Available online at www.sciencedirect.com
SCIENCE DIRECT®
Nuclear Science and Techniques, Vol.17, No.5 (2006) 272–275
NUCLEAR SCIENCE AND TECHNIQUES

Study of calf thymus DNA irradiated in vitro with MeV fluorine ions

MIAO Qi[1,2,*] LAI Jiang-Nan[2] XUE Jian-Ming[2] QIN Huai-Li[2] WANG Wei-Dong[1]
QIN Guang-Yong[1] WANG Yu-Gang[2]

([1]Henan Provincial Key Lab of Ionbeam Bioengineering, Zhengzhou University, Zhengzhou 450052, China;

[2]Key Laboratory of Heavy Ion Physics, Peking University, MOE, Beijing 100871, China)

Abstract A study of the fragments of DNA irradiated with MeV ions is important for the understanding of the DNA damage mechanism and the subsequent biological effects (induced by heavy ions). In this experiment, the products of calf thymus DNA (CT DNA) irradiated with MeV fluorine ions were analyzed using agarose gel electrophoresis, modified time-of-flight mass spectrometer (MALDI-TOF), and high-performance liquid chromatography (HPLC). The results showed that the molecular mass of the fragments were concentrated around 831 bp with agarose gel electrophoresis, there was no observable product in the range of 1,000 – 30,000 (m/q) using MALDI-TOF, and small biomolecules were separated from the products. The results of this study indicated that the strand breaks of calf thymus DNA induced by MeV fluorine ions were nonrandom.

Key words MeV ion, Irradiation, DNA, Damage

CLC numbers O571.32+4, Q691.5, Q523

1 Introduction

In recent years, low-energy ion biotechnology has attracted considerable attention because of its practical applications in many fields such as botanic mutation breeding and cancer therapy. With the development of new experimental techniques, studies on the bioeffects induced by low-energy heavy ions, especially on DNA irradiation damage, are being carried out extensively at the molecular level because the most critical initial damage is believed to be responsible for subsequent biological effects[1]. In the past decade, several results on both base damage and DNA damage have been reported[1-5], for example, showing the saddle-type of fluence-ssbs curve induced by keV ions[3,4] and the linear fluence-dsbs curve induced by higher energy ions[2], which are different from those induced by electrons, X-rays, and γ-rays. The mechanism of DNA irradiation damage is important but is not well understood. Thus far, only little information has been reported about experimental studies on DNA irradiated in vitro with MeV ions.

The objective of this study was to investigate how heavy ions damage the solid state DNA in vitro. Some preliminary results on CT DNA irradiated with MeV fluorine ions were presented, and the relationships among ion dose, energy, and irradiation product were discussed.

2 Experimental

2.1 Samples preparation

Calf thymus DNA (10 – 15 million daltons) used in the experiment was purchased from Sino-American Company. It was dissolved in 0.01 mol·L^{-1} KPB solution with the concentration of 2 mg·mL^{-1}, and 0.2 mL of the solution was then transferred using a microinjector (accuracy is ±0.1 μL) onto a special metal plate and allowed to dry naturally. Thus, a DNA film with mass thickness of 0.127 mg·cm^{-2} was

*E-mail: miaoqimiaoqi@126.com

Received date: 2005-12-17

formed.

After irradiation, the DNA was dissolved in 0.01 $mol \cdot L^{-1}$ KPB solution again for analysis.

2.2 Irradiation

The irradiation was carried out using a 2×1.7 MV tandem accelerator at Peking University. During irradiation, the density of ion beam current was kept lower than 4 $nA \cdot cm^{-2}$ to ensure that the sample temperature was kept as low as possible. In this experiment, 3 MeV F^+ and 4 MeV F^{2+}, with dose of 11×10^{12} and 30×10^{12} cm^{-2}, respectively, was used.

2.3 Sample analysis

2.3.1 Agarose gel electrophoresis assay

The irradiated sample was separated by agarose gel electrophoresis. The gel containing 1% agarose was run for 30 min at 7 $V \cdot m^{-1}$ electric field and stained by 1% ethidium bromide (EB, 5 $\mu L \cdot (100\ mL)^{-1}$). The gel was finally subjected to UV radiation, and a fluorescence image was recorded using a CCD camera. A DNA ladder marker was used for calibration.

2.3.2 Modified time-of-flight mass spectrometer (MALDI-TOF) measurement

A mass spectrum of the samples before and after irradiation was taken with a BIFLEX III modified time-of-flight mass spectrometer (MALDI-TOF) manufactured by American Bruker Company at Mass Spectrum Center of Beijing. The sample spectrum was measured in the range of 1,000 – 30,000 (m/q).

2.3.3 High-performance liquid chromatography (HPLC) measurement

Part of the samples after irradiation was deviated using HPLC method. The detection of the compounds was achieved using a UV – VIS variable wavelength detector (at 254 nm). AC-18 (4.61 mm × 250 mm) analytical column was purchased from Kromasil. The solvent was a mixture of H_2O and CH_3OH at a ratio of 90:10 (V/V), the flow rate was 1.0 $mL \cdot min^{-1}$.

3 Results and discussion

3.1 Agarose gel electrophoresis

Fig. 1 shows the fluorescence image of the agarose gel electrophoresis. Most of the CT DNA molecules contain about 20,000 bp, which is consistent with the real one (10 – 15 million daltons, that is approximately 15 – 22 kb). The lane of the control sample was scattered in a very wide range, which means that a DNA molecule that was in vacuum for 1 hour could be denatured. For irradiated samples, the fragment's mass was mainly about 831 bp. Compared with lanes *d* and *e*, lanes *f* and *g* were weak, which indicated that the dose had an obvious influence on the product. Moreover, the brightness of the lanes was also dependent on the ion energies. The 4 MeV fluorine ions were more efficient at producing the fragments with molecular weight around 831 bp. It was shown that the higher energy increased the amount of the products whose molecular weight was around 831 bp. Besides the fragments of 831 bp and the original CT DNA molecules, there were no other fragments observed with agarose gel electrophoresis, indicating that the damage to DNA molecules was nonrandom.

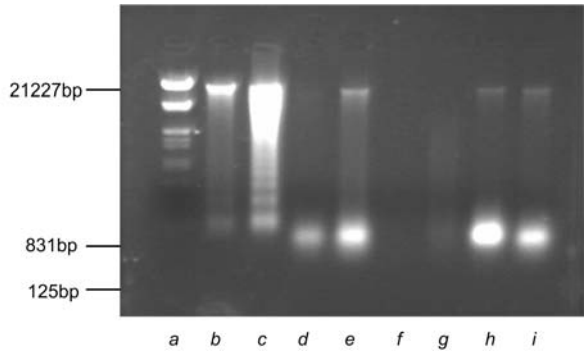

Fig. 1 Fluorescence image using agarose gel electrophoresis.

a, DNA ladder marker; *b*, original sample; *c*, control sample; *d*, *e*, sample irradiated with 3 MeV F^+ at the dose of 11×10^{12} cm^{-2}; *f*, *g*, sample irradiated with 3 MeV F^+ at the dose of 30×10^{12} cm^{-2}; *h*, *i*, sample irradiated with 4 MeV F^{2+} at the dose of 11×10^{12} cm^{-2}.

3.2 Modified time-of-flight mass spectroscopy (MALDI-TOF)

A mass spectrum of the sample irradiated with 3 MeV F^+ at the dose of 11×10^{12} cm^{-2} is shown in Fig. 2. No obvious mass peak was observed, which implied that the fragment mass was distributed randomly between 1,000 and 30,000, or the mass peaks did not exist at all, which implied that the fragment mass was

distributed nonrandomly.

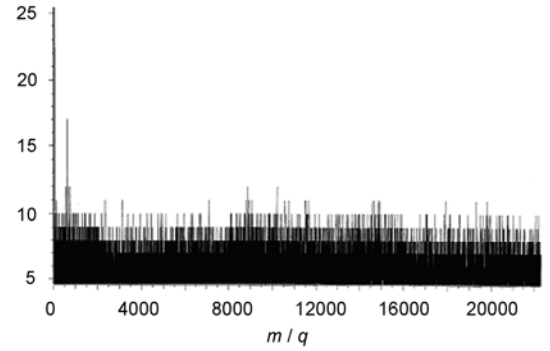

Fig. 2 Mass spectrum of CT DNA sample irradiated with 3 MeV F^+ at the dose of 11 × 10^{12} cm^{-2} using MALDI-TOF.

3.3 High-performance liquid chromatography (HPLC)

HPLC was used to detect the small fragment that has a mass lower than 1,000. The results are shown in Fig. 3. The spectrum of the sample irradiated at the dose of 11×10^{12} cm^{-2} had a new peak at 2.7 min, which was similar to the peak observed for retention time of thymine; however, it is difficult to confirm that this is really the thymine itself. At doses up to 30 × 10^{12} cm^{-2}, the peaks of small biomolecules were undetected because the small biomolecules produced by implanted ions were destroyed again[6]. The fact that only one kind of small biomolecule was produced showed that DNA molecules were not damaged at random as pointed out by the results of agarose gel electrophoresis.

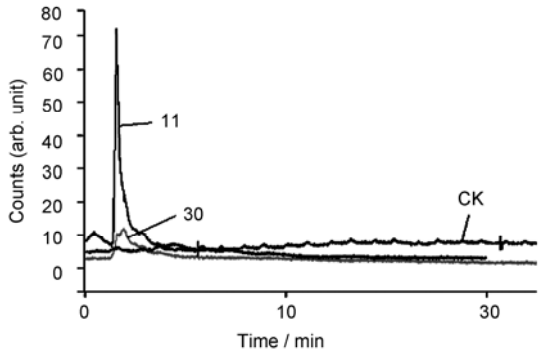

Fig. 3 HPLC spectrum of CT DNA sample irradiated with 3 MeV F^+.

CK, control sample; 11, sample irradiated with 3 MeV F^+ at the dose of 11 × 10^{12} cm^{-2}; 30, sample irradiated with 3 MeV F^+ at the dose of 30 × 10^{12} cm^{-2}.

The possible reason for the nonrandom distribution of the products of DNA after irradiation is that the energy that is mostly deposited by electron collisions causes the vibration of the chemical bond in the DNA molecule and transfers along the DNA chain, leading to the breaking of the weaker chemical bond because the energy deposition through electron collisions is usually several eV and much smaller than bond energy [7]. This form of energy migration is multiplicate. Energy can be deposited directly on the DNA molecule and migrate along the DNA chain, creating ionized and excited states of various molecules (sugars, bases, phosphates, etc). As a result, the distribution of damage is nonrandom [8, 9]. However, after initial energy deposition, the liberated electrons with excess kinetic energy are thermalized over a distance of 5 – 100 bp and randomly attached to purine and pyrimidine bases in DNA molecules. Subsequent to their attachment to DNA, electrons can then migrate to and attack the bases having the highest electron affinity and lowest ionization potential [10,11].

In this experiment, the products were concentrated around molecular weights of 831 bp, as shown by agarose gel electrophoresis, which showed that the fragments whose molecular weights were 831 bp on CT DNA might be a particular site that appears periodically. As can be seen from the result obtained using HPLC, certain bases and small biomolecules are vulnerable to the implanted ions.

4 Conclusion

Our experimental results show that low-energy heavy ions do cause DNA damage. It was observed that both dose and energy of implanted ions had an effect on the amount but not on the type of the products. It is proposed that the process of DNA irradiation damage is nonrandom: the products are concentrated around the molecular weight of 831 bp and specifically small biomolecules such as thymine are induced. But it is difficult to give an accurate description of the structure and properties of the product. Further experiments are needed to obtain systematic information on the cellular response to the radiation-induced DNA damage at phenomenological level and to further understand the damage mechanism of DNA molecules.

Acknowledgement

The authors thank Ma Hongji for his kind help in

the irradiation of the samples.

References

1 Zhou Guangming, Wei Zengquan, Li Wenjian, *et al*. Acta Biophysica Sinica, 1998, **14**(1):145-149 (in Chinese)

2 Zhou Guangming, Li Wenjian, Wang Jufang, *et al*. Acta Biophysica Sinica, 2000, **16**(1):56-61 (in Chinese)

3 Du Yanhua, Huang Shenghai, Tan Zheng, *et al*. Chinese Science Bulletin, 1999, **44**(1): 56-61 (in Chinese)

4 Du Yanhua, Qiu Guanying, Li Guohong, *et al*. Acta Biophysica Sinica, 1997, **13**(2): 267-272 (in Chinese)

5 Wang Xiangqin, Han Jianwei, Yu Zengliang, Acta Biophysica Sinica, 1998, **14**(2):337-340 (in Chinese)

6 Li Lingyun, WANG Yugang, XUE Jianming, *et al*, Nuclear Science and Techniques, 1999, **10**(4):221-224

7 Huang Weidong, Yu Zengliang, Zhang Yuheng. Nuclear Instruments and Methods in Physics Research B, 1998, **134**:202-208

8 Chatterjee A, Holley W. R., Int J Quantum Chem, 1991, **39**(5):709-727

9 Milligan J. R., Ng J. Y-Y., Wu C. C.L., *et al*. Radiatat Res, 1995, **143**(3):273-280

10 Denifl S., Ptasinska S., Cingel M., *et al*. Chem Phys Lett, 2003, **377**:74-80

11 Fuciarelli A.F., Sisk E.C., Miller J.H., *et al*. Int J Radiat Biol, 1994, **66**(5):505-509