A PIXE METHOD TO STUDY BIOLOGY AT THE DNA LEVEL*

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ABSTRACT

An external proton beam system with a detection sensitivity of 10^{-11}g for ruthenium was used to study DNA. A metal- organic compound tris (4,7- diphenylphenanthroline) ruthenium (Ⅱ) [$Ru(DiP)_3^{2+}$] was chosen as a metal marker. The target DNA labeled with $Ru(DiP)_3^{2+}$ was electrophoresed in an agarose gel and then was analysed by PIXE. The DNA sample with the metal marker showed clearly Ru peaks in the PIXE spectra, while the control showed no Ru peak at all. This method can be used to study biology at the DNA level.

Keywords: Deoxyribonucleic acid (DNA) Tris (4,7- diphenylphenanthroline) ruthenium (Ⅱ) Electrophorese PIXE

Ⅰ. INTRODUCTION

Particle Induced X- ray Emission (PIXE) has been widely used in the study of biology and medicine, especially to analyse trace elements of hair, tissues and cells[1,2]. The recently developed micro- PIXE technique has been applied to scan a cell to get the elemental distributions of the cell with the beam spot of φ 1μ m or even smaller[3-5]. But there are few papers on the application of PIXE in the study of biology directly at molecular level- especially at the DNA level.

As the most important genetic material, DNA determines the development of organism and affects their whole process of life. In many cases metal ions such as Ca^{2+}, Zn^{2+}, Cu^{2+} and Cd^{2+} are involved in gene transcription. For example, the RNA polymerase Ⅱ transcription factors H4TF- 1 and H4TF- 2 requires metal to bind specific DNA sequences[6]; the important protein factor GALA for the gene regulation contains zinc and requires zinc to carry out its function[7]; the commonly used model of the regulation of DNA transcription is the "cysteine- zinc DNA binding finger" [8-10]; and the regulation of MT gene is directly affected by metal ions[11-13]. Barton J.K. has proved that $Ru(DiP)_3^{2+}$ and $Co(DiP)_3^{3+}$ can be used as the metal probe to distinguish the different conformations of DNA and to locate the particular DNA structure which usually is critical to the regulation of the DNA transcription[14,15]. Therefore, a system

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which can detect metal in a DNA sample is required and will help a lot to the study of molecular biology.

II. EXPERIMENTAL

The metal marker of $Ru(DiP)_3^{2+}$ was synthesized according to Lin et al[16]. Concentrations of this compound were determined spectraphotometrically using $\varepsilon_{460} = 2.95^4 \times 10^{-1} M^{-1} cm$. The pBR322 DNA was prepared and electrophoresed with the comonly used method[17].

A proton beam of 2.3 MeV from the 3 MV Van de Graaff accelerator at Fudan university was used. A beam size of φ 0.8 mm was chosen and the beam current was usually kept to about 2– 10 nA. A 28 $mm^2 \times$ 5.2 mm ORTEC Si(Li) detector with an energy resolution of 165 eV at 5.9 keV was located at an angle of 90° with respect to the proton beam. A 110 μ m Mylar absorber was used, and a tantalum sheet with 2mm $\times$ 6mm slit at its center was placed in front of the detector for shielding. A very tight geometry was adopted to get as large as possible the solid angle subtended by the detector.

The PIXE spectra were analysed with the FORTRAN program AXIL[18] which was based on a numerical correction method for accurate peak– shape description and is incoporated in a nonlinear iterative least– squares fitting procedure.

The MDL (minimum detection limit) of this system was measured using a Ru standard sample (Ru:$10^{-7} g/cm^2$) manufactured in Ruhr University, Bochum, West Germany.

The DE– 81 paper (DEAE paper) was used as a supporting film since the DNA sample in the agarose gel can be efficiently transferred onto the DEAE paper without changing the position of the DNA bands.

About 20μ g pBR322 DNA was labeled by enough $Ru(DiP)_3^{2+}$ as described by J.K.Barton et al[19] with some modifications. Then the DNA sample was electrophoresed in a 0.7% agarose gel at 60V for 2 hours. The DNA sample was extracted from the gel and condensed to a very small fraction. After the sample was prepared onto an acetic cellulose paper with the target spot of about 0.6mm in diameter, it was analysed by the external beam PIXE system[20].

In the scanning experiment, The DNA was Labeled by $Ru(DiP)_3^{2+}$ in the same way and then was electrophoresed in a 0.7% agarose gel at 80V for 1 hour. After the DEAE paper of the same size as the agarose gel was put on top of the gel, the transfer process was carried out according to Sothern Blot[21] with modifications. Then the DEAE paper with transferred DNA sample was scanned in one– dimension by the same system.

Ⅲ. RESULTS AND DISCUSSION

The MDL of this PIXE system was measured and estimated to be 10^{-11}g for ruthenium. Fig.1 shows the spectrum of the Ru standard. As shown in Fig.2, the DNA

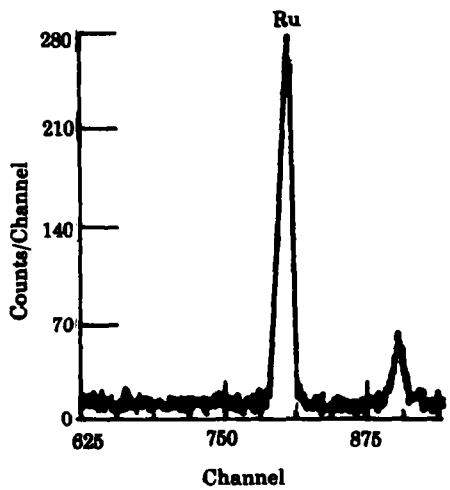

Fig. 1 The PIXE spectrum of the ruthenium standard

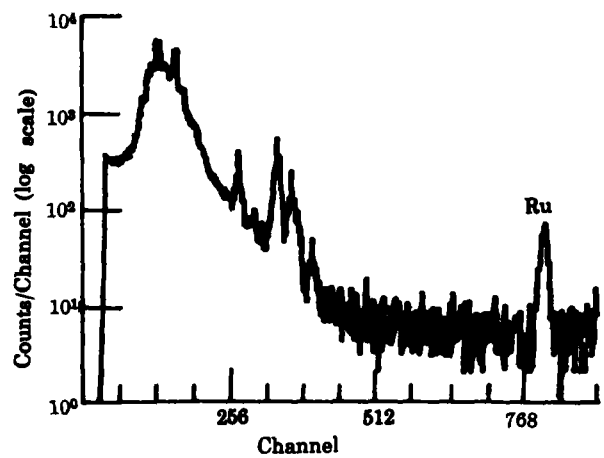

Fig. 2 The PIXE spectrum of the Ru(DiP)$_3^{+}$– DNA on the acetic cellulose paper

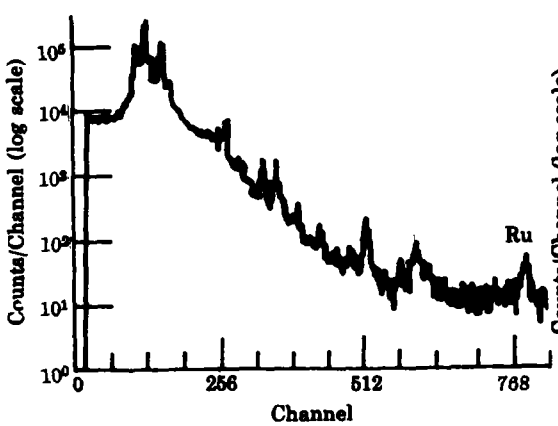

Fig.3 (*a*) The PIXE spectrum of the Ru– DNA (DEAE) from the scanning analysis

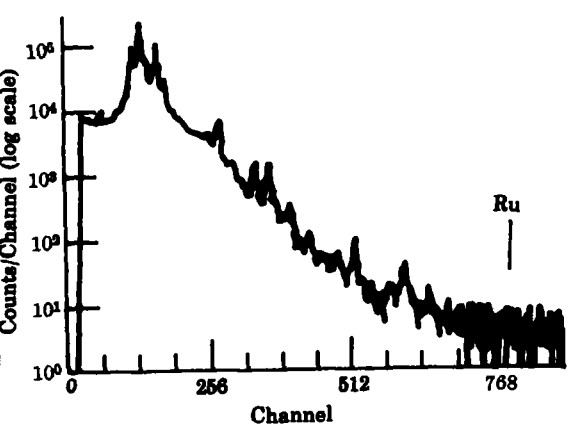

Fig.3 (*b*) The control of the DNA (DEAE paper) from the scanning analysis

Table 1

Relation of the positions of the beam spots and the Ru area counts in the PIXE analysis by scanning

No.	1	2	3	4	5	6	7	8	9
Position of the beam spot (mm)	1.5	5.0	7.5	9.0	10	11	12	15	19
Ru area counts	35	28	89	256	362	319	170	4	18

sample with the metal marker shows clearly Ru peak while the control shows no Ru

peak at all. So this PIXE system is able to detect the metal marker of Ru in the DNA sample with ease.

In Fig.3, two spectra obtained in the scanning PIXE analysis are shown. Obviously, with this system the DNA sample labeled with $Ru(DiP)_3^{2+}$ can be easily located and the interesting DNA can be found. As shown in Fig.4 and Table 1, the scanning analysis is processed in a way in which the position of the labeled DNA is located easily.

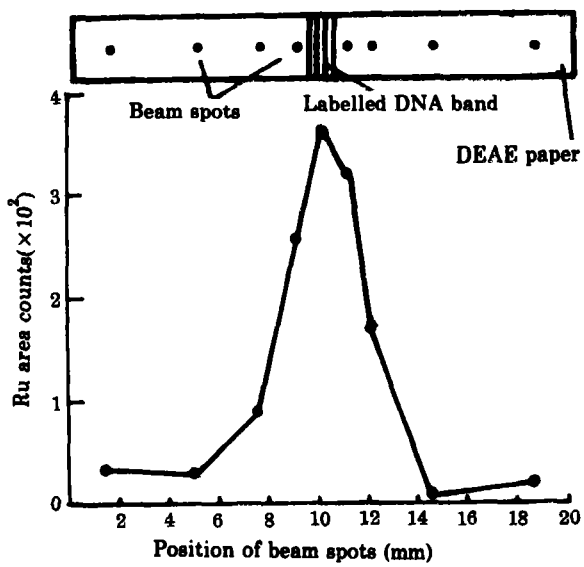

Fig. 4 The diagram of ruthenium area counts versus the position of beam spots

Since it has been proved that the $Ru(DiP)_3^{2+}$ is a useful tool to study the DNA structure and function, this system may offer a new way of studing DNA. The multiple markers can also be developed, meanwhile, the multi- element analysis advantage of PIXE can be most efficiently used. As it is well- known that the regulation of most of the genes requires more than one transcription factors to act on[22], and the descriptions in detail of the way in which these regulatory factors function is always the exciting part in the study of revealing the mechanism of life. A lot of research work in molecular biology have been carrying out in this subject[23,24], the multiple metal marker system may offer a new way in this research field.

IV. CONCLUSIONS

This PIXE system has proved to be a reliable and a handy means in the analysis of the DNA samples labeled with $Ru(DiP)_3^{2+}$. Combined with some molecular biology techniques. This system can be used to study biology at the DNA level.

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