Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1004 (2003) 47–50
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Handling and detection of 25 amol of near-infrared dye deoxynucleotide conjugates by capillary electrophoresis with laser-induced fluorescence detection

Guodong Li, Jianxin Gao, Xiaojuan Zhou, Olga Shimelis, Roger W. Giese*

Department of Pharmaceutical Sciences in the Bouve College of Health Sciences, Barnett Institute, and Chemistry Department, Northeastern University, Boston, MA 02115, USA

Abstract

Near-infrared dyes are attractive as labeling reagents to enhance sensitivity in trace analysis largely because background fluorescence is low in this spectral region. Here we demonstrate, towards a goal of detecting DNA adducts in small biological samples, that some near-infrared (IR) dye-labeled deoxynucleotides can be separated and detected with high sensitivity by capillary electrophoresis (CE)–laser-induced fluorescence detection (LIF) in a realistic way (handling detection limit of 25 amol) for near-IR dye-labeled deoxynucleotides. This detection limit is achieved by polarity-switching injection of 2.0 μl from a volume of 5.0 μl, in which the compounds are $5{\cdot}10^{-12}$ mol/l in 50% aqueous methanol. Although the adenine and cytosine-containing conjugates co-migrated, the other three (guanine, N^2-ethylguanine and thymine) were resolved.

Keywords: Deoxynucleotides; Nucleotides; Dyes

1. Introduction

Towards a goal of detecting DNA adducts (carcinogen-modified nucleotides) in small biological samples, we have continued to develop a technique in which the DNA adducts are labeled with a fluorescent dye and then detected by capillary electrophoresis with laser-induced fluorescent detection (CE–LIF). For the labeling, we employ a dye conjugated to an imidazole functional group. The imidazole links onto the phosphate of a nucleotide after this latter moiety has been activated with a carbodiimide. While we employed a BODIPY dye in our earliest studies [1–3], more recently we are studying a near-infrared cyanine dye. However, the large size of such dyes potentially is an obstacle for their use as chemical tags. This is because it might be difficult to separate small analyte molecules after tagging. This problem was encountered in more general terms, for example, when a bulky radioactivity tag was used to label nucleosides [4].

Previously we demonstrated that as little as 0.8 amol of a cyanine near-infrared dye could be handled and detected by CE–LIF [5]. Here we move on to dye-nucleotide conjugates, and establish CE–LIF conditions that accomplish, simultaneously, the following: (1) large volume, methanol-rich, polarity-switching injection, to take advantage of the high organic solubility of the analytes; (2) a handling detection limit of 25 amol; and (3) separation of four

*Corresponding author. Tel.: +1-617-373-3227; fax: +1-617-373-8720.
E-mail address: r.giese@neu.edu (R.W. Giese).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00855-0

of the five conjugates that we injected, including high resolution of deoxyguanosine-5′-monophosphate and N^2-ethyldeoxyguanosine-5′-monophosphate.

A handling detection limit refers to the minimal amount that one can actually handle prior to injection. Such a detection limit is important in general since trace analytes commonly undergo losses during handling. It is particularly important for the detection of substances such as DNA adducts because the amounts are limited. As we have discussed before, method development in trace analysis by CE–LIF tends to proceed through a sequence of ‘‘peak’’, ‘‘injection’’, ‘‘handling’’ and ‘‘real sample’’ detection limits [5].

2. Experimental

Synthesis of the near-IR dye, construction of the near-IR LIF detector (now available as the ZETALIF near-IR detector from Picometrics, Ramonville, France), and set up of the CE–LIF system were described before [5]. The dye was conjugated to a histamine-containing leash as described for a corresponding BODIPY dye [2,6]. Nucleotides were conjugated to the dye as described for a BODIPY-histamine reagent [7] and purified by HPLC: Vydac C_4-silica, 250×4.6 mm, gradient from 20 m*M* triethylammonium acetate pH 7.0–methanol (1:1, v/v) (as A–B) to A–B (2:8) in 20 min. N^2-Ethyldeoxyguanosine-5′-monophosphate was synthesized as described [8]. New capillaries (75×0.10 mm) were conditioned with flowing (50 mbar) 0.1 mol/l NaOH for 1 h, then rinsed with water for 10 min. Before each injection, the capillary was flushed with 0.1 mol/l NaOH for 5 min, water for 5 min, 90% methanol for 5 min, and running buffer (see Fig. 1) for 10 min.

3. Results and discussion

Many DNA adducts comprise modifications of the nucleobase moiety, which also is the group that ionizes at moderately low and high pH values (pH 3–4.5 and 9–12 ranges). Thus we focused on electrolytes in these pH ranges for the CE separation of our dye-nucleotide conjugates. We also included ≥20% methanol in the electrolytes since this organic solvent enhances both the solubility and brightness of the dye. Five dye-nucleotides (A, G, T and C, plus an N^2-ethyl-G adduct) were tested. The latter adduct forms in alcoholics [9]. Only three, partly-separated peaks could be obtained for the mixture of the five compounds in the low pH range (data not shown). Although a single peak for the mixture was observed (not surprisingly, considering their pK_a values) at pH 8.5 (10 m*M* sodium borate–methanol, (1:1, v/v)), four peaks were observed (A and C co-migrated) starting at pH 9.0 (partial resolution), that were fully resolved throughout the pH range of 9.0–10.0; pH 9.8 was optimal (data not shown). This concentration of methanol also turned out to be optimal: less methanol reduced the migration time at the expense of resolution, while more methanol degraded resolution largely as a consequence of prolonging the migration time, which yielded broader peaks. Increasing or decreasing the buffer concentration had a similar effect. Although 20 m*M* borate increased the resolution somewhat, it doubled the migration time.

We next turned our attention to practical sensitivity, which means how much of the analyte one can handle for detection. For detection by CE–LIF, this demands an enrichment injection of a large volume. By employing polarity-switching injection conditions [1,10], as illustrated and described in Fig. 1, we were able to measure a $5 \cdot 10^{-12}$ mol/l solution of the dye-nucleotide mixture by injecting 2 μl as a methanol–water (90:10) solution into the capillary filled with the 50% aqueous methanol solution of the buffer (10 m*M* borate, pH 9.8). However, the reproducibility was very poor in terms of resolution and recovery. We assumed that the origin of this problem, at least in part, was the difference in the concentration of methanol between the injection solvent and electrolyte, creating a variable disturbance at this interface, which in turn made it difficult to optimize resolution and recovery simultaneously. Thus we changed the injection solvent to 50% aqueous methanol, which gave a much more reproducible separation. A representative electropherogram is shown in Fig. 1, in which 2.0 μl of $5 \cdot 10^{-12}$ mol/l sample was injected from a sample volume of 5.0 μl, giving a handling-detection limit of 25 amol (amount in the 5 μl). The efficiency

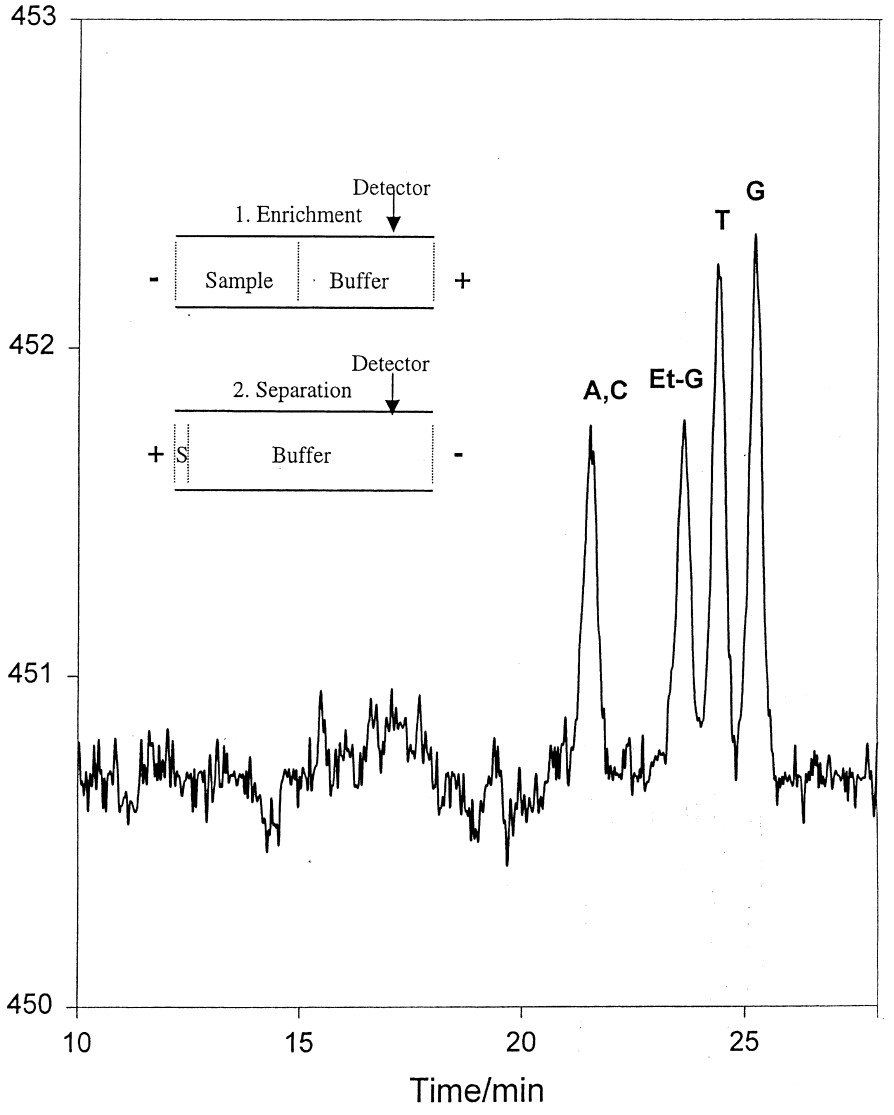

Fig. 1. Electropherogram of near-IR dye deoxynucleotide conjugates of 5′-dAMP (A), 5′-dCMP (C), N^2-ethyl-5′-dGMP (Et-G), 5′-TMP (T) and 5′-dGMP (G). Detector: ZETALIF (Picometrics), with excitation at 785 nm. Capillary: 75 cm (60 cm to the detection window)×100 μm. Running buffer: methanol–10 m*M* sodium borate pH 9.8 (1:1, v/v). Injection: from a 5 μl volume of $5 \cdot 10^{-12}$ mol/l of each analyte in methanol–water (1:1, v/v), was pressure-injected (50 mbar) 2 μl. After −20 kV was applied at the sample end (see inset) until the current reached >95% of its value for running buffer, the polarity was switched and separation commenced at +20 kV.

varies from 18 000 to 28 000 when multiple runs are made.

While dye-labeled nucleotides of A and C are not resolved in the electropherogram shown in Fig. 1, the resolution of the G and N^2-G dye-labeled nucleotides is high, as is the overall sensitivity. Ultimately we plan that overall selectivity will come from a combination of pre-labeling HPLC followed by post-labeling CE–LIF, e.g. A and C deoxynucleotides are readily separated by reversed-phase HPLC [11]. High sensitivity is extremely important in studies of DNA adducts, since large populations of people need to be tested to produce meaningful epidemiology data. This in turn means that a practical biological

sample, such as a fingerprick of blood, needs to be sufficient for such testing.

4. Conclusion

We have demonstrated the resolution and handling-detection of low amol amounts of near-IR dye-labeled deoxynucleotides by CE–LIF. This includes the resolution of a small DNA adduct (N^2-ethyl-G) in spite of the presence of a large dye tag. Our primary motivation for studying such dyes as labeling reagents is the lack of interference from background fluorescent signals even at low amol levels with no special precautions to purify the other reagents involved. While work remains to bring this dye-labeling technique to the analysis of real samples, such work is encouraged by the results presented here.

Acknowledgements

This work was supported by contract 340811E3119 from the Massachusetts Department of Public Health, and NIEHS grant ES10539. Contribution No. 827 from the Barnett Institute.

References

[1] P. Wang, R.W. Giese, Anal. Chem. 65 (1993) 3518.
[2] Z.-H. Lan, X. Shen, R.W. Giese, Anal. Chem. 70 (1998) 3978.
[3] P. Wang, R.W. Giese, J. Chromatogr. A 809 (1998) 211.
[4] F.Z. Sheabar, M.L. Moriningstar, G.N. Wogan, Proc. Natl. Acad. Sci. USA 91 (1994) 1696.
[5] C. Yang, O. Shimelis, X. Zhou, G. Li, C. Bayle, M. Nertz, H. Lee, L. Strekowski, G. Patonay, F. Couderc, R.W. Giese, J. Chromatogr. A 979 (2002) 307.
[6] J. Gao, R.W. Giese, in preparation.
[7] Z.-H. Lan, P. Wang, R.W. Giese, Rapid Commun. Mass Spectrom. 13 (1999) 1454.
[8] M. Sako, H. Kawada, K. Hirota, J. Org. Chem. 64 (1999) 5719.
[9] J.-L. Fang, C.E. Vaca, Carcinogenesis 18 (1997) 627.
[10] R.-L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046.
[11] C. Leuratti, R. Singh, C. Lagneau, P.B. Farmer, J.P. Plastaras, L.J. Marnett, D.E.G. Shuker, Carcinogenesis 19 (1998) 1919.