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# The influence of DNA concentration in the experiment of DNA damage induced by $^{7}$Li ions and γ rays

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## ABSTRACT

**To evaluate the influence of the DNA concentration in the aqueous solution on DNA radiation damage, the plasmid DNA in the presence or absence of Mannitol (scavenger of free radical $OH^{\cdot}$) was irradiated by $^{7}$Li ions and γ rays at various DNA concentrations. Gel electrophoresis analysis revealed that the DNA damage of single and double strand breaks induced by irradiation was dependent on DNA concentration and became more severe at lower DNA concentration in the radiation experiment when all others parameters are the same. In the condition of γ-ray irradiation, most of double strand breaks (DSB) damage was neutralized and less associated with DNA concentration in the presence of mannitol. However, under $^{7}$Li irradiation, the DSB damage could not be cleared by mannitol but was gradually aggravated with decreasing DNA concentrations. Our study sheds light on the underlying mechanisms in the DNA radiation damage process. And there are potential significances for the human space flight, cancer therapy by heavy ions as well as the radiation security assessment.**

**Keywords:** Heavy Ions; Plasmid DNA; DNA Concentration; γ Rays; Strand Break

## 1. INTRODUCTION

DNA is considered to be the most important biomacromolecule and target responsible for most of the biological effects in the cells. Many kinds of damage can be induced by radiation, e.g., base damage, single strand breaks (SSB), double strand breaks (DSB) and crosslink of DNA and protein [1]. DNA double strand breaks are considered to be the most important initial damage initiating serious biological consequences post-radiation. In particular, unrejoined DSBs may result in cell mutation or cell death. The DNA damage could be affected by radiation dose, quality of radiation, dose rate, etc. [2]. In contrast with the low Linear Energy Transfer (LET) radiations, the heavy-ion irradiation with higher LET and Bragg peak of dose distribution can produce more complicated track structure and stimulates biological havoc in organisms, tissues, cells, and DNA [3,4].

In previous experiments, it has been noticed that the DNA strand-break damage is related to DNA concentration. Milligan *et al*. have measured the yield of single strand breaks G(SSB) induced by γ rays ranging from 0 Gy to 100 Gy and assayed the DNA samples by agarose gel electrophoresis in the presence or absence of scavengers at various DNA concentrations. The result showed that the G(SSB) had small fluctuation over a wide range of DNA concentration in the absence of scavenger. In the presence of DMSO, G(SSB) was proportional to the DNA concentration, rising as the concentration increasing. At higher DNA concentration, the G(SSB) approached a plateau value[5,6]. The similar result was obtained by SHAO *et al*. [7] in 2000. However, these researches were focused on DNA damage induction at low-LET radiation. So in order to evaluate the influence of the DNA concentration in the aqueous solution on DNA radiation damage induced by low-LET and high-LET radiation, in this work, the high-LET heavy ions of $^{7}$Li was first employed in the plasmid DNA damage in addition to the low-LET γ rays with various DNA concentrations in the presence or absence of free radical scavenger (Mannitol).The distribution of DNA conformations and the number of DSB per DNA were statistically analyzed. It is found that the degree of DNA radiation damage has an obvious relationship with the DNA concentration, whereas this effect could be partially attenuated by scavenger at low-LET radiation. This work will redound to understanding of ionizing

radiation damage to the organisms and provides basic experimental data for the human space flight, cancer therapy by heavy ions as well as the radiation security assessment.

## 2. MATERIALS AND METHODS

### 2.1. DNA Sample

The purified DNA sample, double-stranded pUC19 plasmid (2686-bp long) was purchased from TaKaRa Biotechnology Co., Ltd., (Dalian, China) at a concentration of 500 ng/μl in TE buffer. The original forms of DNA sample are a mixture of supercoiled (SC) form (~90%) and open circular (OC) form. Mannitol was supplied by Beijing 306 Hospital at a concentration of 1.1 M and was diluted to 600 mM for experiment.

### 2.2. γ Rays and $^{7}$Li Ions Irradiation

DNA samples in a series of concentrations were irradiated by γ rays of $^{60}$Co source located in China Institute of Atomic Energy (CIAE) at the dose of 482 Gy (dose rate = 10 Gy/min) at room temperature.

Appling 37.3 MeV $^{7}$Li ions (LET = 70.2 keV/μm, in aqueous solution) generated by HI-13 heavy ion tandem accelerator of CIAE, the DNA samples in various concentrations were irradiated at the dose of 500 Gy (48.4 Gy/min) at room temperature. The range of $^{7}$Li ions in water is about 307 μm. For all irradiations, a 20 μl aliquot of DNA solution was dropped onto the middle of a 5 μm-thick Mylar film and sealed with another Mylar film. An Au-Si surface barrier semiconductor detector was deployed to monitor the number of incident ions, then radiation dose can be determined by formula (1). All presented values of energy, LET and dose are at the entrance surface of the solution. After irradiation, the samples were preserved in the micro centrifuge tube at –20˚C prior to be used. The irradiated dose is defined as follows:

$$\text{Dose(Gy)} = 1.6 \times 10^{-9} \times \text{LET(keV/μm)} \times \text{F(ions/cm}^2\text{)} \times 1/\rho \ \text{(g/cm}^3\text{)} \quad (1)$$

where F is the fluence of ions and ρ is the density of the medium.

### 2.3. Agarose Gel Electrophoresis

The control and irradiated DNA samples were loaded on 1% agarose gel and electrophoresed for 90 min at 4V/cm. After electrophoresis, the gel was stained with ethidium bromide and visualized on a UV-transilluminator. The DNA bands were photographed and analyzed by AlphaImager Imaging System (Alpha Innotech Corporation, San Leandro, CA) to quantitate the fraction of the three kinds of DNA conformation (*i.e.*, supercoiled, open circular and linear form of the DNA molecules).

## 3. RESULTS

### 3.1 The Result of γ Rays Irradiation

DNA samples were irradiated by γ rays at a series of concentration from 10 to 100 ng/μl by the dose of 482 Gy. In **Figure 1**, lanes 1, 8 are unirradiated control samples. From lane 2 to lane 7, the DNA concentrations are 100、50、40、30、20 and 10 ng/μl, respectively ( without mannitol) and samples in lane 9 to lane 14 are arranged in the same order (in the presence of 600 mM mannitol). It is shown that at lower DNA concentration, the SC form DNA disappears and the fraction of linear (L) form DNA increases. Meanwhile, the fraction of OC form DNA reduces with the decreasing concentration in the absence of mannitol in DNA solution. Moreover, the short linear DNA fragments appears when the concentration is lower than 50 ng/μl, until all of DNA molecules are broken into very short fragments at the concentration of 10 ng/μl. This tendency indicates that DNA is damaged more seriously at lower DNA concentration. In **Figure 1(b)**, 600 mM mannitol was added in the DNA solution. Comparing to **Figure 1(a)**, the linear form disappears, on the contrast, most of the original SC form molecules are preserved and its fraction dropped by decreasing DNA concentration. Nevertheless, the SC molecules tends to transform into OC conformation under DNA concentration of 30 ng/μl, indicating that the SSB damage could not be eliminated by scavenger at lower DNA concentration. Generally, it is confirmed that DNA damage can be neutralied by mannitol to a great extent but is still depends on DNA concentration.

The fractions of three kinds of DNA forms versus different DNA concentrations without or with 600 mM mannitol are shown in **Figure 2** and **Figure 3** respectively. It is revealed in **Figure 2** that the fraction of OC form dramatically rises from 0% to 75% and the fraction of linear form decreases from 100% to 25% with the increasing DNA concentrations in the absence of mannitol. In the presence of 600 mM mannitol (**Figure 3**),

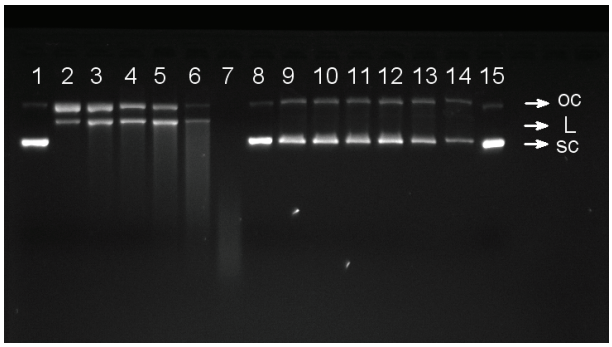



**Figure 1.** Gel electrophoresis image of DNA after irradiated by γ rays at various concentrations.

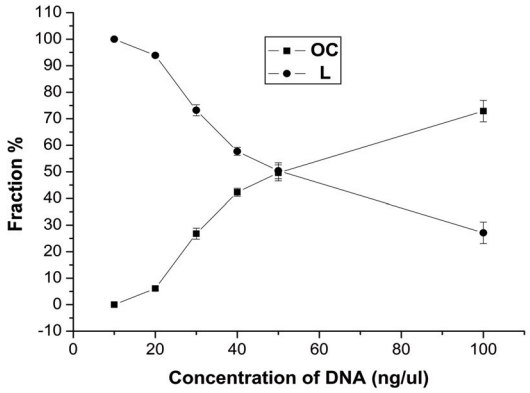



**Figure 2.** Distribution of DNA OC form (■) and L form (●) irradiated by γ rays at various DNA concentrations without mannitol.

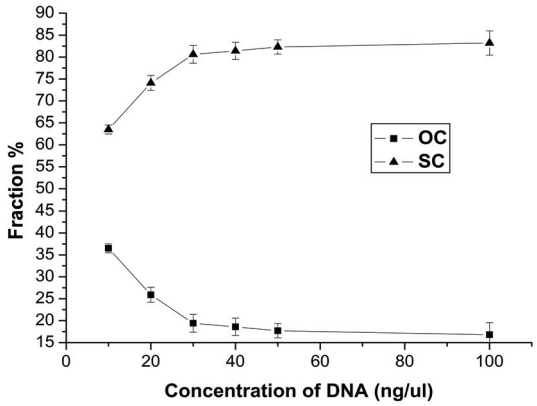



**Figure 3.** Distribution of DNA OC form (■) and SC form (▲) irradiated by γ rays at various DNA concentrations with 600 mM mannitol.

linear form of DNA molecule disappears, which means there is no DSB damage when $OH^{\cdot}$ is eliminated. The SC form is maintained at the concentration above 30 ng/μl. The conformational change from SC to OC only occurs at lower DNA concentrations (20 ng/μl and 10 ng/μl). Even though, there is still 60% of original SC form at the concentration of 10 ng/μl. Therefore, it can be postulated that almost all DNA damage by γ rays irradiation is caused by diffusion of free radical $OH^{\cdot}$ and associated with DNA concentration, which is consistent with related reports [8-11].

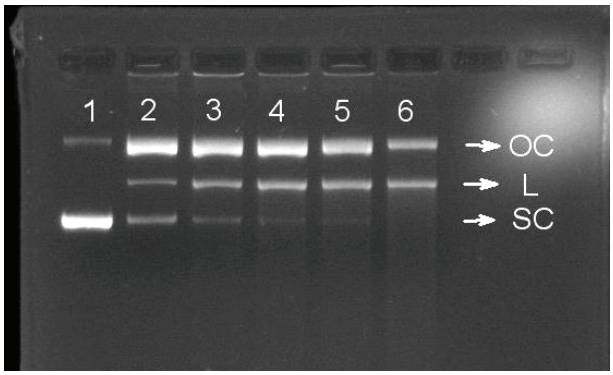
## 3.2. The Result of $^{7}$Li Ions Irradiation

### 3.2.1. The Result of DNA Damage in the Absence of Mannitol

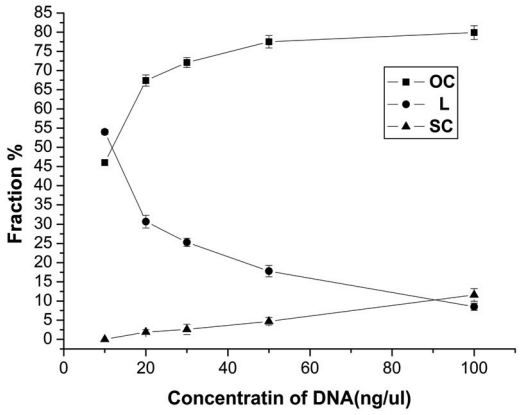
**Figure 4** shows the gel electrophoresis image and form distribution of DNA irradiated by $^{7}$Li ions at different concentrations in the absence of mannitol. In **Figure 4(a)**, lane 1 is the control sample and DNA concentrations are 100, 50, 30, 20 and 10 ng/μl from lane 2 to lane 6, respectively. It is shown in **Figure 4(a)** that the OC form decreases but the linear form increases with the decreasing DNA concentrations, which is similar with the result irradiated by γ rays. Different from the disappearing of SC form irradiated by γ rays, the SC form decreases irradiated by $^{7}$Li ions with the decreasing DNA concentration. The fraction of SC is less than 15% and the fraction of linear form is only 10% at the DNA concentration of 100 ng/μl. The maximum fraction of linear form is up to 55% at the DNA concentration of 10 ng/μl in the absence of mannitol(shown in **Figure 4(b)**).

### 3.2.2. The Result of DNA Damage in the Presence of 600 mm Mannitol

**Figure 5** shows the gel electrophoresis image and form distribution of DNA irradiated by $^{7}$Li ions at different concentrations in the presence of 600 mM mannitol. It is shown in **Figure 5(a)** that, since DNA are protected by




(a)




(b)

**Figure 4.** (a) Gel electrophoresis image after irradiated by $^{7}$Li ions at various DNA concentrations without mannitol; (b) Distribution of DNA OC form (■), L form (●) and SC form(▲) after irradiated by $^{7}$Li ions at various DNA concentrations without mannitol.

mannitol compared to the **Figure 4(a)**, the influence of DNA concentration is still exit. However, different from the γ-ray result, the linear form still exists in the presence of 600 mM mannitol, indicating that part of DSB damage comes from the direct ionizing energy deposition onto the DNA molecules and does not disappear because of any radical scavenger. The data in **Figure 5(b)** shows that the severity of DNA damage is attenuated since the most of OH˙radicals are eliminated by mannitol. Hence the maximum fraction of undamaged SC form reaches about 70% at higher DNA concentrations. However the DNA damage is still aggravated with decreasing DNA concentration from the decreasing of SC form and increasing of linear form.

### 3.3. The DSBs/DNA Induced by γ Rays and $^{7}$Li Ions

Based on the data presented in above, the number of DSB per single DNA molecule (DSBs/DNA) is calculated by **Eq.(2)** [12].

$$\text{DSBs/DNA} = f(LI)/[1 - f(LI)] \quad (2)$$

where $f(LI)$ *is* the fraction of linear form of DNA.

#### 3.3.1. The DSBs/DNA Induced by γ Rays

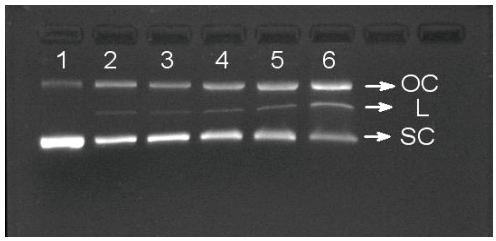
In **Figure 6**, the curve shows the DSBs/DNA at different DNA concentrations in the absence of mannitol except the concentration of 10 ng/ul since the fraction of linear form is 100% at this concentration. It is indicated that the yield of DSBs per DNA averagely increases as the decreasing DNA concentration, especially at the very lower DNA concentration. In the presence of 600 mM mannitol, the $f(LI)$ is zero (shown in **Figure 1** and **Figure 3**) so the DSBs/DNA can not be calculated from the formula (2) which means that there is no DSB in DNA samples after irradiated by γ rays. It is indicated that the major damage from γ rays irradiation is the free radical and most free radicals can be neutralized by mannitol. So one DNA was attacked by more free radicals at very lower DNA concentration without mannitol.

#### 3.3.2. The DSBs/DNA Induced by $^{7}$Li Ions

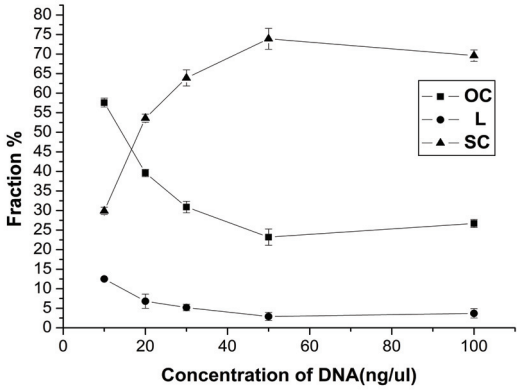
In **Figure 7**, it is shown that the DSBs/DNA increase when the DNA concentrations decrease either with or without scavenger. Since the yield of DSB decreases in evidence when DNA samples are added 600 mM mannitol compared with the yield of DSB without mannitol. But the DSB damage still considerable in the presence of mannitol, which implies that these DSB sites are resulted from the local multiple damage induced by direct ionizing of $^{7}$Li ions, thus cannot be prevented completely by scavenger [13] and is dependent on DNA concentration.

## 4. DISCUSSIONS

After irradiated by γ rays, the DNA concentration influences the experimental result when all others parameters are the same. It would probably be result of interaction between DNA and free radical OH˙. The plentiful yield of OH˙is generated by the radiolysis of water when the DNA aqueous solution is irradiated by γ rays. During irradiation, the concentration of free radical in water could be assumed roughly steady and may be higher than the concentration of DNA molecules. At lower




(a)




(b)

**Figure 5.** (a) Gel electrophoresis image after irradiated by $^{7}$Li ions at various DNA concentrations with 600 mM mannitol; (b) Distribution of DNA OC form (■), L form (●) and SC form(▲) after irradiated by $^{7}$Li ions at various DNA concentrations with 600mM mannitol.

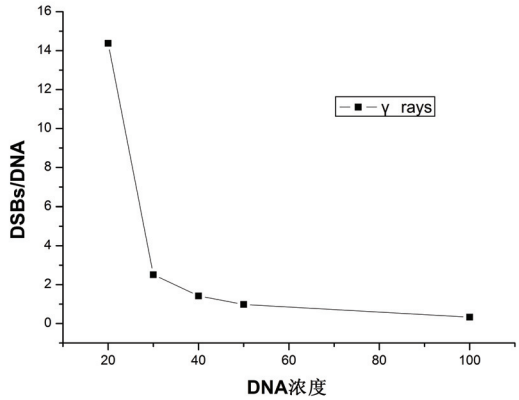



**Figure 6.** The DSBs/DNA at various DNA concentration irradiated by γ rays.

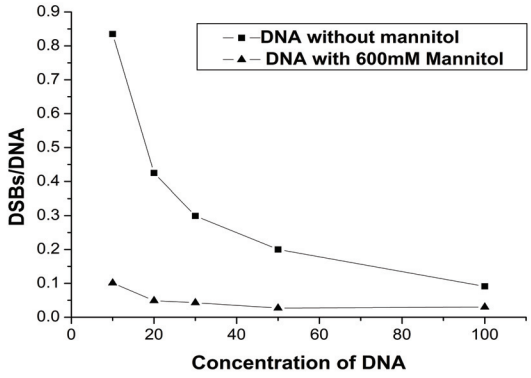



**Figure 7.** The yield of DSBs/DNA at various DNA concentration irradiated by $^{7}$Li ions. ■ and ▲ show the DSBs/DNA without mannitol and with 600 mM Mannitol, respectively.

DNA concentration, more number of free radicals reacting with one DNA molecule, *i.e.* a single DNA molecule is under attack statistically by more free radicals and DNA is damaged seriously as well as the change of DNA forms is obvious. On the other hand, the gross amount of free radicals is much lower than that of DNA molecules especially at higher DNA concentration in the presence of mannitol, which result that the number of free radicals reacting with DNA is reduced. Therefore the effect of DNA concentration associated with strand break can be neglected. Thus the DNA form is less affected by DNA concentration when the DNA concentrations larger than 20ng/μl as shown in **Figure 3**. At the same time, the linear DNA fragments vanished, only the small variety of OC form occurs at the lowest DNA concentration. It is indicated that the influence of free radical is main reason of DNA damage. Once cleared by scavenger, the number of residual free radicals is not enough to produce double strand breaks. And this implies that DNA damage largely come from indirect effects during DNA damage induced by γ rays.

Comparing **Figure 2** and **Figure 4(b)**, it is found that the change of DNA forms after irradiating by γ rays is more obvious than that irradiated by $^{7}$Li ions when the DNA concentration decreases in the absence of mannitol. In the presence of 600mM mannitol, there are no L form but only SC form and OC form existence after irradiated γ rays (shown in **Figure 3**). However, there are still some linear molecules left despite the existence of mannitol after irradiated by $^{7}$Li ions and their fraction increases with the decrease of DNA concentrations (shown in **Figure 5**). These parts of DNA damage should be the result of direct ionizing energy deposition of $^{7}$Li ions and thus cannot be eliminated by scavenger. Under γ rays irradiation, the DNA, γ photon and the free radicals induced by photons distribute uniformly in water so that the probability of interaction between DNA and the free radicals is relatively high, hence DNA damage is severe. Under $^{7}$Li ions radiation, the situation is different due to the presence of track structure of high-LET $^{7}$Li ions. A lot of secondary electrons and free radicals induced by $^{7}$Li ions aggregate near the tracks and even form complex track structure [14]. The concentrations of secondary electrons and free radicals reduce exponentially along the radial distance of track. Thus the probability of interaction between DNA and ions reduces exponentially along the radial distance of track in the same tendency and only DNA close enough to the track structure can be damaged. So DNA damage induced by $^{7}$Li ions is alleviated than that of γ rays as the decreasing DNA concentrations. At higher-LET radiation, the interaction between DNA and heavy ions is a combined effect of direct ionizing and indirect free radical, [15,16] which is different from that of the low LET irradiation. When the DNA concentration increases, there are more DNA molecules around the track of ions, the probability of interaction between a DNA molecule and an ion track decreases accordingly. So the yield of strand breaks decreases as the increasing DNA concentration. Based on the same reason, in the presence of scavenger, even though most of free radicals have been eliminated，the SSBs and DSBs induced by direct ionizing still exist and decrease at higher DNA concentration. It is shown that DNA is damaged more severe by $^{7}$Li ions on the contrast to γ ray irradiation as shown in **Figure 3** and **Figure 5**, further verifying that DNA damages induced by heavy ions are the common results of direct and indirect interaction. So the DSBs/DNA still increases with decreasing DNA concentrations regardless of the presence of scavenger, because of direct interaction of $^{7}$Li ions (shown in **Figure 7**). But the influence in the conditions of without mannitol and with 600 mM mannitol is different. In **Figure 7**, when the DNA samples without mannitol, any decrease in DNA concentration makes the yield of DSBs increase apparently within the range of concentration of this experiment. However the yield of DSBs changes less at the higher DNA concentration when DNA with 600 mM mannitol. It is indicates that DNA concentration influence deeper in the course of DNA damage induced by indirect free radical than by direct ionizing.

## 5. CONCLUSIONS

In this paper, it is found that the DNA concentration is an important parameter in the course of DNA damage induced by $^{7}$Li ions and γ rays. Different DNA concentration may be result in dissimilar results, which are neglected in previous experiment and theory. These effects might be caused by associated factors including the free radical, the track structure of heavy ions, the probability of interactions as well as aggregation of DNA. This

finding there are potential significances for the human health especially for the space flight, cancer therapy by heavy ions as well as the radiation security assessment and would be a challenge to the theoretic research and strongly calls for further experiments.

## 6. ACKNOWLEGEMENTS

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