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Analysis of laser-induced plasmid DNA photolysis by capillary electrophoresis

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Abstract

Capillary electrophoresis (CE) was used to monitor the laser-induced conversion of supercoiled pKOL8UV5 plasmid DNA into nicked conformers. The plasmid samples (0.1 mg/ml) were incubated in the absence or presence of 110 μmol/l ethidium bromide (EB) and then exposed to 110 J of argon laser radiation (488 nm). The nicked, open circular conformers were separated from the supercoiled DNA by a 15% increase in retention time. Approximately 90% of the control DNA was in the supercoiled form. Laser radiation in the presence of EB caused complete conversion of the supercoiled plasmid DNA into nicked conformers. Laser-induced fluorescence CE (LIF–CE) was about 100-fold more sensitive than UV–CE in the detection of these conformers. Agarose gel electrophoresis confirmed these findings and showed the presence of the nicked plasmid conformers. Based on these comparisons, CE is an efficient analytical tool for the identification of laser-induced conformational changes in plasmid DNA.

Keywords: DNA photolysis; Plasmid DNA; Capillary electrophoresis

1. Introduction

Laser-induced destruction of tumors, usually with minimal side-effects, is a cornerstone of photodynamic therapy [1]. Effective treatment depends on selective targeting of DNA through the appropriate combination of chromophore and wavelength-matched laser. An understanding of the mechanisms of laser-induced DNA photolysis in different model systems will aid in the development of more advanced treatment protocols. Previous studies using plasmids have shown that DNA is unaffected by laser radiation, even at high doses [2]. Instead, laser-induced photoscission occurs due to energy transfer from a bound chromophore to DNA [1,2]. EB is a widely used intercalating chromophore and is known to facilitate laser-induced DNA photoscission by this mechanism [2].

Agarose slab gel electrophoresis has previously been used to measure laser-induced plasmid DNA photolysis in the presence of EB and other chromophores [2]. We have chosen an analytical tool, CE, to measure the nicked, circular and linear conformers of supercoiled plasmid DNA photolysis. CE offers better resolution, speed, sensitivity and reproducibility relative to slab gel electrophoresis. CE has been used to great advantage in the fields of pharmacy and forensic sciences, as well as molecular biology [3].

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Recombinant DNA technology has also benefited from the rapid CE analysis of plasmid DNA [4,5] and CE screening of cosmid libraries [6]. Specific DNA damage studies using CE include the measurement of oxidative damage to fluorescein-labeled deoxyadenylic acid [7], the determination of point mutations in DNA [3] and analysis of ionizing radiation-induced DNA damage [8].

In the present study CE was used to monitor laser-induced damage to the pKOL8UV5 plasmid in the presence or absence of EB. This intercalating chromophore has an excitation wavelength of 482 nm which is well-matched to the argon laser (488 nm) employed and thus allows efficient fluorescence energy-excitation transfer to induce DNA photoscission. CE was used in combination with two different detection modes; i.e. UV absorbance at 260 nm and LIF to monitor structural changes in plasmid DNA resulting from laser radiation.

2. Experimental

2.1. Chemicals

A 15 mmol/l stock solution of EB (in distilled water) was kept in the dark at 4°C. A stock solution of thiazole orange (in distilled water; No. 477409) was obtained from Beckman (Columbia, MD, USA). The pKOL8UV5 plasmid and *Hin*dIII-digested linear plasmid DNA were gifts from Dr. K. McKenney (NIST, Gaithersburg, MD, USA) and stored at −21°C as a 1 mg/ml preparation in 10 mmol/l Tris, 1 mmol/l EDTA (pH 8).

2.2. Laser radiation of samples

Plasmid DNA was diluted to 0.1 mg/ml with distilled water prior to radiation and EB added to a final concentration of 110 μmol/l to one set of samples. Samples were irradiated in 0.5-ml Eppendorf tubes by an argon laser (Coherent Innova 200, Sunnyvale, CA, USA). A sample holder and optical train were constructed so that the beam illuminated the sample from the top, and down through the center of the open Eppendorf tube. No focusing optics were used; the beam diameter at the sample was approximately 2.1 mm. Approximately 50% of the sample volume was illuminated by the beam for a total of 250 s. The beam time was divided into three 60 s cycles, allowing for 30 s cooling between each illumination, followed by a final 70-s exposure. The measured intensity of the argon laser (488 nm) was 0.44 W at the sample tube (approximately 110 J).

2.3. Agarose gel electrophoresis

A 1% agarose gel containing 0.7 μmol/l EB in 0.04 mol/l Tris–acetate; 0.001 mol/l EDTA buffer (pH 8) was run using an Owl Scientific Plastics (Cambridge, MA, USA) apparatus. The running conditions were 103 V for 80 min with 100–300 ng of DNA loaded per well.

2.4. Capillary electrophoresis

A BioFocus 3000 Electrophoresis system (Bio-Rad, Hercules, CA, USA) with a UV absorbance detector set at 260 nm was used to perform the first series of CE analyses. A coated capillary (50 cm×50 μm I.D., Bio-Rad No. 148-3033) was filled with a polyacrylamide mixture (Beckman eCap ds 1000 No. 477411) containing 100 μmol/l EB. This chromophore served as a DNA detection agent in the UV–CE system. Plasmid DNA samples (0.1 mg/ml) were pressure injected for 1 s at 1.4×10^5 Pa, resulting in the loading of 0.2 ng DNA. Runs were conducted at 10 kV for 90 min at a temperature of 25°C.

The P/ACE system 2200 LIF system (Beckman) was used to perform the second series of CE analyses. This system employed an argon ion laser source (488 nm) set at 3 mW. The Beckman eCap ds 1000 separation polymer containing 10 μmol/l thiazole orange was used in a coated capillary (Beckman No. 477412; 37 cm×100 μm I.D.). Thiazole orange, a chromophore with an excitation wavelength of 509 nm and an emission wavelength of 533 nm, served as a DNA detection agent in the LIF–CE system. Plasmid-DNA samples (0.1 mg/ml) were pressure injected for 20 s at 3.4×10^3 Pa, resulting in the loading of 2.0 ng of DNA. The samples were run at 7.4 kV for 30 min at a temperature of 20°C. Data from each instrument were collected at 5 Hz using the matching software.

Post-run analyses of both CE data sets were done using SigmaPlot (Jandel Scientific, San Rafael, CA, USA).

3. Results and discussion

Fig. 1A–D shows CE separations with UV detection of pKOL8UV5 plasmid DNA before and after exposure to 110 J of laser radiation. A typical profile of control plasmid DNA is shown in Fig. 1A. Approximately 90% of the pKOL8UV5 DNA is in the supercoiled form (3890 bp) and migrates at a retention time of 46 min. The smaller peak migrating at 53 min corresponds to nicked plasmid DNA. Linearized DNA, shown here, is produced by digestion of the supercoiled plasmid with the *Hin*dIII restriction enzyme and migrates at 39 min. It is not observed in the control and irradiated samples under these conditions. Fig. 1B shows the CE separation of plasmid DNA which has been irradiated in the absence of EB. The supercoiled DNA peak is prominant and the nicked DNA peak is not visible. This is due to radiation-induced conformational changes not visible by UV–CE (LIF–CE, a more sensitive detection technique, confirms the formation of radiation-induced conformational changes to the nicked DNA as will be discussed). Unirradiated plasmid DNA, which has been incubated in the presence of 110 μmol/l EB, is shown in Fig. 1C. The addition of EB to the samples prior to loading on the CE, results in an increase in the amount of bound dye and a shift in the retention times for the supercoiled and nicked DNA [6]. Therefore the supercoiled DNA peak retention time changed from 46 to 43 min, while the nicked DNA peak retention time shifted from 53 to 49 min. It should be noted that the sieving polymer which is used in the UV–CE separations also contains EB which has previously been shown to increase signal strength and

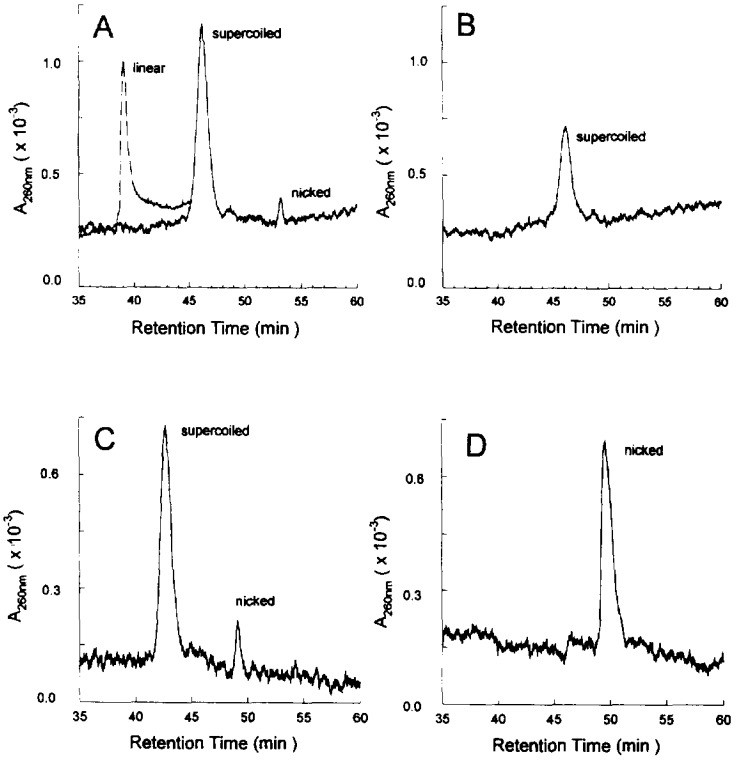

Fig. 1. UV–CE separations of pKOL8UV5 plasmid DNA. Samples were irradiated in the absence or presence of EB. (A) control DNA; (B) irradiated DNA; (C) control DNA +110 μmol/l EB; (D) DNA laser-irradiated in the presence of 110 μmol/l EB. Measurements were made using Beckman eCap ds1000 sieving buffer containing 100 μmol/l EB (see Experimental).

improve DNA peak separation [9]. Fig. 1D shows the complete conversion of supercoiled plasmid into nicked DNA when incubated in the presence of 110 μmol/l EB and then subjected to 110 J of laser radiation.

LIF–CE shows a similar pattern of DNA damage induction as UV–CE, except that the relative proportions of the supercoiled and nicked peaks are almost reversed (Fig. 2A–D). Thiazole orange was used in place of EB for the LIF–CE separations since its fluorescence properties are particularly well suited for the Beckman LIF–CE system. Previous studies have shown that thiazole orange differs from EB in that it has a higher binding density to double-stranded DNA and, unlike EB, also binds to single stranded DNA [9]. This may account for the higher peak signal observed for nicked DNA in LIF–CE (Fig. 2A). Supercoiled plasmid DNA migrates as a single-small peak with a retention time of 21 min, while the nicked DNA migrates as a single, large peak at a retention time of 23 min. Apparently the thiazole orange binds the nicked circular DNA with a much higher binding avidity than supercoiled DNA. This is most likely due to conformational differences in the base stacking of these two forms of DNA [9]. By comparing the relative peak areas in the supercoiled and nicked DNA of the UV–CE data to that of the LIF–CE, we estimate that the LIF–CE is about 100-times more sensitive than UV–CE at detecting the nicked DNA. Plasmid DNA subjected to 110 J of laser radiation in the absence of EB is shown in Fig. 2B. The nicked DNA peak in Fig. 2A represents the induction of strand breaks in plasmid DNA, while the presence of multiple peaks at retention times of 22–23 min (Fig. 2B) may represent conformational changes of the nicked plasmid DNA which can only

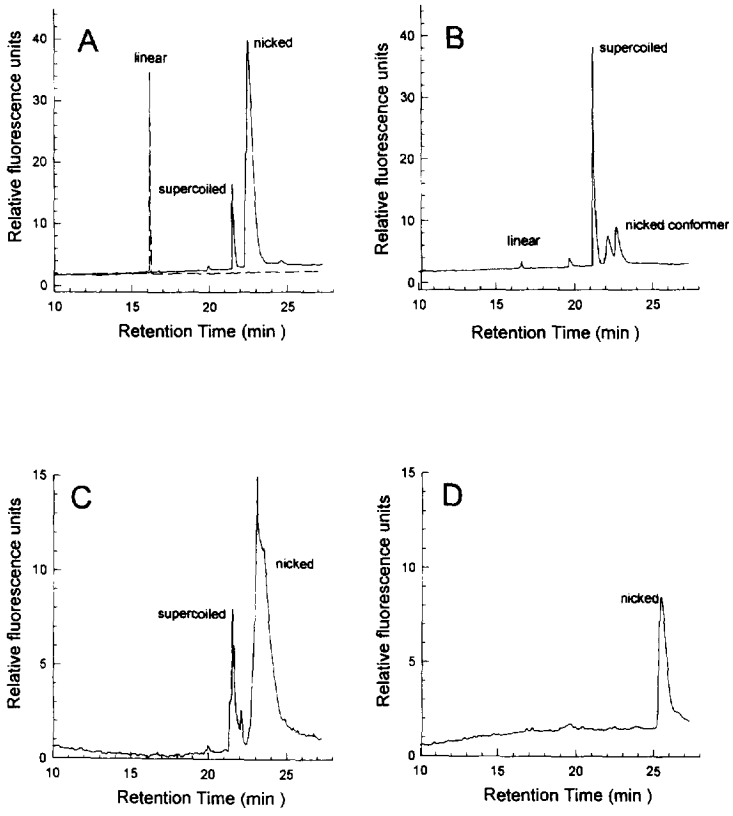

Fig. 2. LIF–CE of control and laser-irradiated pKOL8UV5 plasmid DNA. (A) control DNA; (B) irradiated DNA; (C) control DNA+110 μmol/l EB; (D) DNA laser-irradiated in the presence of 110 μmol/l EB. Measurements were made using the Beckman eCap ds1000 buffer containing 10 μmol/l thiazole orange (see Experimental).

be detected by the more sensitive LIF system. Plasmid DNA irradiated in the presence of 110 μmol/l EB (Fig. 2D) shows complete conversion to the nicked form without formation of linearized DNA which migrates at about 16.2 min. This is in agreement with results obtained with UV–CE.

Agarose slab gel electrophoresis confirmed the pattern of laser-induced DNA photoscission observed with CE. Fig. 3 shows 1% agarose gels of control and laser-irradiated pKOL8UV5 plasmid DNA samples described in Fig. 1 and Fig. 2. Lane 1 in Fig. 3A shows control pKOL8UV5 supercoiled plasmid DNA. Lane 2 (Fig. 3A) shows plasmid DNA which has been linearized with *Hin*dIII. Fig. 3B shows control and irradiated plasmid samples loaded at a threefold higher concentration than in Fig. 3A. At this concentration, additional bands corresponding to nicked and linearized DNA conformers are visible in the control (lane 1). Laser-irradiated plasmid DNA (lane 2) exhibits the same band pattern as the control. The addition of 110 μmol/l EB (lane 3) does not affect the band migration patterns. However, lane 4 clearly indicates that laser radiation in combination with EB induces complete conversion of the supercoiled plasmid into nicked DNA conformers.

Laser-induced photoscission of supercoiled plasmid DNA in the presence of EB was observed with all three techniques, in agreement with previous studies using agarose gel electrophoresis [2]. We have shown differences in the three techniques, i.e., UV–CE, LIF–CE and agarose gel electrophoresis, in the measurement of laser-induced plasmid DNA conformers. UV-CE can be used to detect and quantify laser-induced nicked conformers of supercoiled plasmid DNA. LIF–CE is shown to be about 100-times more sensitive than UV–CE in the detection of these nicked plasmid DNA conformers. However, high DNA concentration loading of the agarose gel showed additional nicked and linear DNA conformers not clearly visible by UV– and LIF–CE. Thiazole orange, the chromophore employed to detect DNA in the LIF–CE experiments, emphasizes the nicked DNA conformers and this binding mechanism awaits further investigation.

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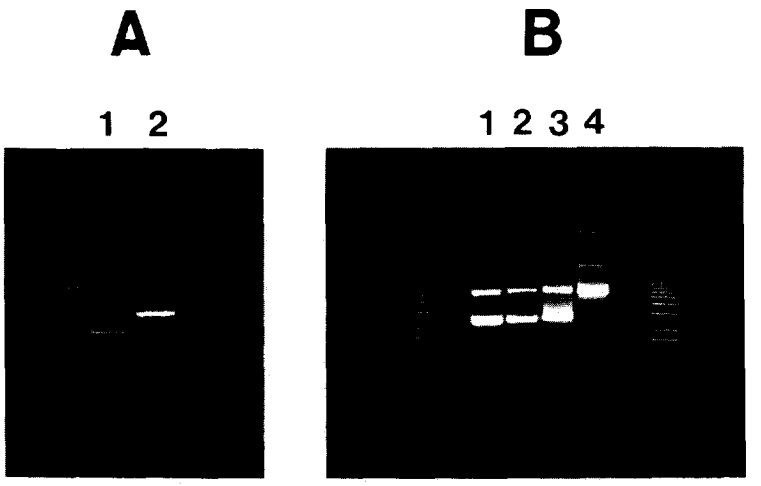

Fig. 3. Agarose gel electrophoresis of pKOL8UV5 plasmid DNA. (A) Lanes: 1, control DNA; 2, pKOL8UV5 plasmid DNA linearized with *Hin*dIII; (B) plasmid samples were irradiated in the absence or presence of 110 μmol/l EB. Lanes: 1, control DNA; 2, irradiated DNA; 3, control DNA + 110 μmol/l EB; 4, DNA irradiated in the presence of 110 μmol/l EB. 1 kb DNA ladder was run for comparison on right and left sides of the gel (fragment sizes in bp from top to bottom of each gel = 12 216, 11 198, 10 180, 9162, 7126, 6108, 5090, 4072, 3054, 2036, 1018, 517 and 506; smaller unresolved fragments have not been identified).

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.

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