); however,

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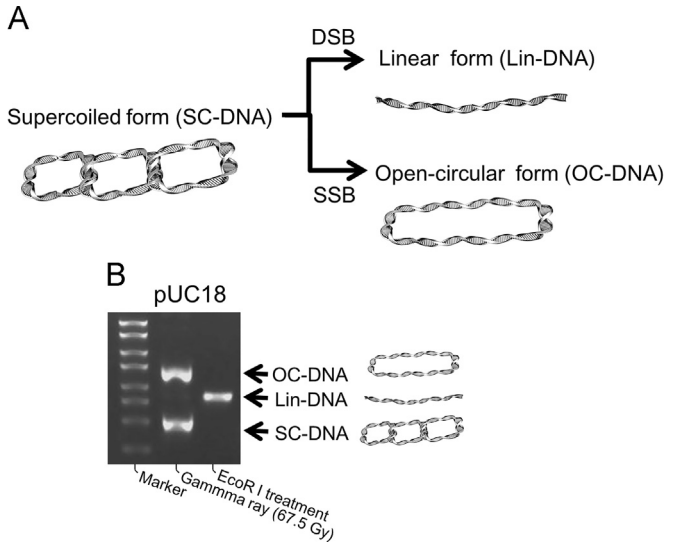

Fig. 1. (A) Conformational changes in plasmid DNA caused by DSBs and SSBs. (B) Gel electrophoresis of a control sample with plasmid pUC18.

this technique has not yet been applied to BNCT. Therefore, the objective of our study was to evaluate DSB independent of the DNA repair mechanism and to assess the relationship between the number of DSBs and the radiation dose in BNCT.

2. Materials and methods

2.1. Materials

Plasmid DNA, pUC18 (2686 bp; Takara Bio Inc., Shiga, Japan), was diluted with 1 × Tris–EDTA (TE) buffer (10 mM Tris–HCl, 1 mM EDTA · 2Na, pH 8.0) to make a 0.25 g L^{-1} solution. We used standard boron solution (1000 mg B L^{-1}), which contained 19.9% of ^{10}B (Wako Pure Chemicals, Tokyo, Japan). As a control for the Lin-DNA produced by DSBs, nonirradiated pUC18 solution was treated with *Eco*RI (Fig. 1B).

2.2. Irradiation experiments

Neutron irradiation was performed at the Heavy Water Facility of the Kyoto University Research Reactor. To elucidate the relationship between the number of DSBs and the dose of neutron beam irradiation, a plasmid DNA solution (5 μL) with boron solution (5 μL) containing 100 mg L^{-1} of ^{10}B in a polypropylene tube (0.2 mL; NIPPON Genetics Co., Ltd., Tokyo, Japan) was irradiated for different times (90, 180, and 270 min). The total absorbed doses are shown in Table 1. To assess the relationship between the number of DSBs and the ^{10}B concentration, plasmid DNA solution (5 μL) was mixed with boron solution (5 μL) containing different concentrations of ^{10}B (0, 20, 50, and 100 mg L^{-1}) and irradiated for 90 min. The total absorbed doses are shown in Table 2. The absorbed dose rates of thermal (< 0.5 eV), epithermal (0.5eV – 10 keV), fast neutrons (> 10 keV), and gamma rays in the neutron mixed beam were 1.3×10^{-2}, 1.3×10^{-3}, 9.56×10^{-3}, and 1.44×10^{-2} Gy min^{-1}, respectively. The dose rate of ^{10}B (n,α)^{7}Li was 7.1×10^{-3} Gy min^{-1}mg^{10}B L^{-1}. As a control for the OC-DNA produced by SSBs, a plasmid DNA solution (10 μL) in a polypropylene tube was irradiated with ^{137}Cs gamma rays (Gamma Cell-40) at a dose rate of 7.4×10^{-1} Gy min^{-1} (Fig. 1B).

2.3. Post-irradiation analysis

The irradiated samples were mixed with 1 μL of loading dye (TOYOBO, Osaka, Japan) and then placed into the wells of a 1% agarose gel (Reliant Gel System, Lonza Japan, Tokyo) containing 0.5 mg L^{-1} ethidium bromide. The samples were typically run at 100 V in TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA2 Na, pH 8.0) for 35 min at room temperature. After electrophoresis, the gel images were captured with an imaging system (Tyhoon FLA 7000; GE Healthcare Japan Corporation, Tokyo, Japan). The separated band intensities were measured by gel analysis software (ImageQuant TL; GE Healthcare Japan Corporation). The amounts of Lin-DNA, OC-DNA, and SC-DNA relative to total plasmid DNA were calculated. The data were analyzed by Tukey's honestly significant difference test. Differences with p values of less than 0.05 were considered significant.

3. Results

Gel analysis of the samples containing 100 mg L^{-1} of ^{10}B with irradiation doses of 0, 67.5, 135, or 202.4 Gy showed that the relative amount of Lin-DNA in pUC18 increased with the total physical dose (Fig. 2). The maximal value of Lin-DNA was approximately 2.3% with a dose of 202.4 Gy (Fig. 2B). In addition, the relative amount of OC-DNA increased with the total physical dose (Fig. 2). Significant increases in OC-DNA were observed at 135 and 202.4 Gy (Fig. 2B).

Analysis of the samples containing different concentrations of ^{10}B (0, 20, 50, and 100 mg L^{-1}) after a 90-min irradiation revealed that the relative amount of Lin-DNA did not differ significantly among the ^{10}B concentration, although there was a tendency to increase with the ^{10}B concentration (Fig. 3). The relative amount of OC-DNA was significantly higher at a ^{10}B concentration of 100 mg L^{-1} than at a ^{10}B concentration of 0 mg L^{-1} (Fig. 3B).

4. Discussion

In the present study, the relative amount of Lin-DNA increased with the dose of neutron beam irradiation. Sèche et al. (2002) reported that the number of DSBs per plasmid increases linearly with the dose of fast neutron beam irradiation (with a range of 0–15 Gy) using plasmid DNA (pOC203, 4565 bp) in the presence of

Table 1
Total doses of neutron irradiation for different irradiation times.

Irradiation time (min)	^{10}B Concentration (mg L^{-1})	Thermal neutron flux (cm^{-2} s^{-1})	Dose (Gy)					Total dose (Gy)
			Thermal neutrons	Epithermal neutrons	Fast neutrons	Gamma rays	^{10}B (n,α)^{7}Li	
0	100	0	0	0	0	0	0	0
90	100	5.8×10^{12}	1.2	0.12	0.86	1.3	64	67.5
180	100	2.9×10^{12}	2.4	0.24	1.72	2.6	128	135.0
270	100	1.9×10^{12}	3.6	0.36	2.58	3.9	192	202.4