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Short communication

Measurement of ^{60}Co-γ ray-induced DNA damage by capillary electrophoresis

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Abstract

Capillary electrophoresis was employed in this study to monitor ^{60}Co-γ ray-induced damage to a 1 kb DNA ladder which consists of restriction fragments ranging from 75 to 12 000 bp. DNA samples (0.5 mg/ml) were exposed to 0–60 Gy of γ-radiation in the presence and absence of 110 μmol/l ethidium bromide (EB). The analysis showed peak broadening without significant changes in the size distribution of irradiated fragments. Radiation-induced conformational changes may account for this peak broadening. EB addition caused small increases in the retention times of DNA fragments without affecting the overall DNA damage. This indicates that the presence of intercalated EB during radiation will not stabilize the DNA against ^{60}Co-γ ray-induced damage.

Keywords: DNA; ^{60}Cobalt

1. Introduction

Ionizing radiation produces a spectrum of DNA damage ranging from base lesions to strand breaks and cross-links [1,7]. Few analytical tools exist to monitor the chemical nature of DNA damage at either low or high doses of radiation. Strand break assays are the most popular, with alkaline centrifugation and unwinding assays as prominent examples [2,8,9]. Radiation-induced DNA conformational changes are frequently monitored with the nucleoid or "comet assays" [2,3]. These assays are extremely sensitive at low doses of radiation, provided lysis conditions and other experimental variables are stringently controlled. High-performance liquid chromatography (HPLC) and gas chromatography–isotope dilution mass spectrometry (GC–MS) provide powerful analytical alternatives to these tools [10]. Novel tools that can augment these analytical methods are crucial in understanding the chemical nature of radiation-induced DNA damage.

Capillary electrophoresis (CE) is a relatively new tool which has been used with success in the fields of analytical chemistry, pharmacy, forensic sciences and molecular biology [6]. Its enhanced resolution, speed, sensitivity and reproducibility over slab gel electrophoresis have been used to great advantage in applications such as the study of human diseases [11,15–17], DNA sequencing and the sizing of DNA fragments [4,6]. DNA damage studies using CE include the determination of point mutations in DNA [11] and the measurement of oxidative damage to fluorescein-labelled deoxyadenylic acid [14].

In the present study high resolution CE with a UV detector was used to monitor radiation-induced damage to a commercial preparation of a 1-kb DNA

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ladder in the presence or absence of ethidium bromide (EB). The choice of DNA substrate and mode of analysis enabled damage detection in double-stranded DNA fragments over a wide range of size and sequence. The possibility that EB may be a DNA protectant when added prior to radiation was also investigated.

2. Experimental[1]

2.1. DNA samples

The 1-kb standard DNA ladder (Gibco-BRL, #5615SB, lot #EJY702) was used as the starting material for the CE analysis. The ladder is a mixture of linear double-stranded DNA restriction fragments from 500 to 12 216 bp. It also contains Hinf I restriction fragments of the PBR322 vector DNA resulting in fragments ranging from 75 to 1636 bp. In addition, the ladder contains from 1 to 12 copies of a 1018 bp DNA fragment [19]. The standard is supplied at a concentration of about 1 mg/ml in 10 mmol/l Tris–HCl (pH 7.5), 50 mmol/l NaCl, 0.1 mmol/l EDTA.

2.2. Sample irradiation

The 1-kb DNA ladder was diluted in distilled water to 0.5 mg/ml either in the presence or absence of 110 μmol/l EB for all experiments. Samples were irradiated in a ^{60}Co-γ source while their container was kept at room temperature. The dose rate as determined by Fricke dosimetry was 60 Gy/min [12].

2.3. Capillary electrophoresis

The CE analyses were performed using a BIO-RAD BioFocus 3000 electrophoresis system with a 50 cm×50 μm I.D., coated capillary cartridge and a set cartridge temperature controlled at 25°C. Hydroxyethylcellulose (0.5 g/100 ml) served as the separation polymer. This sieving buffer solution was prepared by dissolving 0.132 g of hydroxyethyl cellulose in 25 ml of a solution of 100 mmol/l Tris–borate, 0.1 mmol/l EDTA and titrating with KOH to pH 8.7.

A 200-μl volume of each standard was placed in the CE sample vials. Samples were pressure-injected (1 s) at 137 kPa and run at 10 kV for 30 min. This resulted in the loading of 1.4 ng of DNA. The detector was set at a wavelength of 260 nm. CE separations were further analyzed by PeakFit non-linear curve-fitting software to determine area and heights of incompletely resolved peaks.

3. Results and discussion

Fig. 1A is a CE separation of the 1 kb DNA ladder with each labelled peak denoting a separate DNA fragment. The 75–1636 bp fragments are clustered in the 11–16 min region. The 2036 and 3054 bp fragments appear as large peaks with retention times of 16.31 and 16.47 min. The present conditions were designed to enhance the separation of fragments in the 75–3054 bp range. The 4072–12 216 bp fragments are not resolved and emerge as one large peak at a retention time of about 16.6 min.

Fig. 1 compares CE separations of control and irradiated DNA fragments in the absence of EB. Radiation effects are minimal at 30 Gy and enhanced at 60 Gy. The 1636, 2036 and 3054 bp fragments are distinct in Fig. 1A (control) and unresolved in Fig. 1B and C due to radiation effects. The 506 and 517 bp fragments appear as a distinct doublet of peaks in Fig. 1A (control) which becomes broader after exposure to 30 and 60 Gy of ionizing radiation (Fig. 1B and 1C). The 75–396 bp fragments appear less distinct in the 30 Gy (Fig. 1B) and 60 Gy (Fig. 1C) samples compared to Fig. 1A. The 1018 bp fragment in the control (Fig. 1A) is similarly affected. DNA fragments $\geq$1018 bp show larger peak broadening effects than smaller fragments, presumably due to the greater number of the same radiation damage sites in these fragments.

We examined the effect of adding EB to the DNA before irradiation. This fluorescent dye intercalates

[1]Certain commercial equipment or materials are identified in this paper in order to adequately specify experimental procedures. Such identification does not imply endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

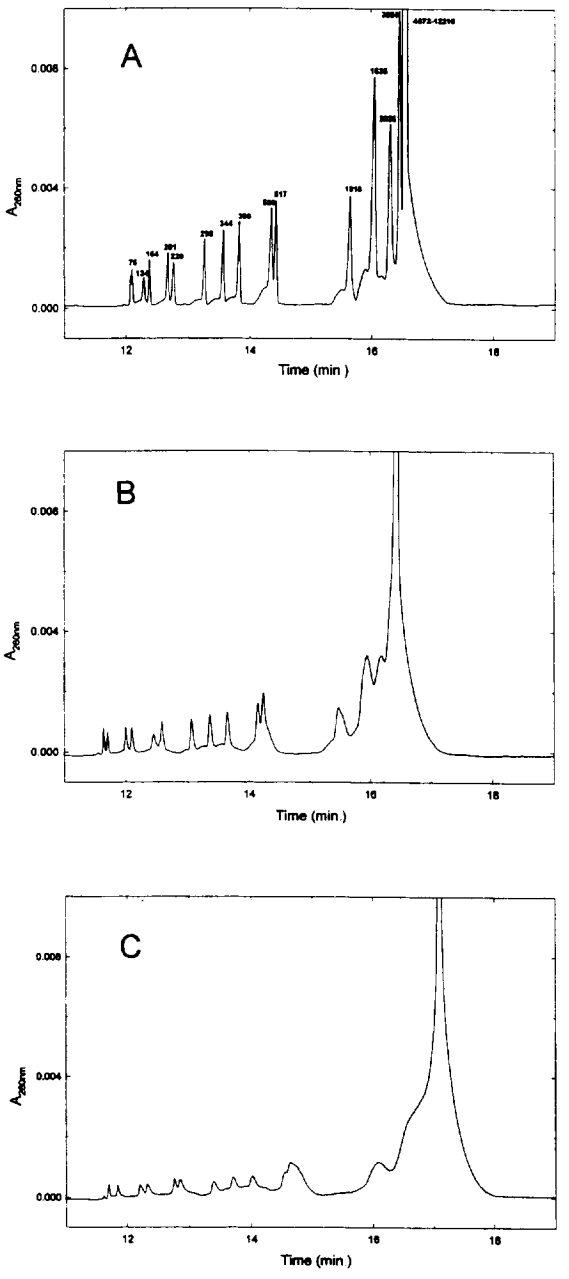

Fig. 1. CE separations of the 1-kb DNA ladder before and after exposure to ^{60}Co-γ radiation. Separation was performed at 25°C using a hydroxyethylcellulose sieving buffer as described in Section 2. (A) Control; (B) 30 Gy; (C) 60 Gy.

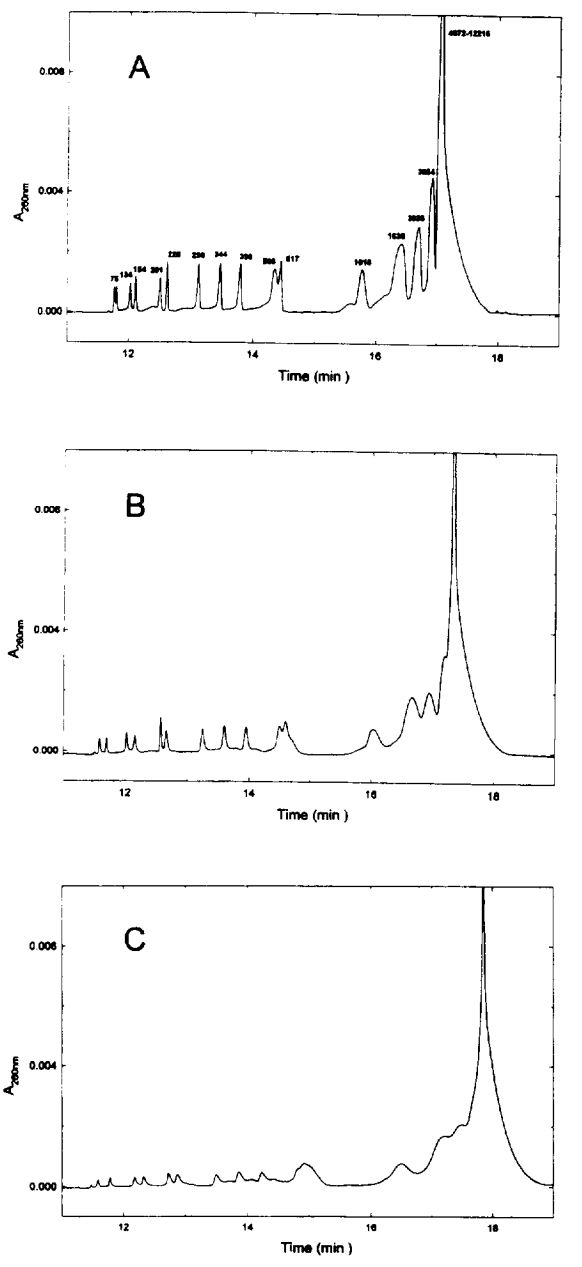

Fig. 2. CE separations of the 1-kb DNA ladder ^{60}Co-γ irradiated in the presence of 110 μmol/l EB. Separations were performed as described in Section 2. (A) control; (B) 30 Gy; (C) 60 Gy.

between adjacent bases, thereby unwinding and lengthening the DNA helix [18]. Fig. 2A shows a CE separation of the 1-kb DNA fragments in the presence of 110 μmol/l EB. In general, peak heights in Fig. 2A appear lower and broader in the presence of EB when compared to the non-irradiated control (Fig. 1A), especially in the 1636–3054 bp region.

Although fluorescent dyes such as EB usually enhance peak resolution of DNA fragments, these separations typically employ much lower DNA and dye concentrations [5,13,18]. Our measurements are consistent with other observations that high EB concentrations (>100 μmol/l) result in an increased polydispersity and broader peaks [20].

Under experimental conditions of high DNA and dye concentration, complete saturation of DNA binding sites by EB would be expected during radiation exposure. Under these conditions, the peak broadening produced by radiation (Fig. 2B and C) is similar to that produced in the absence of EB (Fig. 1B and C). This indicates that the EB does not have a protective effect on the radiation-induced changes. In Fig. 3 the retention times are plotted versus DNA fragment size to further examine the radiation effects noted in the presence of EB. An increased dose response measured as increased retention time is visible up to 60 Gy. Doses larger than 60 Gy (≥100 Gy) caused extensive broadening of all peaks and the areas could not be quantified (data not shown). In order to evaluate whether or not the ^{60}Co-γ radiation breaks both DNA strands into smaller fragments, the peak areas of control and irradiated DNA fragments are plotted as a function of size (bp) in Fig. 4. Peak areas of DNA fragments ≥1018 bp overlap and have not been included in this graph. A linear relationship is expected since peak area is proportional to the concentration of each fragment in the 1-kb DNA restriction enzyme digest. Although there is some error associated with peak areas corresponding to 506/517 bp, no systematic shift in size distribution could be seen as a result of radiation.

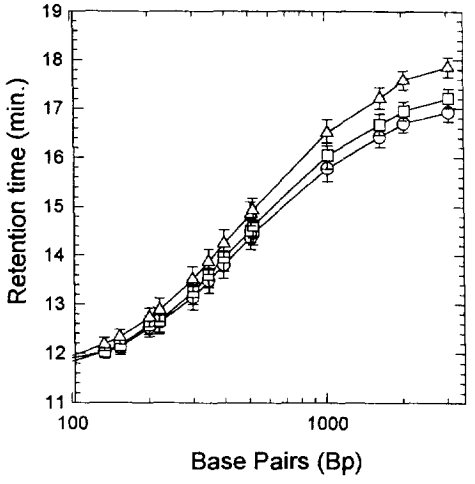

Fig. 3. Effect of ^{60}Co-γ radiation on peak retention times of the 1 kb DNA ladder in the presence of 110 μmol/l EB. Symbols: (○) control; (□) 30 Gy and (△) 60 Gy. Peak retention times were obtained from the data in Fig. 2.

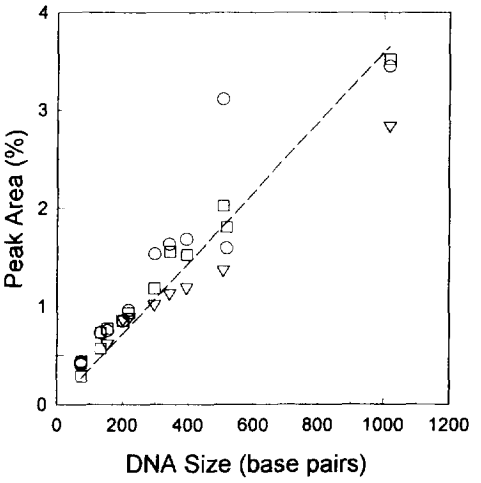

Fig. 4. Analysis of DNA size distribution before and after radiation (in the presence of 110 μmol/l EB). Peak areas were obtained from the data in Fig. 3. The dashed line is shown for reference (see Section 3). Symbols: (▽) control; (□) 30 Gy and (○) 60 Gy.

We have shown that CE can be a sensitive tool in the screening of physical changes in DNA which occur with radiation. The high resolution of CE is demonstrated by the efficient separation of smaller double-stranded DNA fragments differing by 11–20 bp (506/517, 134/154 and 201/220 bp; Fig. 1) as well as the separation of larger DNA fragments (1018–3054 bp). We have shown an increase in dose response (shift in retention time) in the range of 30–60 Gy. These radiation effects were all observed at much lower doses than the 166–581 Gy employed to monitor ^{60}Co-γ ray-induced DNA damage to polyadenylic acid [14]. CE analysis apparently is more sensitive to the conformational changes that occur in the larger fragments. This may be due to the larger DNA fragments (1018–3054 bp) being statistically more prone to radiation damage than the smaller fragments. Radiation-induced modifications in individual DNA bases may also cause charge changes in DNA fragments which would affect CE

separation [14]. Unrepaired DNA base modifications are known precursors for single and double-strand breaks [10]. Strand breaks may contribute to the conformational changes underlying the peak broadening and corresponding decrease in peak height observed in the irradiated samples. However, the average size distribution of the irradiated DNA fragments remained the same indicating that essentially all of the strand breaks were rejoined. Future experiments using CE in combination with mass spectrometry of homogeneous fragments will shed light on the chemistry underlying these physical changes.

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