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Development of New Dosimetry Using Extended DNA Fibers

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We applied fluorescent microscopy to monitor the damage of DNA upon exposure to γ radiation. Our developed dosimetry demonstrated that the number of breaks in DNA is proportional to the dose of the irradiation but is not dependent on dose rate of the irradiation and the GC content of DNA.

[**Key words:** DNA fiber, radiation, dosimetry, fluorescent microscopy]

The fundamental method for the detection of the external radiation has remained largely unimproved for a long time. Passive personal dosimeters comprised of photographic emulsions had been used since the beginning of the 20th century. Recent developments of various types of the dosimeter such as a glass dosimeter (1), an electronic dosimeter (2) and an optically stimulated luminescence dosimeter (3), have made them more attractive for use in routine personal dosimetry. The reliability of the devices has also been improved but the cost of their personal dosimeters remains expensive. If a novel personal dosimeter, whose price is more competitive with the simpler passive dosimeters, is developed, the use of personal dosimeters will explosively increase and become normal for all radiation workers in the world. Since dosimetry using biological materials can evaluate the influence without the specificity of radiation species, various types have been developed (4). Particularly because the main target of irradiation is DNA, some researchers proposed some dosimeters based on conformational changes of DNA using capillary electrophoresis (5) and ligation-mediated PCR (6). Recent development of visualization methods enable us to see genetic materials directly even by optical microscopy (7). Optical mapping is a convenient method in the construction of restriction maps by the direct confirmation of restriction sites of individual DNA molecules using a fluorescent microscope (8). This method has been used to prepare restriction maps of λ bacteriophage DNA (9), bacterial artificial chromosomes (BACs) (10), and, more recently, the whole genome of *Deinococcus radiodurans* (11). Therefore, we embarked on the development of a new dosimetry of absorption dose using DNA double strand breaks (DSBs) in conjunction with fluorescent microscopy.

Lambda bacteriophage DNA (Takara, Otsu) and five human bacterial artificial chromosome (BAC) clones (Nigorikawa Rikakogyo, Tsukuba) including RP4-650H14 (accession no. AL158217), RP11-156A19 (accession no. AL139392), RP3-511E16 (accession no. AL050328), RP11-145L22 (accession no. AL023694) and RP1-302G2 (accession no. AL391258), were used for this study. Purification and isolation of λ bacteriophage DNA and BAC clones were performed by Quntum Prep Maxi kit (BioRad, Hercules, CA, USA) and Large-Construct kit (Qiagen, Valencia, CA, USA), respectively. Purified BAC clones were digested with *Not*I for 2 h at 37°C. Ten μl aliquot of 5 μg/ml DNA solution was put onto a slide glass which was derivatized with 3-aminopropyltriethoxysilane (Matsunami, Osaka). DNA molecules were elongated and fixed using the flow and adhesion forces generated when a fluid sample is compressed between a coverglass and the slide glass. A cover glass was put on the aliquot and pulled at a speed of 200 μm/s using a handmade motor. DNA fibers were air-dried for 6 h. Preparations were irradiated with 0 to 30 Gy of γ ray at the dose rate of 0 to 15 Gy/h by the Cobalt-60 irradiation. DNA was stained with 1,1′-(4,4,7,7-tetramethyl-4,7-diazaundecamethylene)-bis-4-[3-methyl-2,3-dihydro-(benzo-1,3-thiazole)-2-methylidene (YOYO-1) (12). DNA images were observed by blue excision at 490 nm and yellow emission at 520 nm under a BX 52 fluorescence microscope (Olympus, Tokyo). The fluorescent images were captured by a cooled CCD camera (Cool-SNAP HQ; Olympus) and stored into a computer. DNA break numbers per DNA molecule were counted by a program, AutoCal, which is written in the C programming language. This program consists of three processes including making to binary, removal of unsuitable fragments and measurement of spots for each length.

Figure 1A shows a fluorescent image of a typical extended λ bacteriophage DNA stained with YOYO-1 before irradiation. Since the λ bacteriophage DNA is 48,502 bp in length, the extended DNA has a 16.5 μm. Before irradiation, artifacts by physical stress through the preparation of extended DNA were automatically removed in calculation of the gap by our developed software AutoCal. The soft-

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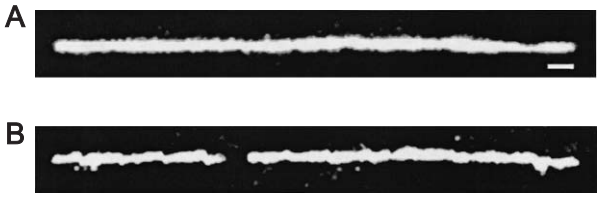

FIG. 1. Digital fluorescent micrographs of extended DNA. (A) Extended λ bacteriophage DNA on the 3-aminopropyltriethoxysilane-treated glass surface before irradiation. (B) A DNA molecule after irradiation of 6 Gy γ ray. Gaps represent cleavage sites by irradaition of gamma ray. A scale bar indicates 1 μm.

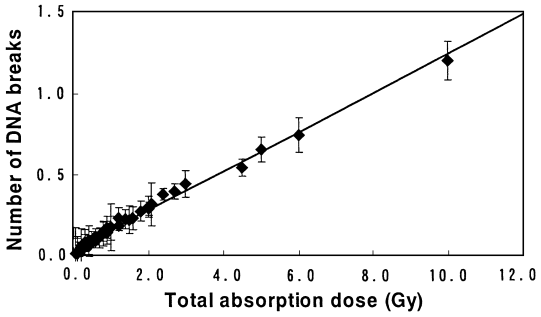

FIG. 2. Relationship between DNA breaks of extended λ bacteriophage DNA and total absorption dose.

ware can remove fragments with the total length beyond the limits from 15 μm to 18 μm and intermittent fragments that are not one straight line. After irradiation, the number of gaps within an irradiated area on the slide glass was automatically calculated by AutoCal. When 6 Gy γ ray was irradiated to the DNA fiber, a break of DNA appeared as a gap (Fig. 1B). The size of gaps that ranged from 0.2 μm to 1.0 μm is consistent with that of gaps by digestion with restriction enzymes in optical mapping (8). To test a linear relationship between the total absorption dose and numbers of DNA breaks, the extended λ bacteriophage DNA on the slide was irradiated with γ ray from 0 Gy to 6.0 Gy. Figure 2 shows that the average numbers of DNA breaks was plotted against the total absorption dose. Each number of breaks was generated from 50 images. The linearity between total absorption dose and the number of DNA breaks is seen in the range of total dose of 0 Gy to 10 Gy ($y=0.1196x+0.0413$, $r^2=0.9898$). The relationship between the amount of energy deposited in the DNA helix and the subsequent production of DNA breaks has been investigated (13, 14). For example, by agarose gel electrophoresis, the dose effect curve for DSB induction in the 4.3 kb of a pBR322 plasmid was linear in the range from 0 Gy to 15 Gy (15). Our automatic measurement using AutoCal also indicated that the number of breaks of the λ bacteriophage DNA is proportional to the dose of γ ray.

Next, in order to asses the relationship between the DNA breaks and dose rate, the extended λ bacteriophage DNA on the slide was irradiated with γ ray at the dose rate from 0.05 Gy/h to 1.0 Gy/h. The dose was fixed to 3 Gy but the exposure time was changed. Each number of breaks in these 11 samples was generated from 50 images. Figure 3 shows that the number of DNA breaks is roughly 0.6 even if the

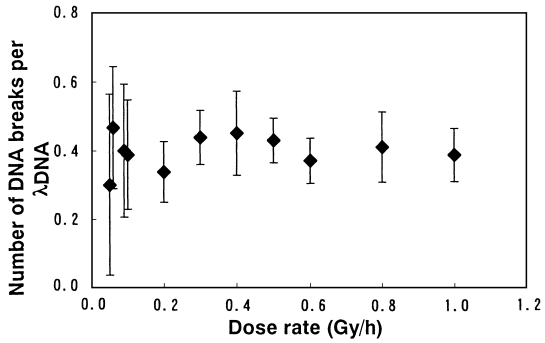

FIG. 3. Relationship between DNA breaks of extended λ bacteriophage DNA and dose rate.

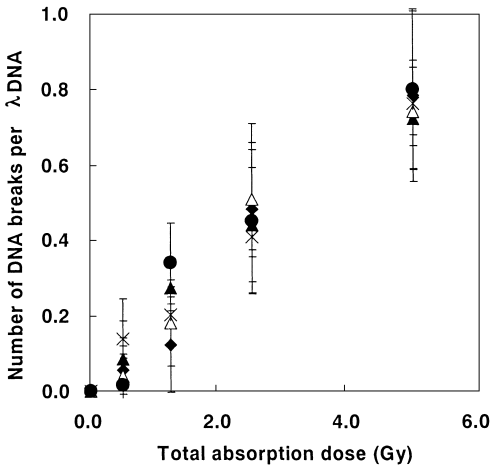

FIG. 4. Relationship between DNA breaks of five extended DNAs with different GC contents and total absorption dose. Closed squares, closed triangles, opened triangles, closed circles and asterisks show the number of breaks per λDNA using RP3-511E16, RP11-145L22, RP1-302G2, RP4-650H14 BAC clones and λDNA, respectively.

ratio changes. It also clearly demonstrates that the DNA breaks were not correlated with dose rate in the range between 0.05 Gy/h and 1.0 Gy/h, although the variance of the data at the low dose rate is fairly high. The independence of dose rate is reasonable because of our dosimetry using purified DNA without a DNA repair system.

Figure 4 shows the relationship between the total absorption dose and numbers of breaks of four different BAC clones as shown in Table 1. The maximum difference of GC contents in these BAC clones is approximately 20%. The number of DNA breaks in these BAC clones is proportional to the total absorption from 0 Gy to 5.0 Gy. The difference of the inclination among BAC clones was a range of average errors. This indicates that the difference of GC contents in these BAC clones give no effect on DNA breaks.

TABLE 1. GC contents of DNA

Clone no.	GC content (%)	Length (kb)
RP3-511E16	41	56.6
RP11-145L22	46	54.1
λDNA	50	48.5
RP1-302G2	54	61.2
RP4-650H14	60	54.0

Our results demonstrate that extended DNA fibers are suitable and simple biological materials for dosimetry mainly in the following three points. First, the number of breaks in DNA is proportional to the dose of the irradiation but is not dependent on dose rate of the irradiation and the GC content of DNA. This result indicates that all DNAs from various organisms are useful for this dosimetry. Second, our procedure is based on easy and rapid visualization methods. Finally, our developed method makes it possible to evaluate the influence of radiation on the biological material directly. Although the sensitivity of our dosimetry using extended DNA fibers is lower than that of conventional dosimetry, the dosimetry will give a new measurement to directly estimate the damage of the biological material. In conclusion, dosimetry using extended DNA fibers meets the proposed requirements for a personal biological dosimeter and it is applicable to the practical evaluation of personal dose of irradiation.

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