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Short Communication

Capillary electrophoresis with laser fluorescence detection for profiling damage to fluorescein-labeled deoxyadenylic acid by background, ionizing radiation and hydrogen peroxide

Wenni Li[☆], Adel Moussa[☆☆] and Roger W. Giese*

Department of Pharmaceutical Sciences in the Bouve College of Pharmacy and Health Sciences, and Barnett Institute of Chemical Analysis and Materials Science, Northeastern University, Boston, MA 02115 (USA)

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ABSTRACT

Fluorescein–ethylenediamine–deoxyadenosine-5′-monophosphate, as an aqueous solution (including 10% acetonitrile), was exposed, as separate aliquots, to ^{60}Co and H_2O_2. Analysis of the resulting samples by capillary electrophoresis with laser fluorescence detection showed a complex but significantly resolved array of peaks for each, even when a relatively concentrated sample was injected (the peak for the parent compound was 100× off-scale). The pattern of peaks was qualitatively similar for both samples, and also for a "non-exposed" sample, but much more intense for the exposed sample. This indicated the predominant role of degradation by oxidation in both exposures as well as from background. At least one peak was caused only by the ^{60}Co exposure. The technique therefore may lead to a specific biomarker for exposure of DNA to ionizing radiation.

INTRODUCTION

We are interested in combining fluorescence labeling and capillary electrophoresis (CE) with laser fluorescence detection to study chemical damage to DNA. One strategy, investigated here, is to first label a nucleotide (X) with fluorescein (F) yielding the conjugate F–X. Due to the high separation efficiency and sensitivity of CE, even subtle chemical degradation of the X part of F–X can be readily visualized as resolved new peaks.

For this purpose, it is attractive to form an F–X conjugate in which the fluorescein moiety, F, is attached via an alkyldiamine spacer, such as ethylenediamine (ED), to the phosphate moiety of the nucleotide, X. This opens up a mechanism to differentiate damage to the nucleobase, which is our concern, from uninteresting damage to the F moiety: since a phosphoramidate linkage undergoes facile hydrolysis at low pH, damaged F–ED can be removed in this way, followed by replacement with fresh, undamaged F–ED. Damage to F can also be sorted out by the more severe change in fluorescence characteristics of F–X, or, ideally, goes un-

* Corresponding author.

☆ Present address: American Cyanamide Co., Agriculture Research Division, Princeton, NJ 08540-0400, USA.

☆☆ Present address: Covalent Associates, Inc., Woburn, MA 01801, USA.

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detected because of the significant loss in fluorescence.

Here we demonstrate some promise for the first part of this technique by comparing background, radiation and oxidative damage to a fluorescein–ethylenediamine–deoxyadenylic acid conjugate by CE. While F–X also might be subjected to HPLC with laser fluorescence detection for this purpose, HPLC is a surface-intensive technique, increasing the likelihood of band broadening, losses and interferences, which can complicate this kind of analysis.

EXPERIMENTAL

Chemicals and reagents

Trizma base [tris(hydroxymethyl)aminomethane, or Tris] and catalase (C-100) were purchased from Sigma (St. Louis, MO, USA). Hydrogen peroxide (30%) was from Aldrich (Milwaukee, WI, USA). Boric acid, HPLC-grade acetonitrile and 0.22-μm MSI Cameo filters were from Fisher Scientific (Bedford, MA, USA). The fluorescein–ethylenediamine–deoxyadenosine-5′-monophosphate (F–ED–dAMP) was prepared as described [1] using Isomer II FITC (fluorescein-6-isothiocyanate) from Molecular Probes (Eugene, OR, USA). All solution compositions were v/v unless indicated otherwise.

Capillary electrophoresis with laser fluorescence detection

A laboratory-built apparatus was used for CE. The laser fluorescence detector was designed and built by Dr. Edward S. Yeung at Iowa State University. It utilized an argon ion laser (Model 2211-30SL; Cyonics, CAS, USA) to give excitation at 488 nm. A Model PS/EH60R01.5 regulated high-voltage d.c. power supply (Glassman High Voltage, NJ, USA) was used. Capillary electrophoresis was performed in a 95 cm long fused-silica capillary (75 μm I.D.; PolyMicro Technologies, Phoenix, AZ, USA). The polyimide coating of the capillary was burned off to form a flow cell 50 cm from the injection end (grounded anode end) of the capillary. Samples were injected hydrodynamically: anode end 5 cm higher for 20 s.

New capillary columns were cleaned initially by syringe-filling (until 4 drops emerged, which was done for all syringe steps) with methanol–water (1:1), followed by 0.1 *M* NaOH, with standing for 30 min each. The buffer of interest was then subjected to 30 min of electrophoresis followed by standing overnight. Periodically (about once a week) the capillary was cleaned by syringe injection of 0.1 *M* NaOH, standing for 30 min, similarly injecting buffer, and then operating the electrophoresis for one hour before samples were injected.

Solutions

pH 8.7 Buffer. This was prepared by combining 0.8 ml of stock buffer (0.5 *M* Tris, 0.5 *M* boric acid, stored at room temperature), 35 ml of water, and 4 ml of acetonitrile, giving pH 8.7. This buffer (10 m*M* Tris–borate, 10% acetonitrile) was filtered (0.22 μm) and degassed (bubbling with helium for 15 min) prior to use as diluent to prepare solutions for injection. The other buffers were prepared by titrating the 10 m*M*, pH 8.7 solution to the desired pH with 2 *M* NaOH.

F–ED–dAMP Stock. The stock solution of F–ED–dAMP was obtained as an HPLC peak from a C_{18}-silica column in a mobile phase of 5 m*M* acetic acid with an acetonitrile gradient. Immediately after collection, the pH was adjusted to 8.7 by the addition of 0.5 *M* Tris–borate. The concentration of F–ED–dAMP was 90.7 μM based on the absorbance of the solution at 486 nm relative to that of a standard solution of fluorescein in the same solvent. This solution was kept in a polypropylene tube, and all dilutions, unless indicated otherwise, were made in polypropylene tubes comprising Corning Brand 15- and 50-ml polypropylene tubes, and Fisher Brand 1.5-ml polypropylene snap cap tubes. All dilutions were made using polypropylene tips. All solutions were kept dark by wrapping the tubes with aluminum foil.

^{60}Co Irradiation of F–ED–dAMP

F–ED–dAMP Stock was diluted 1:10 with pH 8.7 buffer into a glass vial and exposed at a dose rate of 830 rad/min to a Gammacell 220 ^{60}Co γ-ray source (Atomic Energy of Canada Ltd.). The solution was injected directly (or after dilution with pH 8.7 buffer) into the CE system.

H_2O_2 Reaction of F–ED–dAMP

F–ED–dAMP Stock was diluted 1:10 with pH 8.7 buffer and 97 μl were treated with 3 μl of 0.03% H_2O_2 (prepared by diluting 30% H_2O_2 with water).

After 5 min the solution was treated with 5 μl (as supplied) of catalase followed by vortexing, to quench the residual H_2O_2, and then injected directly into the CE system. A control experiment showed that the catalase did not modify the CE profile.

RESULTS AND DISCUSSION

To demonstrate this technique for studying chemical damage to DNA nucleobases, we prepared a F–ED conjugate of deoxyadenosine-5′-monophosphate (dAMP). An electropherogram by CE of the resulting compound, F–ED–dAMP, is shown in Fig. 1A, where the peak for the F–ED–dAMP is kept on scale. Injecting a 100 × more concentrated solution of the compound, under the same instrumental conditions, gives the "off-scale" electropherogram shown in Fig. 1B. It is impressive that the base of the peak for F–ED–dAMP is so narrow given that the actual height of this peak is 100 × the part that is observed. The extreme sharpness for this peak in turn, makes it possible to observe multiple trace impurities in the sample.

Exposure of a portion of the sample to a ^{60}Co source, and a second portion to H_2O_2, and similarly injecting in the off-scale mode for the parent compound, yields the electropherograms in Fig. 1C and D, respectively. Aside from much stronger peak intensities in Fig. 1C and D, the three patterns of peaks (Fig. 1B–D) are seen to be very similar. This was confirmed by injecting combined samples (data not shown). The profiles were reproducible, aside from the absolute migration times for the peaks (which can vary in CE due to fluctuations as in elec-

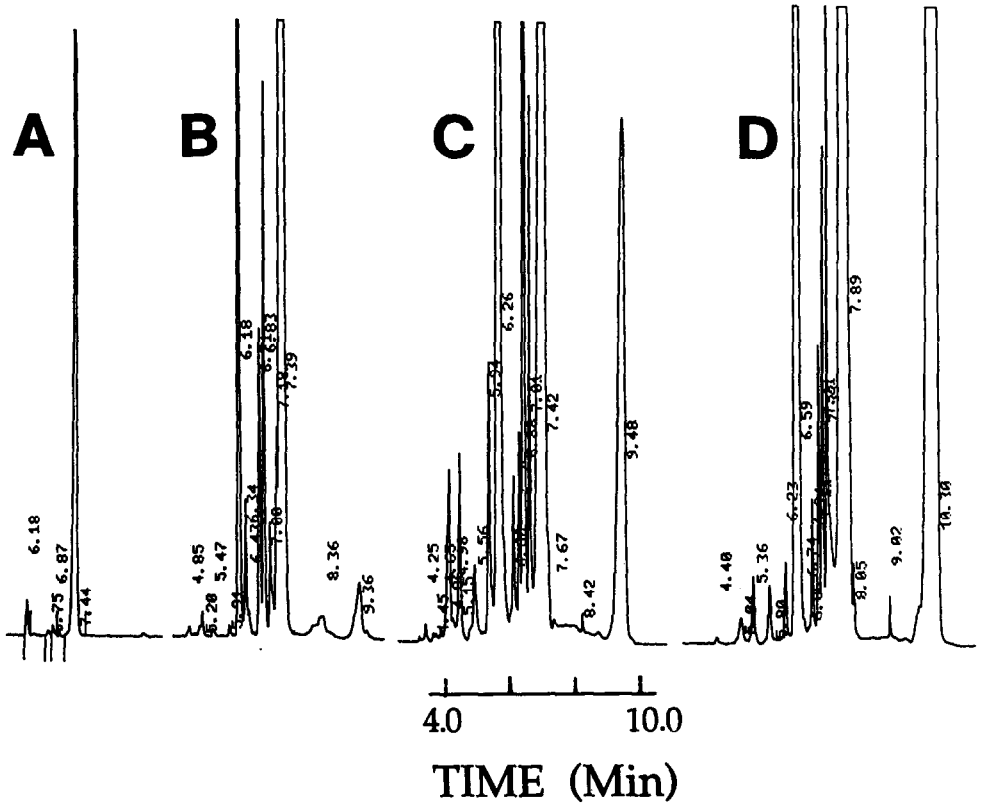

Fig. 1. Electropherograms from capillary electrophoresis at pH 8.7 with laser fluorescence detection of F–ED–dAMP samples: (A, B) unexposed; (C) after exposure to 581 Gy from a ^{60}Co source; (D) after reaction with H_2O_2. Sample (A) was 100 × more dilute than the other samples. The values give the migration times in minutes for the peaks; the major peak at 7.39–7.89 min (depending on the injection) corresponds to F–ED–dAMP. Injection time: 15 s; applied voltage: 30 kV.

troendosmosis and column temperature) throughout the period (one week) for the overall experiment.

The qualitative similarity of the three patterns of peaks (Fig. 1B–D) is interesting, especially the close resemblance of the Fig. 1B profile to the other two. It is reported that both ionizing radiation and H_2O_2 produce similar base products from aqueous DNA, apparently via attack especially by hydroxyl radicals [2–4]. Based on our data, such oxidative degradation apparently is important for this compound (or the precursors for its formation) as well under ordinary storage conditions, where H_2O, H_2O_2 and background radiation all exist. Of course, one cannot claim at this stage that all of the co-eluting peaks among the three electropherograms represent the same products. Some of the products probably correspond to those observed when polyadenylic acid [5], adenosine-5′-monophosphate [6], or DNA [2] are irradiated.

There is interest in establishing a specific biomarker for human exposure to ionizing radiation [7]. To examine the damage to F–ED–dAMP in this context by ^{60}Co *vs.* H_2O_2, we subjected samples of this exposed compound to CE at a higher pH to enhance the resolution. A higher pH was selected since molecules differing in charge tend to separate in CE, and oxidation of adenine is anticipated to form products having pK_a values more in the alkaline range. For example, deoxyguanosine-5′-monophosphate, possessing a purine base that is more highly oxidized than adenine, has a pK_a of 9.7 [8], and 8-oxoadenosine has a pK_a of 8.7 [9]. No pK_a exists for deoxyadenosine-5′-monophosphate throughout the pH range of 8–12 [8,10,11].

Separate ^{60}Co and H_2O_2 exposures of F–ED–dAMP, followed by off-scale CE separation at pH 10.4, yields the electropherograms shown in Fig. 2A and B, respectively. A combined sample was injected to obtain the profile shown in Fig. 2C. As before, similar patterns are observed aside from some of the relative peak ratios. Highlighted in Fig. 2A (and also in Fig. 2C from the combined injection) is a peak that is caused by ^{60}Co but not H_2O_2 exposure.

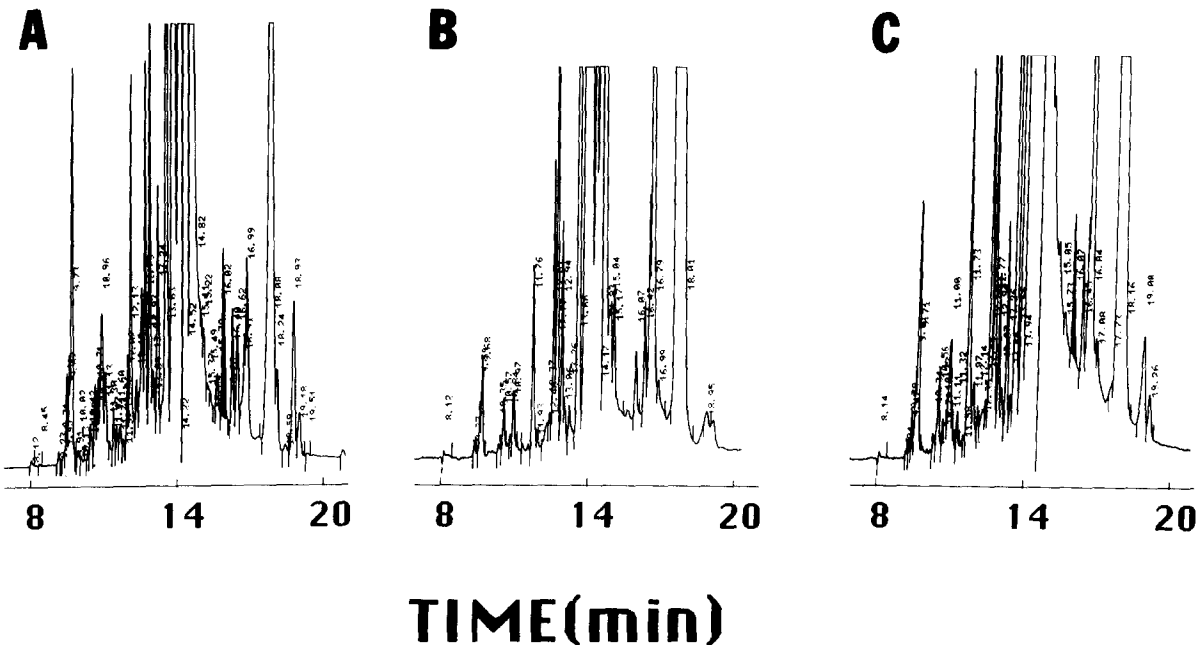

Fig. 2. Electropherograms by capillary electrophoresis at pH 10.4 with laser fluorescence detection of F–ED–dAMP after exposure to (A) ^{60}Co (166 Gy; the 581 Gy-exposed sample was exhausted); (B) H_2O_2; (C) combined injection of (A) and (B) samples. Injection time: 20 s; applied voltage: 15 kV.

It will therefore be interesting to elucidate the structure of this compound.

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