Double-strand breaks in genome-sized DNA caused by photo-irradiation, gamma-rays and ultrasound

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Abstract. We evaluated double-strand breaks (DSBs) in genome-sized DNA (T4 DNA; 166 kbp) caused by photo-irradiation, gamma-rays and ultrasound through a single DNA observation by fluorescence microscopy in a quantitative manner. Based on experimental results, DSBs were induced by two-step mechanism for the photo-irradiation. On the other hands, one-step mechanism led to DSBs for the irradiations of gamma-ray and ultrasound. Regarding the effect of ultrasound, it was found that DSBs were generated above a threshold power. It is suggested that the experimental methodology of single DNA observation serves as a useful tool for studying DSBs of genome-sized DNA.

Keywords: Double-strand break, Genome-sized DNA, Single DNA observation, Photo-irradiation, Gamma-ray, ultrasound

1. Introduction

There is increasing evidence that DNA damages, including single- and double-strand breaks, cross-links and base modifications caused by various environmental factors, induce mutagenic and carcinogenic processes. Among these damages, DNA double-strand breaks (DSBs) are thought to be the most biologically significant lesions in living cells. A number of in vivo and in vitro studies have been performed to detect DSBs. The comet assay, or single-cell gel electrophoresis assay, is a rapid and sensitive method for the detection of DNA strand breaks in individual cells [1, 2]. However, intact cells are too complicated to analyze DNA strand breaks in a quantitative manner. Recently, it has been shown that giant DNA molecules, larger than several tens of kilo base-pairs, are more useful for studying various physico-chemical reactions on DNA by single-molecule observation [3-5]. We have also adapted this technique for detection of DSBs [6-13]. Here, we describe our recent works regarding the applicability of this method for a comparative study of DSBs induced by different...
2. Results and Discussion

Figure 1 indicates the logarithm of percentage of surviving DNA molecules, $X$, in photo-irradiation as a function of square of irradiation time, $t^2$ [6]. Experiential results shows that $\ln X$ decrease linearly with increasing $t^2$.

![Figure 1](image1.png)

Figure 1: Left: Real-time monitoring of DSB of a T4 DNA molecule in solution by fluorescence microscopy. Right: Probability of double-strand breaks in photo-irradiation as a function of $t^2$ [6].

Figure 2 shows the average number of DSBs caused by gamma-ray irradiation, $<n>$, as a function of irradiation dose of gamma-ray [10]. We found that the average number increases linearly as irradiation dose increase.

![Figure 2](image2.png)

Figure 2: Left: Fluorescence microscopic images of DNA molecules after irradiation with different doses of gamma-ray. Right: The number of DSBs vs. the irradiation dose of gamma-ray at different DMSO concentrations [13].
Shown in Fig.3 (a) are the average number of DSBs caused by ultrasound with frequency of 30 kHz in the absence and presence of ascorbic acid (AA) [11]. It is known that AA suppress the generation of reactive oxygen species (ROS) in aqueous solutions. We found a threshold pressure on the DSBs around 40 kPa and the average number of DSBs increase linearly with increasing the pressure of ultrasound above the threshold pressure, and the addition of AA does not contribute to the number of DSBs significantly. Figure 3 (b) shows the protective effect on DSBs of AA for phot-, gamma-ray and ultrasound irradiations. The experimental results have indicated that the addition of AA lead to the largest amount of reduction on the number of DSBs in the irradiations. This mean that ROS cause dominant contribution on DSBs in photo-irradiation. Meanwhile, mechanical stresses results in DSBs in ultrasound irradiation instead of ROS.

Based on the experimental results, we reveled that DSBs induced by gamma-ray and ultrasound have the linear relationship for the irradiation dose of gamma-ray and pressure of ultrasound above the threshold, respectively. Meanwhile, DSBs caused by photo-irradiation depend on the square of irradiation duration, $t^2$. We have described the dependence of $t^2$ as follows: first, the amount of increase on the number of nicks along a single DNA molecule is given by

$$\frac{dn}{dt} = \alpha t,$$

(1)
where \( n \) is the number of nicks in a single DNA molecule, \( \alpha \) is a constant and \( I \) is an intensity of photo-irradiation [9, 11]. One obtain the number of nicks in the irradiation duration as \( n = \alpha It \) with the initial condition of \( n = 0 \) at \( t = 0 \). Considering the probability of surviving DNA molecules against double-strand damage, \( X \), the probability is described by the following equation with a rate constant \( k \).

\[
\frac{dX}{dt} = -knX = -\alpha ItX. \tag{2}
\]

Then we obtain

\[
\ln\left(\frac{X}{X_0}\right) = -(1/2)\alpha It^2. \tag{3}
\]

After some arrangement with the initial condition of \( X = X_0 \) at \( t = 0 \), the probability of \( X \) is represented by

\[
\ln(X) = -At^2, \tag{4}
\]

where \( A = (1/2)\alpha \alpha \). As the next, we will discuss the plausible mechanism on DSBs caused by gamma-ray and ultrasound irradiations and propose the following model on DSBs on gamma-ray and ultrasound irradiations. The number of DSBs in gamma-ray and ultrasound irradiations, \( m \), will be described as,

\[
dm/dP = \beta, \tag{5}
\]

where \( P \) is a dose of gamma-ray or pressure of ultrasound and \( \beta \) is a constant. After integration of eq.(5) with the initial condition of \( m = 0 \) at \( P = 0 \), the numbers of DSBs in gamma-ray and ultrasound irradiations are expressed by \( m = \beta P \). The observations in Figs.2 and 3 indicate such linear relationship, suggesting that single-step mechanism in gamma-ray and ultrasound damages.[8-14] Based on the above argument on DSBs due to photo-irradiation, we consider a two-step mechanism as a mechanism due to DSBs in photo-irradiation. The two-step mechanism is described as follows: First, a single-strand break is caused in DNA molecules, then another single-strand break is occurred nearby the broken paired strand. Meanwhile, one-step mechanism leads to DSBs in the irradiations of gamma-ray and ultrasound. The one-step mechanism means that two single-strand breaks both sides of double-stranded helix are induced by the irradiations simultaneously.

3. Conclusion

We have evaluated double-strand breaks of genome-seized DNA (DSBs) caused by pho-
to-irradiation, gamma-ray and ultrasound adopting the methodology of single DNA observation with fluorescence microscopy. Based on experimental results, the one-step mechanism leads to DSBs in irradiations of gamma-ray and ultrasound. While, DSBs due to photo-irradiation are induced by the two-step mechanism. Regarding DSBs due to ultrasound irradiation, we found the threshold pressure around sound pressure of 40 kPa. The methodology of single DNA observation with fluorescence microscopy is useful tool to estimate double-strand breaks of genome-sized DNA in a quantitative manner. ROS takes an active role on DSBs in photo-irradiation. While mechanical stress cause DSBs in ultrasound irradiation.

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**References**


