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Evanescent Fluorobiosensor for the Detection of Polyaromatic Hydrocarbon Based on DNA Intercalation

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ABSTRACT

A flow-injection analysis (FIA) system coupled with an evanescent wave (EW) biosensor employing total internal reflection of fluorescence radiation (TIRF) for the detection of polyaromatic hydrocarbon that intercalates into DNA is reported. A highly fluorescent intercalator, "ethidium bromide," has been used as the reference compound for detection. The EW biosensor was developed according to the procedure described earlier (*1,2*). Data on the analysis of Naphthalene, 3-methylcholanthrene, 7,12-dimethylbenz(a)anthracene, 1,2-benzanthracene, and some standard reference materials supplied by the National Institute of Standards and Technology are reported. The relative ability of the polyaromatic hydrocarbon to displace ethidium bromide, based on the relative binding ratio, is found to be on the order of 7,12-dimethylbenz[a]anthracene > 3-methylcholanthrene > 1,2-benzanthracene > napthalene.

Index Entries: Evanescent wave; polyaromatic hydrocarbon; PAH; DNA intercalation; biosensor; fluorobiosensor.

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INTRODUCTION

The intercalation or aromatic residues into the DNA double helix (*1–18*) has attracted a significant amount of attention, because such studies are useful in the development of technologies for the detection and quantitation of several varieties of organic carcinogens and environmental toxins, and also for detecting DNA hybridization (*1,2,8,14,17,18*). The selective detection of a DNA intercalating species has been used for the detection of other intercalating residues that competitively bind with the DNA double helix. As a first approach, we used a photoelectroactive derivative of anthraquinone as the intercalator. The detection of the intercalated anthraquinone is based on the electrochemical oxidation of photoreduced anthraquinone at the surface of a chemically modified electrode. The decrease in anodic current determines the quantity of intercalated anthraquinone, since it cannot be oxidized at the modified electrode surface. This is because of the restricted diffusion of DNA intercalated anthraquinone (*1*). In a second method, the double-stranded DNA (dsDNA) was immobilized at the surface of a cylindrical optical wave guide. The dsDNA was probed by intercalation of ethidium bromide and monitored by measurement of the evanescent wave (EW), employing total internal reflection of fluorescence radiation. The change in fluorescence owing to competitive displacement of the fluorescent intercalator by the nonfluorescent intercalator determines the unknown concentration (*2*). As an example, the detection of 9,10-anthraquinone-2,6-disulfonic acid, Remazol brilliant blue, Decacyclene, and 4′,6-diamidiono-2-phenylindole dihydrochloride (DAPI) was reported.

The present article describes the data on the detection of some polyaromatic compounds based on the approach reported earlier (*2*). The relative binding affinity of these compounds with respect to ethidium bromide, the selected fluorescent DNA intercalator, is also reported.

EXPERIMENTAL SECTION

Materials

The materials used in this investigation and their sources were as follows: Calf thymus dsDNA and ethidium bromide were obtained from Sigma Chemical Company (St. Louis, MO); 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, 1,2-benzanthracene, and naphthalene were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). A standard reference material (SRM, #1587) containing the solution of polyaromatic hydrocarbon in methanol was obtained from the National Institute of Standards and Technology. All other chemicals used were of analytical reagent grade. The procedure for the immobilization of dsDNA over the optical fiber was similar to that described in an earlier publication (*2*).

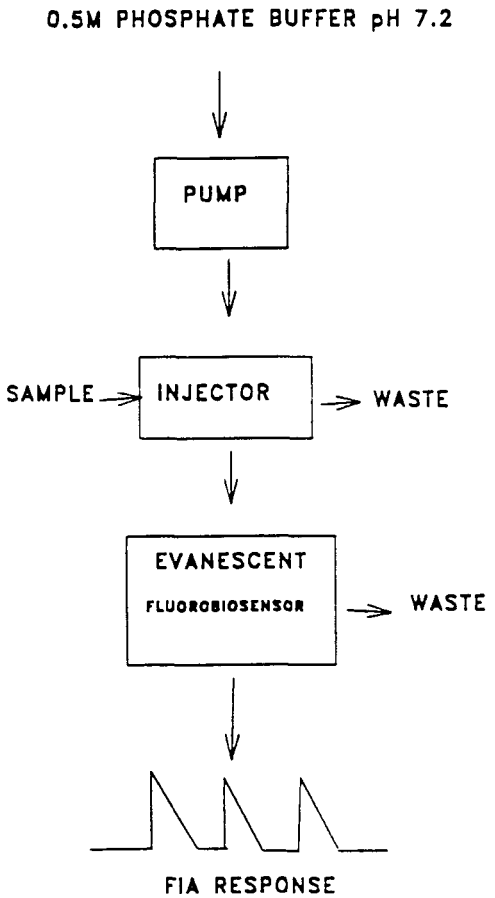

Fig. 1. A schematic diagram of the FIA fluorobiosensor.

Flow-Injection System

A diagram of the flow-injection system is shown in Fig. 1. The phosphate buffer (0.5*M*, pH 7.2) was pumped by an HPLC pump (Waters 501 HPLC pump, Waters, Milford, MA) to the evanescent fluorometer designed and built at ORD Inc. (North Salem, NH) through an autosampler (Spectra-Physics, SP 8880, Freemont, CA) equipped with a 20-μL sample loop. A sample in the full loop injection mode was introduced, unless otherwise stated. The evanescent fluorometer was connected to an x-t recorder (Linear™ Instrument Corporation, model 1200, Reno, NV). The flow rate was 24 mL/h unless otherwise stated. The temperature of the carrier buffer stream was maintained at 25°C with a Brookfield thermostat (Stoughton, MA). The phosphate buffer was sterilized prior to use by autoclaving and subsequently filtered through a 0.45-μ filter.

A schematic diagram of fiber optic evanescent fluorometer is shown in Fig. 2. Ethidium bromide was excited just outside the wave guide boundary of a patented optical fiber at the excitation wavelength of 499/82 nm

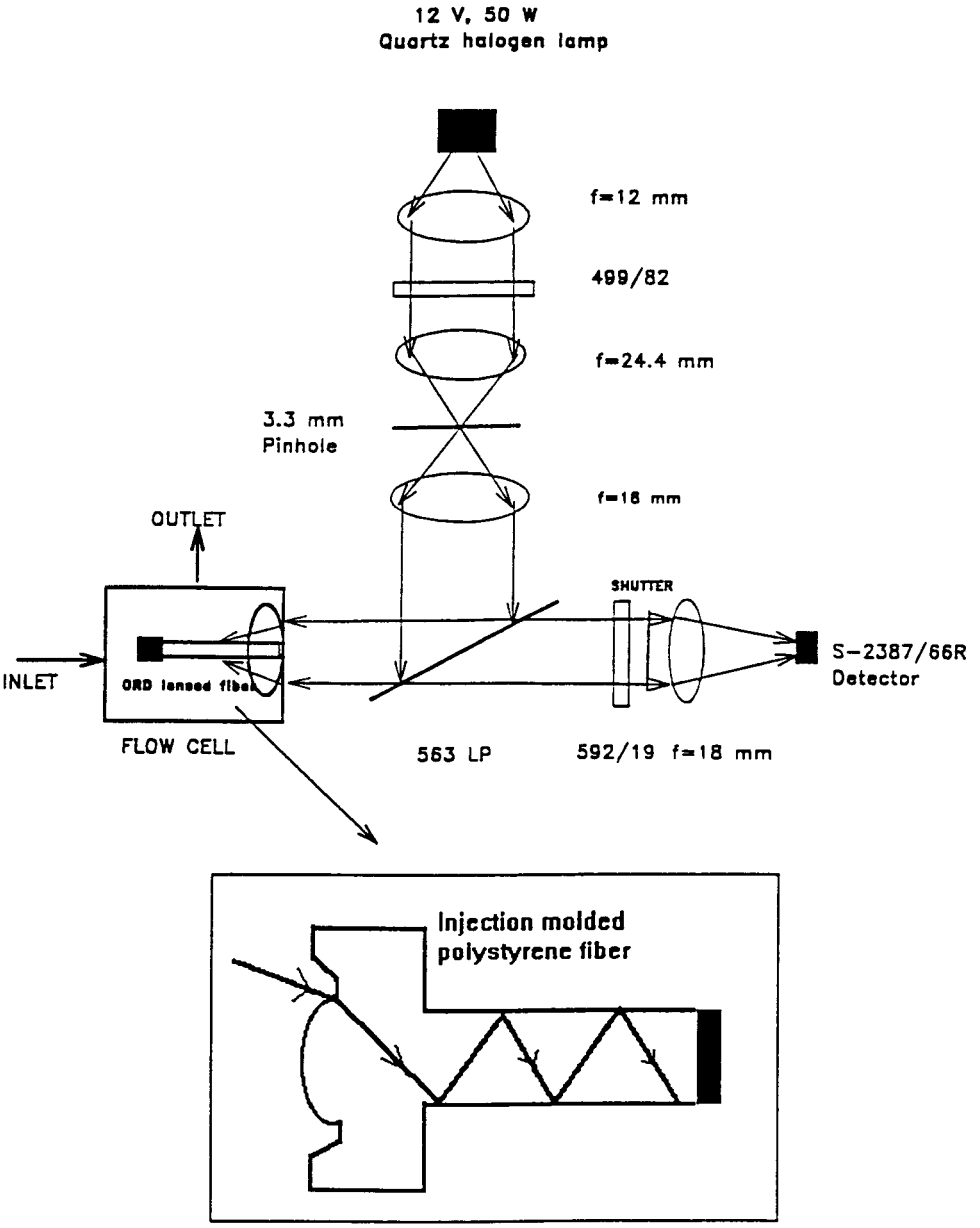

Fig. 2. Schematic diagram of the optical system for the fluorescence measurement.

(center wavelength, 499 nm; FWHM, 82). A part of the emitted fluorescence re-entered the wave guide and was transmitted back up the fiber for the detection by a Hamamatsu S-2387/66R silicon detector, after transmission through 563-nm Long pass at 45° and 592/19-nm (center wavelength, 592 nm; FWHM, 19 nm) filters. The dead volume of the flow cell equipped with the optical fiber was 40 μL.

Measurement of Ethidium Bromide

Ethidium bromide concentrations were measured using:

1. Unmodified optical fiber;
2. Polymer-modified fiber without DNA; and
3. DNA-modified optical fiber in a flow injection system as described earlier (2).

The mobile phase 0.5*M* phosphate buffer, pH 7.2, was pumped at the flow rate of 24 mL/h. Increasing concentrations of ethidium bromide were injected through an autosampler, and the flow-injection analysis (FIA) responses in a peak height mode were used for analytical calculation. The interaction of fluorophore (ethidium bromide) with EW was recorded by collecting the resulting fluorescence to the detector.

Measurement of Competitive Binding Between Ethidium Bromide and Other DNA Intercalators

For the measurement of competitive binding between DNA intercalators and ethidium bromide, a constant concentration of ethidium bromide (4 ng/mL) was pumped at a flow rate of 24 mL/h along with the mobile phase (0.5*M* phosphate buffer, pH 7.2) until the steady-state response of the sensor was observed. While at steady state, varying concentrations of the competitive intercalators containing the concentration of ethidium bromide present in mobile phase were injected. There was a decrease in fluorescence followed by the recovery of the steady state.

RESULTS AND DISCUSSION

The optimum conditions with regard to the concentration of immobilized dsDNA on the optical wave guide, the flow rate, and minimum concentration of intercalated ethidium bromide to obtain stead-state response have been described previously (2). The optimum dsDNA concentration was 0.2 mg/mL, and a steady-state response of the EW biosensor was obtained at a constant (4 ng/mL) concentration of ethidium bromide at a flow rate of 24 mL/h. At the steady-state response of the sensor, varying concentrations of competitive intercalators were injected. Figure 3 shows the calibration curves for 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, 1,2-benzanthracene, and naphthalene.

The standard reference material (SRM, no. 1587) sample containing nitrated polyaromatic hydrocarbons in methanol, obtained from the National Institute of Standards and Technology, was also analyzed. The SRM sample consists of seven nitrated polyaromatic hydrocarbons of known concentration in (ppm), namely 2-nitrofluorene (9.67), 9-nitroanthracene (5.01), 3-nitrofluoranthene (9.24), 1-nitropyrene (8.95), 7-nitrobenz[a]anthracene (9.27), 6-nitrochrysene (8.13), and 6-nitrobenzo[a]pyrene

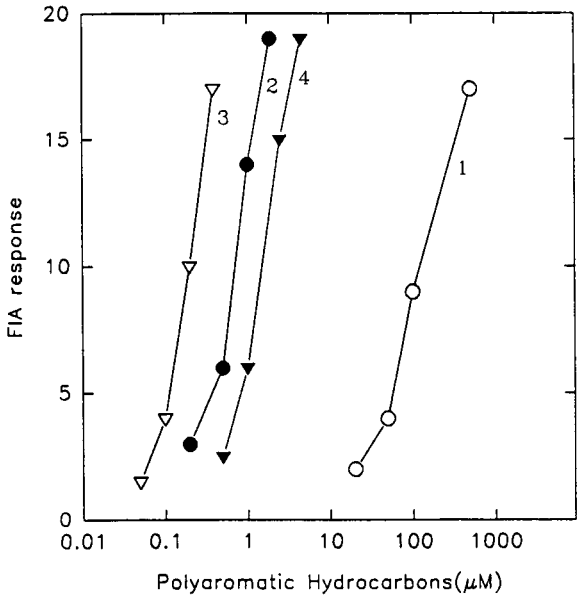

Fig. 3. Calibration curves for analysis of: (1) naphthalene; (2) 3-methylcholanthrene; (3) 1,2-benzanthracene; and (4) 7,12-dimethylbenz[a]anthracene based on the displacement of intercalated ethidium bromide. The competitive intercalators were injected at the steady-state response of the evanescent fluorobiosensor obtained after circulating a constant concentration of ethidium bromide (4 ng/mL) along with the mobile phase (0.5*M* phosphate buffer, pH 7.2).

(6.1). The sample was diluted in the working buffer and injected for the competitive measurement of the ethidium bromide fluorescence. Figure 4 shows the results of the fluorescence measurements. It appears that the nitrated polyaromatic hydrocarbons do intercalate in the dsDNA.

The relative binding ratios (k) of the detected intercalator with dsDNA have been calculated using ethidium bromide as the reference as described earlier (2). The values of the relative binding ratio for different intercalators at the constant concentration of dsDNA are shown in Table 1. The relative ability of the intercalators to displace ethidium bromide based on the relative binding ratios is as follows: 7,12-dimethylbenz[a]anthracene > 3-methylcholanthrene > 1,2-benzanthracene > naphthalene. This technique can be used to detect any molecules that are intercalated into dsDNA that do not fluoresce at the excitation wavelength of the competing fluorescent intercalating eye.

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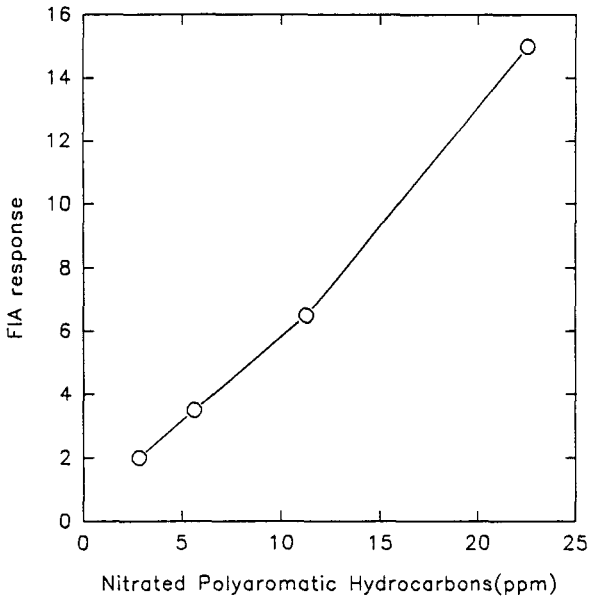

Fig. 4. Analysis of the standard reference mateial sample (SRM, no. 1587) containing nitrated polyaromatic hydrocarbons supplied by the National Institute of Standards of Technology. The SRM sample was injected at the steady-state response of the evanescent fluorobiosensor obtained after circulating a constant concentration of ethidium bromide (4 ng/mL) along with the mobile phase (0.5*M* phosphate buffer, pH 7.2).

Table 1
Relative Binding Ratio of DNA Intercalators with Respect to Ethidium Bromide

Intercalator	Relative binding ratio[a]
7,12-Dimethylbenz[a] anthracene	5.7×10^{-4}
3-Methylcholanthrene	1.2×10^{-4}
1,2-Benzanthracene	5.22×10^{-5}
Naphthalene	4.8×10^{-6}

[a]This represents the ratio $[\delta E/C_{(in)}]/\delta k/c_{(ethidium\ bromide)}$ where δE is the decrease of steady-state response of the sensor on the injection of a known concentration (C[in]) of the intercalator, and δk is the response of the sensor at the concentration of 4 ng/mL ethidium bromide.

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