Comparison of DNA double-strand breaks induced by $^{16}O^{8+}$ in deproteinized DNA and intact cells

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Abstract The yield of DNA double-strand breaks (DSBs) is sure to be influenced by the environment around DNA molecule. Inverse pulsed-field gel electrophoresis (PIGE) has been applied to compare the sensitivity of B_{16} cells and their DNA in DSBs induced by 75 MeV/u $^{16}O^{8+}$ beam. Results show that the percentages of DNA released from the plug(PR) in both kinds of the samples increase with the dose and approach a similar quasi-threshold of about 81%. A simple new equation was presented to calculate the break level of DNA molecules. Within a certain dose, the relationship between the break level and the dose is linear. The yield of DSBs in deproteinized DNA was 1.11 DSBs/100 Mbp/Gy, while that in intact cells was 0.60DSBs/100Mbp/Gy. It is testified that deproteinized DNA is more sensitive to oxygen ions irradiation than intact cells.

Keywords DNA double-strand breaks, 75 MeV/u $^{16}O^{8+}$ beam, Melanomas cells

1 Introduction

Numerous experiments have proved that DSBs are the most important initial damages induced by ionizing radiation[1], whose formation and repair can cause various kinds of biological effects. In eukaryotic cell nucleus, DNA is organized tightly with histone proteins in the form of chromatin. There are many other components in the nucleus, such as nonhistone protein and RNA. The type and frequency of DNA damages induced by ionizing radiation are affected not only by the nature of the radiation, but also by the biomolecules surrounding the DNA fiber. Mee *et al.*[2] found that the DSBs yield in the DNA of chromatin relaxed with low-molarity salt was similar to that of intact mammalian cells. But, Warters *et al.*[3] reported that relaxing nuclear chromatin by exposing nuclei to low concentrations of monovalent salt increased the yield of radiation-induced DSBs by a factor of 4. However, the later report of them[4] belived that the DSBs yield of deproteinized DNA was 70 times greater than in intact cells, and it was 8.3 and 4.5 times to that of DNA in basic nucleosome repeat structure and in condensed chromatin fiber, respectively. Kitayama *et al.*[5] said that a 'cell wall and RNA free' DNA sample was more sensitive than 'packaged' and 'naked' one.

It seems to be necessary to study further the molecular mechanism of the mammalian cell radiosensitivity. This work compared oxygen ion irradiation-induced DSBs in deproteinized DNA and intact cells of B_{16}.

2 Materials and method

2.1 Cell culture

B_{16} cell line was routinely grown in RMPI-1640 medium supplemented with 20% calf serum, 100μg/ml penicillin, and 100units/ml streptomycin. The medium was held at 37°C in humidified atmosphere with 5% CO_2 and was refreshed once every two days. The cells were inoculated every three days.

2.2 Preparation of DNA samples

Cells were harvested with 0.025% trypsin and washed three times with D-Hank's solution, after they were inoculated for two days. The cells were suspended in D-Hank's and adjusted to 1×10^7 cells/ml, then 1% low-melting point agarose (Sigma) of same volume was added. The mixture was poured into plug-mould. When the plugs were formed, they were incubated in lysis buffer (0.5mol/L EDTA, 1% sarkosyl) containing 1 mg/ml proteinase K

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(Sigma) at 50°C for 48 h. Afterwards, plugs were washed twice in TE-buffer (10 mmol/L Tris, 1 mmol/L EDTA; pH8.0) and each time was no less than 2 h. At last, the plugs were kept in 0.5 mol/L EDTA (pH8.0) for irradiation.

2.3 preparation of cell samples

Cells were embedded into plugs as described above and kept in D-Hank's solution for irradiation.

2.4 Irradiation

The two kinds of samples for the same dose were held in a Petri-dish, at the bottom of which a piece of wet filter paper was placed to keep humidity. 75 MeV/u $^{16}O^{8+}$ was accelerated by HIRFL (Heavy Ion Research Facility in Lanzhou) and the samples were irradiated at room temperature.

After irradiation, DNA samples were kept in 0.5 mol/L EDTA (pH8.0) for electrophoresis and the cell samples were treated as the preparation of DNA samples.

2.5 Electrophoresis

The plugs were inserted in 0.8% agarose gel containing 0.5μg/ml ethidium bromide, and were subjected to inversion pulsed-field gel electrophoresis with 1.8 V/cm in 0.5×TBE-buffer (45 mmol/L Tris, 45 mmol/L boric acid, 1.25 mmol/L EDTA; pH8) at about 12°C for 72 h. The time ratio of the electric field alternation in two directions is 5:1.

After electrophoresis, the fluorescence of each lane in the gel analyzed with CS-910 TCL-scanner (Shimazu).

3 Results

The percentages of DNA released from the well (PR) increase and approach a threshold at about 40 Gy as the dose increases (see Fig.1).

Because the controls of both of samples have many DNA fragments eluted into the gel, the background was subtracted from each lane employing the function reported by Cedervall *et al.*[6] The calibrated results were shown in Fig.2. The quasi-threshold of DNA sample is equal to that of the cell sample, which is about 81%.

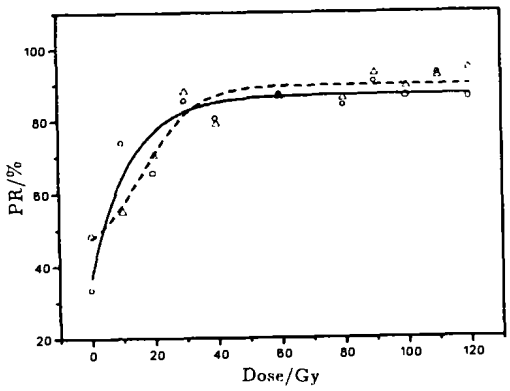

Fig.1 Percentage of DNA released from plug (PR) as a function of dose
o DNA; △ cell

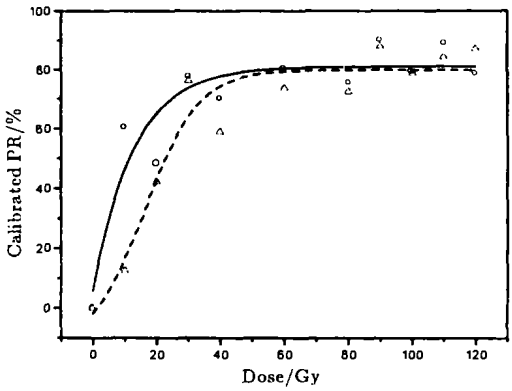

Fig.2 Calibrated PR as a function of dose
o DNA; △ cell

PR value is the total content of the radiation-induced DNA fragments eluted into gel, and it does not show their distribution. Suppose the size of the total DNA in one plug is S Mbp, then the size of the total DNA fragments released from the well is $S\times$ PR Mbp. If the average size of DNA fragments is T Mbp, the number of DNA fragments is $\frac{S\times PR}{T}$ and DSBs are $\frac{S\times PR}{T} - n$. Here n is the number of total chromosome in the plug. The break level (L) is $L = [\frac{S\times PR}{T} - n]/S$. The total size of DNA in a mammalian cell is about 6000 Mbp while the number of chromosome in one cell is no more than 60, so that $n/S \approx 0$. Therefore

$$L = \frac{PR}{T} \quad (1)$$

When the dose is less than 80 Gy, the break level gained with Eq.(1) has linear relationship with the dose. The yield of DSBs obtained in terms of this relationship in deproteinized

DNA is 1.11DSBs/(100Mbp·Gy), and that in intact cell is 0.60DSBs/(100Mbp·Gy) (Fig.3). These results are consistent with the recent acceptable ones that the DSBs yield in mammalian cells induced with X-rays, is 0.32~0.80 DSBs/(100 Mbp·Gy) and RBE of DSBs induced by heavy ions is about 1.[7~9] The sensitivity of deproteinized DNA to oxygen irradiation is 1.9 times as high as that of intact cells. This result located between those of Mee *et al.*[2] and Warters *et al.*[3]

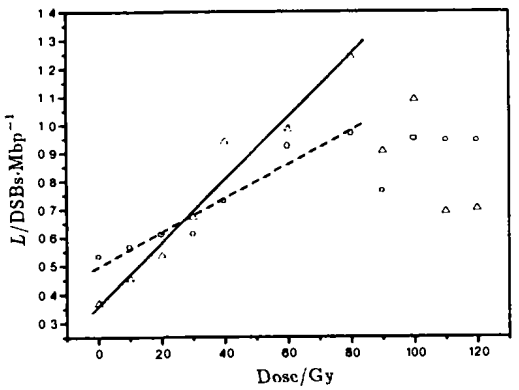

Fig.3 Break level (L) as a function of dose
—△—DNA: L=0.3601+0.0111D, r=0.9810, $P < 0.01$;
--o-Cell: L=0.4980+0.0060D, r=0.9722, $P < 0.01$

4 Discussion

There are two temperature transients during the preparation of DNA sample from irradiated monolayer cells. The first lies in the mixing of cell suspension and low-melting point agarose (about 50°C) to form plugs, and the other is that the plugs are digested with proteinase K in lysis buffer (50°C). Both temperature transients may permit DNA DSBs to be rejoined.[10] In order to avoid these influences and be suitable to compare with deproteinized DNA, we irradiated cells in plugs. But, there are still some difference between these samples, such as that cells are embedded with D-Hank's solution while deproteinized DNA fibers are surrounded by EDTA. But another experiment shows that EDTA does not badly affect the yield of DSBs induced by radiation.

Ager *et al.*[11] and Cedervall*et al.*[6] found that PR increased with the increasing dose and approached a quasi-threshold of ~81%, when they studied DSBs in CHO strain induced by X-rays. As dose increases from ~150 Gy to 500-750 Gy, the PR values approached 95~100%. Even if the dose is as high as 1000 Gy, there are still about 2% DNA retained in the well. The abnormality can be explained as that the overloaden dose causes the cross-linking between DNA and DNA, or DNA and protein. PR values induced by 75 MeV/u $^{16}O^{8+}$ approached the threshold at the dose of about 40 Gy. This may be due to the more serious damages caused by high-LET irradiation than low-LET one[12], or the character of this cancer cell strain.

Warters *et al.*[4] believe that the protection of DNA-binding protein causes the deproteinized DNA more sensitive than the intact cells. And the tighter the protein associated with DNA, the more efficient the protection is. Soluble protein and nonprotein sulfhydryls play a role in the protection, too. However, as the dose increases, PR of cell sample and DNA sample approaches a similar threshold. So, it can be believed that the protection of nuclear components is limited and relative. This may be due to an enhanced accessibility of radiation-induced radicals to DNA and a reduced local capacity of nuclear components to scavenge radicals, which will be completely lost at last.

It was recently reported[13] that DNA fragments induced by high LET particles distributed nonrandomly. The special marker method based on the random suppose did not work well in this experiment (see Fig.4), too. Although PR value reached the threshold at about 40 Gy, DNA fragments maybe shrank and the break level rise with the increasing dose, while PR value kept stable. This could not undertake the non-random distribution, but distinctly, it is necessary to develop a function to fit DSBs induced with high LET radiation. Eq.(1) is very simple in the form, but its principle is reasonable because of its base on the percentage and the size of DNA fragments. The linear relation between the break level obtained with this equation and the dose holds true in a certain dose range. As shown in Fig.3, there is a good linear relation between the break level and the dose only when the dose is less than 80 Gy. Probably, when the dose exceeds 80 Gy, the cross-linking happens and this equation does

not work normally.

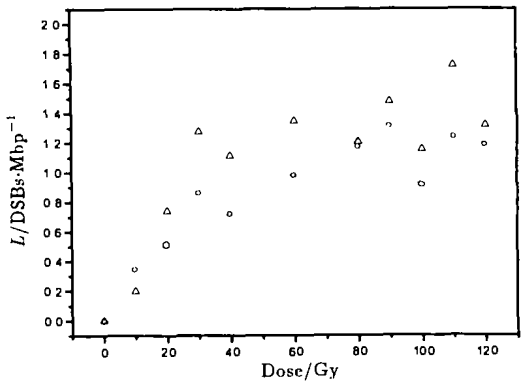

Fig.4 Break level (L) obtained with the special marker method
△ DNA; ○ Cell

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