Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

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Version of record first published: 04 Oct 2006.

To cite this article: D. Zamzow , G. J. Small & R. Jankowiak (1996): Capillary Electrophoresis-Fluorescence Line-Narrowing (CE-FLN) System for DNA Adduct Characterization, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 291:1, 155-162

To link to this article: http://dx.doi.org/10.1080/10587259608042743

Mol. Cryst. Liq. Cryst., 1996, Vol. 291, pp. 155–162
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CAPILLARY ELECTROPHORESIS - FLUORESCENCE LINE-NARROWING (CE-FLN) SYSTEM FOR DNA ADDUCT CHARACTERIZATION

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Abstract Capillary electrophoresis (CE) interfaced with low-temperature (4.2 K) fluorescence line-narrowing (FLN) spectroscopy is used for separation and structural characterization of polycyclic aromatic hydrocarbon-derived DNA adducts. The CE-FLN system is applied to the separation and identification of mixtures of benzo[*a*]pyrene and dibenzo[*a,l*]pyrene DNA adducts. Interfacing CE with FLN spectroscopy allows on-line separation and high-resolution spectroscopic identification of CE-separated DNA adducts, providing a new powerful tool for DNA adduct structure characterization.

INTRODUCTION

Capillary electrophoresis (CE) is a well-established analytical and bioanalytical separation technique. CE is a powerful approach for the analysis of nucleic acid bases and DNA oligonucleotides,[1] for gene mapping,[2] DNA sequencing,[3] and cell analysis.[4,5] In addition to separation methods and protocols, the development of selective detection methods that are capable of providing structural information on separated molecular analytes is an area of active research. Interfacing CE with mass spectroscopy has been demonstrated.[6] Recently, CE has been interfaced with fluorescence line-narrowing (FLN) spectroscopy for the separation and spectral characterization of fluorescent molecular analytes.[7]

FLN spectroscopy is a high-resolution fluorescence-based detection method that provides spectral resolution sufficient for structural characterization of molecular analytes and distinction between structurally very similar species.[8,9] FLNS has been shown to be a powerful technique for the detection and characterization of stable and depurinating polycyclic aromatic hydrocarbon (PAH) - DNA adducts from *in vitro*[10-13] and *in vivo*[14-16] ex-

periments where one is limited to picomole or smaller quantities of bound metabolite. Combining CE with FLN spectroscopy provides an even more versatile methodology, allowing the separation and spectral characterization of complex mixtures of analytes.

This paper further explores the potential of the CE-FLN system, showing the first application of the system to the separation and on-line spectral characterization of CE-separated DNA adducts. We demonstrate that the ability to analyze PAH-DNA adducts in small volumes at femtomole levels, using the CE-FLN system, provides a tool for studying carcinogen metabolism and carcinogen-DNA adduct formation, both of which are important factors in chemical carcinogenesis.

EXPERIMENTAL

The CE-FLN system, consisting of a modular CE system (ATI Unicam model 310), the instrumentation used for low-temperature laser-excited fluorescence spectroscopy, and a specially-designed capillary cryostat (CC, Janis Research), has been described in detail recently.[7] The CC consists of a double-walled quartz cell with inlet and return lines for introducing liquid nitrogen or liquid helium; the outer portion of the CC is evacuated. The capillary, positioned in the central region of the CC, can be cooled to 77 K by a continuous flow of liquid nitrogen through the cryostat or to 4.2 K by a continuous flow of liquid helium for low-temperature laser-excited fluorescence experiments.

Room-temperature fluorescence electropherograms are acquired during CE separations using the UV lines of an argon ion laser (Coherent Innova model 90-6) for excitation. In the current arrangement, a fiber optic is used to deliver ~1 mW laser power to the capillary; the output of the fiber is apertured and re-imaged onto the capillary using a 5-cm focal-length lens, at a demagnification of approximately 4. Fluorescence is collected at a right angle to the laser excitation beam, dispersed by a 1-m monochromator (McPherson model 2061), and detected by a photodiode array (Princeton Instruments IRY-1024/GRB intensified array). To discriminate against scattered and reflected laser light, the CC is tilted 20° with respect to the laser beam. For low-resolution fluorescence measurements, the monochromator is equipped with a 150 G/mm grating, providing a resolution of 1.3 nm and a spectral window of approximately 160 nm at the diode array.

UV-transparent fused silica capillary tubing, 75 μm i.d. and 95 cm in length, is used for electrophoretic separations. The CE buffer is an acetonitrile-water solution (30% v/v) containing 40 mM sodium bis(2-ethylhexyl) sulfosuccinate and 8 mM sodium borate, adjusted to pH 9 using phosphoric acid. Separation is performed at an applied voltage of 30 kV. When the separation is complete, the voltage is turned off and the capillary is cooled to low temperature. The CC is attached to a translation stage (New England Affiliated Technologies model TM-200-SM); translation of the CC and capillary allows the CE-separated analytes to be sequentially characterized by fluorescence spectroscopy. Fluorescence of CE-separated analytes is excited using an excimer laser (Lambda Physik Lextra 100) for non-line-narrowing fluorescence (308 nm excitation at 77 K) or using an excimer-pumped dye laser (Lambda Physik FL-2002) for fluorescence line-narrowing ($S_1 \leftarrow S_0$ excitation at 4.2 K). For FLN measurements, the monochromator is equipped with a 2400 G/mm grating, providing a resolution of 0.8 nm and a spectral window of approximately 10 nm; the diode array is operated in a gated detection mode, using the output of a photodiode to trigger a high-voltage pulse generator (Princeton Instruments FG-100).

RESULTS and DISCUSSION

Dibenzo[*a,l*]pyrene (DB[*a,l*]P), one of the most potent PAH carcinogens, yields a variety of stable and depurinating DNA adducts formed via both the one-electron oxidation and diolepoxide pathways.[17,18] The combination of two-dimensional HPLC and FLN spectroscopy has previously been used for analysis of depurinating and (after DNA digestion) stable adducts. It has been shown that the following adducts are produced *in vitro*: DB[*a,l*]P-10-N3Ade, DB[*a,l*]P-10-N7Ade, DB[*a,l*]P-10-N7Gua, and DB[*a,l*]P-10-C8Gua (one-electron oxidation products), as well as *syn*-DB[*a,l*]P diolepoxide-14-N7Ade and *anti*-DB[*a,l*]P diolepoxide-14-N7Gua (diolepoxide adducts).[12] To illustrate the high-resolution selectivity of FLN spectroscopy, spectra for three one-electron oxidation standard adducts, DB[*a,l*]P-10-N7Ade, DB[*a,l*]P-10-N1Ade, and DB[*a,l*]P-10-N3Ade, are shown in Figure 1. These adducts are identical except for the position of binding of DB[*a,l*]P to adenine. The FLN spectra were obtained for the three adducts, in ethanol at

4.2 K, using selective laser excitation at 416.0 nm. The peaks in the FLN spectra are labeled with their S_1 excited-state vibrational frequencies, in cm^{-1}. Due to differences in some of the excited-state vibrational modes, as well as differences in the intensity distribution of the FLN peaks, it is clear that these adducts can be distinguished by FLNS.

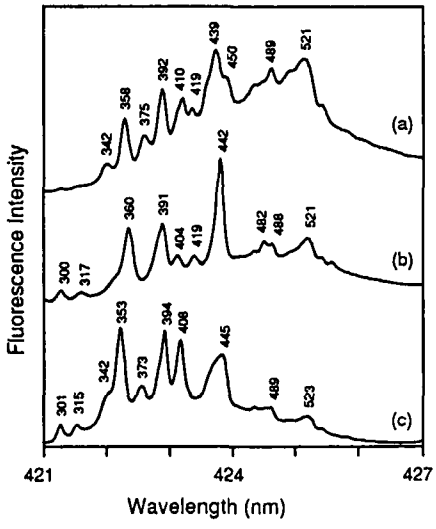

FIGURE 1 FLN spectra of (a) DB[*a*,*l*]P-10-N7Ade, (b) DB[*a*,*l*]P-10-N1Ade, and (c) DB[*a*,*l*]P-10-N3Ade, in ethanol at 4.2 K, for an excitation wavelength of 416.0 nm. The FLN peaks are labeled with their S_1 excited-state vibrational frequencies, in cm^{-1}.

Although these three individual DB[*a*,*l*]P adducts can be distinguished, a mixture of the three would not be resolvable by FLN spectroscopy alone. However, by combining CE (for separation) and FLN (for spectral identification), a mixture of the three adducts can be resolved. This is shown in Figure 2. Panel A of Figure 2 is the room-temperature fluorescence electropherogram obtained during CE separation of (a) DB[*a*,*l*]P-10-N7Ade, (b) DB[*a*,*l*]P-10-N1Ade, and (c) DB[*a*,*l*]P-10-N3Ade. Panel B of Figure 2 shows FLN spectra obtained for the CE-separated adducts, in the CE buffer at 4.2 K, using selective laser excitation at 416.0 nm. The peaks in the FLN spectra are labeled with their S_1 excited-state vibrational frequencies, in cm^{-1}. These and other FLN spectra, obtained for several other excitation wavelengths (data not shown), allowed spectral characterization of these DNA adducts, based on the analysis of vibrational frequencies. A comparison of

the FLN spectra in Figures 1 and 2 shows that there are differences due to the solvent matrices used, ethanol and the CE buffer solution; that is, the FLN spectra for the adducts are not exactly the same in ethanol and in the CE buffer matrix. The 77 K fluorescence spectra for the DB[*a*,*l*]P adducts in ethanol are relatively narrow; in the CE buffer, the spectra are generally broader and red-shifted (data not shown). The FLN spectra for the adducts in the CE buffer matrix are also slightly red-shifted; for a given excitation wavelength, higher frequency vibronic modes are excited more efficiently in the CE buffer than in ethanol. In addition, in the CE buffer the FLN peaks (zero-phonon lines) are superimposed on a larger underlying broad-band fluorescence (phonon sideband emission) than is the case in the ethanol matrix. As a result, for fingerprinting identification of CE-separated adducts, it is necessary to have a library of standard FLN spectra for the adducts dissolved in the CE buffer solution.

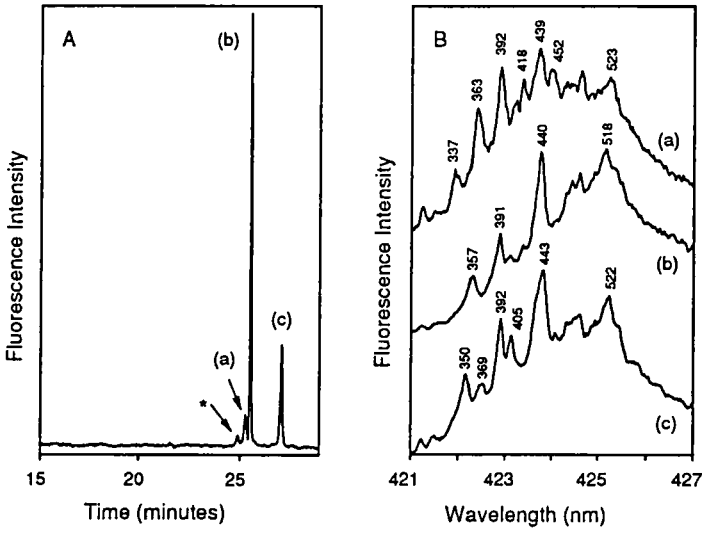

FIGURE 2 (A) Room-temperature fluorescence electropherogram acquired during CE-separation of a mixture of (a) DB[*a*,*l*]P-10-N7Ade, (b) DB[*a*,*l*]P-10-N1Ade, and (c) DB[*a*,*l*]P-10-N3Ade. An unidentified analyte is marked by the asterisk. (B) FLN spectra for the three CE-separated adducts, obtained at 4.2 K using selective laser excitation at 416.0 nm. The peaks are labeled with their S_1 excited-state vibrational frequencies, in cm^{-1}.

A second example of CE-FLN identification of DNA adducts is shown in Figure 3, for a mixture of benzo[*a*]pyrene-derived adducts. Panel A of Figure 3 is the room-temperature fluorescence electropherogram obtained during CE separation of (a) B[*a*]PDE-N7Ade (a diolepoxide adduct) and (b) B[*a*]P-N7Ade (a one-electron oxidation adduct). The upper trace was obtained by integrating the fluorescence emission for the (0,0) transition of B[*a*]PDE-N7Ade at ~378 nm. No peak is seen for B[*a*]P-N7Ade in the upper trace, because the fluorescence emission for this adduct occurs at longer wavelength. The lower trace was obtained by integrating the fluorescence emission for the (0,0) transition of B[*a*]P-N7Ade at ~405 nm. In this case a small peak is observed for B[*a*]PDE-N7Ade, due to vibronic band fluorescence in the region used to generate the electropherogram. Panel B of Figure 3 shows FLN spectra for CE-separated B[*a*]PDE-N7Ade, at 4.2 K, using selective laser excitation at 369.6 nm (upper) and 369.0 nm (lower). The peaks in the FLN spectra are labeled with their S_1 excited-state vibrational frequencies, in cm^{-1}. Although FLN spectra are shown for only two excitation wavelengths, by changing the

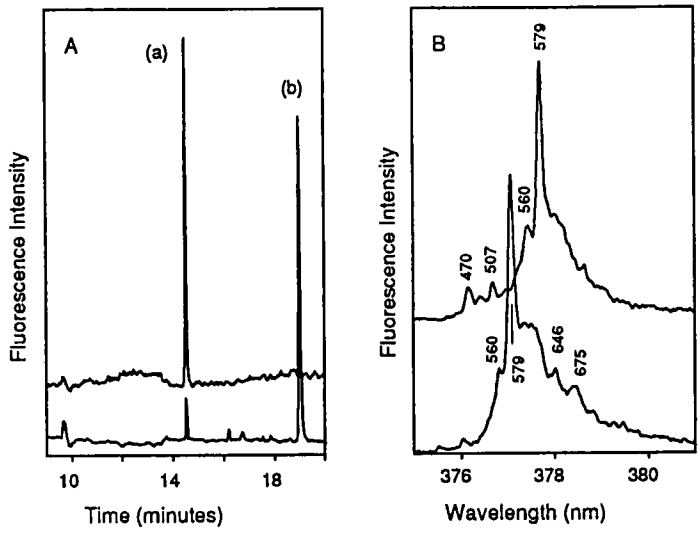

FIGURE 3 (A) Room-temperature fluorescence electropherogram acquired during CE-separation of a mixture of (a) B[*a*]PDE-N7Ade and (b) B[*a*]P-N7Ade (see text for further discussion). (B) FLN spectra for B[*a*]PDE-N7Ade, obtained at 4.2 K using selective laser excitation at 369.6 nm (upper) and 369.0 nm (lower). The peaks are labeled with their S_1 excited-state vibrational frequencies, in cm^{-1}.

laser wavelength, it is possible to map out all the S_1 excited-state vibrational frequencies of the fluorescent chromophore, thereby generating a series of fingerprint FLN spectra that can be used for spectral identification.

CONCLUSIONS

We have demonstrated that mixtures of DNA adducts can be separated and analyzed directly on-line by CE-FLNS. The combination of the separation power of CE and the spectral selectivity of FLN spectroscopy provide a valuable technique for characterizing complex mixtures of DNA adducts, metabolites, PAHs, and other fluorescent analytes. Since the methodology provides excellent selectively and femtomole detection levels, this approach should be particularly valuable in the analysis of PAH-DNA adduct reaction mixtures in *in vitro* and *in vivo* experiments. With improvements in the current CE-FLN system, projected detection levels for many PAH-DNA adducts are in the attomole range, so many applications in biological, medical, and forensic sciences can be anticipated.

ACKNOWLEDGMENT

Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under contract no. W-7405-Eng-82. This work was supported by the Office of Health and Environmental Research, Office of Energy Research.

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