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DNA Biosensor for the Detection of Hydrazines

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A double-stranded (ds) DNA-coated carbon paste electrode is employed as a remarkably sensitive biosensor for the detection of hydrazine compounds. The sensor relies on monitoring changes in the intrinsic anodic response of the surface-confined DNA resulting from its interaction with hydrazine compounds and requires no label or indicator. Short reaction times (1−10 min) are sufficient for monitoring part-per-billion levels of different hydrazines. Applicability to untreated natural water samples is illustrated. The response mechanism is discussed, along with prospects of using DNA biosensors for quantitaing other important molecules and elucidating DNA interactions and damage.

Nucleic acids offer the analytical chemist a powerful tool in the recognition and monitoring of many important compounds.[1] For example, nucleic acid recognition layers can be combined with fiber-optic or electrode transducers to form a new type of affinity biosensors.[2,3] Compared to antibodies or receptors, such biorecognition elements are highly stable and reusable, and they can often be synthesized in the laboratory. Recent activity in this direction has centered upon the design of sequence-selective biosensors based on monitoring of selective hybridization effects.[2,3] Another new and promising avenue is to exploit molecular interactions between the surface-confined DNA and target pollutants or drugs for rapid screening of these compounds. For example, Pandey and Weetall[4] recently reported on the detection of aromatic compounds that intercalate and accumulate onto DNA-coated electrodes.

This note reports on a new DNA biosensor strategy based on monitoring changes in the intrinsic response of the immobilized DNA probe induced by various chemical agents. We have recently demonstrated that DNA- and RNA-modified carbon electrodes display a distinct potentiometric stripping peak associated with the oxidation of the guanine residue.[5,6] Similar to the response of DNA-modified mercury drop electrodes,[7,8] the anodic signal of DNA-coated carbon electrodes is strongly influenced by chemical, structural, or conformational variations in the immobilized probe accrued from DNA−analyte associations. In the following sections we demonstrate that the double-stranded (ds) DNA-coated carbon paste transducer displays an analytically useful response for part-per-billion levels of various hydrazine compounds, based on diminution of the guanine oxidation peak. The resulting DNA biosensor offers a highly sensitive, rapid, and portable tool for field monitoring of these environmentally and toxicologically significant compounds.

EXPERIMENTAL SECTION

Apparatus. A TraceLab potentiometric stripping unit (PSU 20, Radiometer, Denmark) and an IBM PS/2 55SX computer were used to obtain the potentiograms. According to the TraceLab protocol, the potentials were sampled at a frequency of 30 kHz, and the derivative signal (dt/dE) was recorded against the potential. The analytical signal was evaluated using the peak area following baseline fitting.

The three-electrode system consisted of a carbon paste electrode (CPE), a reference electrode (Ag/AgCl, Model RE-1, BAS) and a platinum wire auxiliary electrode, joined at the cell through holes in the Teflon cover. The body of the working electrode was a Teflon sleeve (3.5 mm i.d.) filled with carbon paste. Electrical contact was established with a stainless steel screw. The carbon paste was prepared in the usual way by hand-mixing graphite powder (Acheson 38, Fisher Scientific) and mineral oil (Sigma, Catalog No. M5904, free of DNase, RNase, and protease). The ratio of graphite powder to mineral oil was 70:30. A diode array spectrophotometer (Model 8452A, Hewlett Packard) was employed for measuring the concentration of the DNA solution (at 260 nm). All measurements were carried out in a BAS VC-2 cell, containing 1.0 or 2.0 mL solutions.

Chemicals. Double-stranded calf thymus DNA (dsDNA, Catalog No. D4522), guanine (Catalog No. G6779), O^6-methylguanine (Catalog No. M9287), N^7-methylguanine (Catalog No. M0502),

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(1) McGown, L. B.; Joseph, M.; Pitner, J.; Vonk, G.; Linn, C. *Anal. Chem.* **1995**, *67*, 663A.
(2) Piunno, P.; Krull, U. J.; Hudson, R. H.; Damha, M.; Cohen, H. *Anal. Chim. Acta* **1994**, *288*, 205.
(3) Mikkelsen, S. *Electroanalysis* **1996**, *8*, 12.
(4) Pandey, P. C.; Weetall, H. H. *Anal. Chem.* **1994**, *66*, 1236.
(5) Wang, J.; Cai, X.; Jonsson, C.; Balakrishnan, M. *Electroanalysis* **1996**, *8*, 22.
(6) Wang, J.; Cai, X.; Wang, J.; Jonsson, C.; Palecek, E. *Anal. Chem.* **1995**, *67*, 4065.
(7) Palecek, E. *Electroanalysis* **1996**, *8*, 1.
(8) Palecek, E. *Anal. Biochem.* **1988**, *170*, 421.

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and phenylhydrazine (Catalog No. P6926) were received from Sigma. Hydrazine was purchased from J.P. Baker (Catalog No. 21771), while methylhydrazine (Catalog No. M5000-1), 1,2-dimethylhydrazine (Catalog No. D16,180-2), diphenylhydrazine (Catalog No. 11,459-6), and formaldehyde (Catalog No. 25,254-9) were products of Aldrich. ***Caution*:** The toxicity of hydrazine compounds requires appropriate care.

The stock solution of DNA was prepared in a 10 mM Tris-HCl solution (pH 8.0) containing 1 mM EDTA (TE solution). Solutions of guanine and its methyl derivatives were prepared by dissolving in 0.1 M sodium hydroxide and diluting with water to the desired concentration. Double-distilled autoclaved water was used in the preparation of all solutions.

River water samples were collected from the Rio Grande River at the Picacho Bridge (Las Cruces, NM). The groundwater sample was received from the Hanford Site (Richland, WA).

Procedure. Measurements of hydrazines at the DNA-coated electrode involved nucleic acid immobilization, interaction with the hydrazine species, and PSA transduction of the recognition event. Prior to each medium exchange, the electrode was washed carefully with water for a short time (5 s).

DNA Immobilization. A freshly smoothed carbon paste surface was first pretreated by applying a potential of +1.7 V for 1 min in a stirred acetate buffer solution (0.2 M, pH 5.0) containing 5 mg/L dsDNA. The nucleic acid was subsequently immobilized onto the activated electrode surface by adsorptive accumulation for 2 min at +0.50 V.

Interaction. The DNA-coated electrode was transferred to the stirred hydrazine sample solution (0.2 M acetate buffer solution, pH 5.0) for the desired time (1−10 min, depending on the target concentration) while holding the potential at +0.20 V.

Transduction. Potentiometric stripping analysis (PSA) was performed in the blank electrolyte solution (0.2 M acetate buffer solution, pH 5.0) with an initial potential of +0.5 V and a constant current of +8 μA.

The analytical signals of the target were based on the decrease of the potentiometric stripping peak area of the immobilized DNA probe. Thus, the PSA signal of the sensor in the absence of the analyte serves as "blank", or 100%. Repetitive measurements were carried out by renewing the surface and repeating the above assay protocol (including readsorption of a fresh DNA layer). All experiments were performed at room temperature (23.0 ± 0.5 °C).

Each measurement was performed using a fresh solution and properly cleaned cells (i.e., thoroughly rinsed with diluted nitric acid followed by deionized water).

RESULTS AND DISCUSSION

Figure 1 displays potentiograms recorded in the blank solution after immersing the DNA-coated electrodes in 1,2-dimethylhydrazine solutions of increasing concentrations in 1.2 μg/L (ppb) steps (b−f). A well-defined oxidation peak (at +1.04 V), inherent to PSA at DNA-modified carbon paste electrodes,[5] is observed in the absence of dimethylhydrazine (a). This DNA−guanine peak decreases nearly linearly upon increasing the hydrazine concentration; a short (10 min) reaction time thus offers convenient quantitation of ppb levels of dimethylhydrazine. These traces are a part of a series of thirteen 0.6 μg/L increments in the dimethylhydrazine concentration. The resulting "titration curve"-like calibration plot (shown as inset) consists of two portions. The first one involves a gradual decrease of the response, with the second portion (above 4.8 μg/L) where the signal nearly disappears and hence levels off.

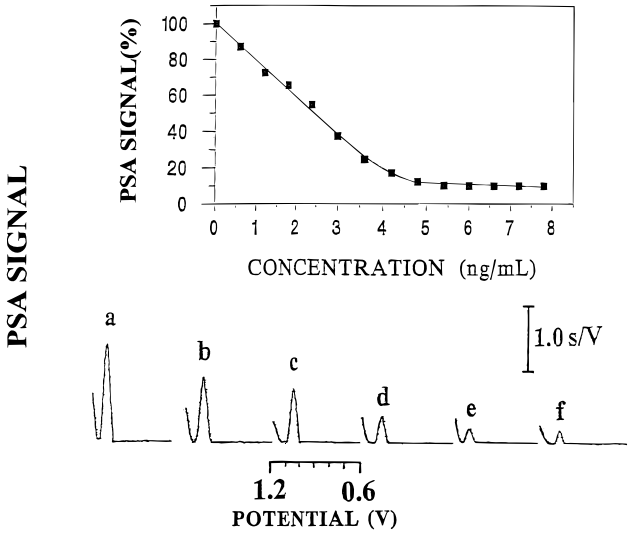

Figure 1. Potentiograms at the dsDNA-coated electrode for 1,2-dimethylhydrazine solutions of increasing concentrations: 0 (a), 1.2 (b), 2.4 (c), 3.6 (d), 4.8 (e), and 6.0 (f) μg/L. Also shown is PSA signal (%) vs concentration plot. Electrode pretreatment and DNA immobilization, 1 min at +1.7 V, followed by 2 min at +0.5 V in the supporting electrolyte (0.2 M acetate buffer, pH 5.0) containing 5 mg/L dsDNA; interaction, 10 min at +0.2 V in the electrolyte containing 1,2-dimethylhydrazine; measurement, in the blank electrolyte using a stripping current of 8 μA.

The reason for the suppression of the DNA−guanine peak appears to reflect the toxicological action of hydrazine compounds. Administration of different hydrazines to small animals resulted in the formation of N^7-methylguanine and O^6-methylguanine.[9,10] Hydrazine-induced strand scission of DNA has also been reported.[11] As shown in Figure 2, methylguanines display a significantly lower redox activity compared to the electrochemical oxidation of guanine. The cyclic voltammetric anodic peak height for O^6-methylguanine (B) is only 8% of the corresponding guanine peak (A); N^7-methylguanine displays a similar (small) peak height at a more positive potential (C). While the endogenous formaldehyde appears to play a role in the in vivo methylation of DNA−guanine by hydrazine compounds,[9] we did not observe any effect of formaldehyde upon the suppression of the DNA−guanine response.

The dynamics of the hydrazine−DNA reaction can be followed by immersing the probe in the stirred hydrazine solution for different reaction periods. Figure 3 displays the influence of the reaction time (with different hydrazine compounds) upon the PSA response of the DNA guanine. These compounds display different temporal profiles. While hydrazine (A) and methylhydrazine (B,a) result in a sharp suppression and near disappearance of the guanine peak above 6 min, more gradual peak diminutions are observed for dimethylhydrazine (B,b), phenylhydrazine (C,a), and diphenylhydrazine (C,b). Note that extremely low (ppb) concentrations are sufficient for such dramatic changes in the DNA−guanine response. Only hydrazine requires a higher (ppm) level to induce similar effects.

(9) Bosan,W.; Shank, R.; Macewen, J.; Gaworski, C.; Newberne, P. *Carcinogenesis* **1987**, *8*, 439.

(10) Mathison, B. H.; Murphy, S.; Shank, R. *Toxicol. Appl. Pharmacol.* **1994**, *127*, 91.

(11) Runge-Morris, M.; Wu, N.; Novak, R. *Toxicol. Appl. Pharmacol.* **1994**, *125*, 123.

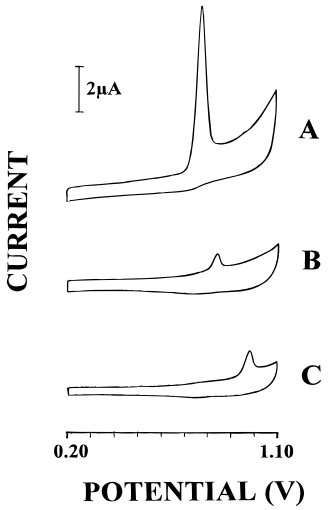

Figure 2. 2. Cyclic voltammograms for 5×10^{-6} M guanine (A), O^6-methylguanine (B), and N^7-methylguanine (C) at the pretreated carbon paste electrode. Supporting electrolyte, 0.2 M acetate buffer, pH 5.0; electrode pretreatment, 1 min at +1.7 V; preconcentration, 2 min at +0.2 V with stirring; equilibration time, 5 s; scan rate, 20 mV/s.

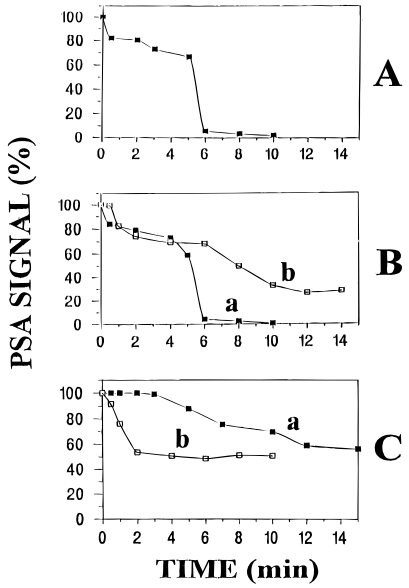

Figure 3. **3.** Effect of interaction time on the chronopotentiometric response (%) to 10 mg/L hydrazine (A), 1.4 μg/L methylhydrazine (B,a), 3.0 μg/L dimethylhydrazine (B,b), 25 μg/L phenylhydrazine (C,a), and 40 μg/L diphenylhydrazine (C,b). Other conditions as in Figure 1.

Such diminutions of the DNA−guanine peak resulted in concentration-dependent calibration plots (similar to the one shown in Figure 1). For example, using a 5 min reaction time, the probe response decreased gradually for increasing dimethylhydrazine and diphenylhydrazine concentrations (over the 0.5−8.0 and 10−110 μg/L ranges, respectively; not shown). For methylhydrazine and phenylhydrazine, the response decreased slowly at first (up to 2 and 30 μg/L, respectively), and then more rapidly. As expected (from Figure 3), hydrazine led to changes in the DNA−guanine response only at the ppm level, with a slow decrease up to 10 mg/L, and a sharper one at higher concentrations; the peak disappeared above 25 mg/L hydrazine. Overall, the sensitivity trend, methylhydrazine > dimethylhydrazine > diphenylhydrazine > phenylhydrazine > hydrazine, appears to

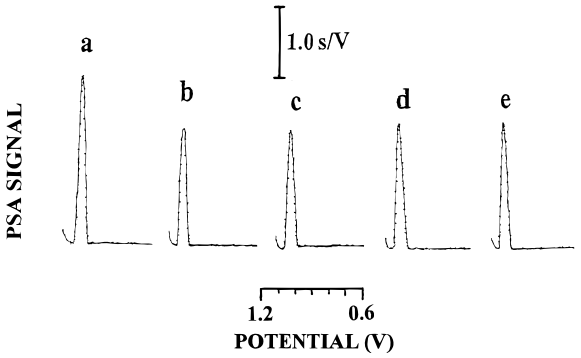

Figure 4. Discrimination among hydrazine compounds. (a) Potentiograms for the blank solution (0.2 M acetate buffer, pH 5.0); (b) same as (a) but after adding 1.5 μg/L methylhydrazine; (c−e) same as (b) but after additions of 50 μg/L hydrazine. Interaction time, 2 min; other conditions as in Figure 1.

reflect the extent of hydrazine−DNA interactions and damage. The detection limits, 0.5 μg/L for methyl- and dimethylhydrazines, 10 and 15 μg/L for phenyl- and diphenylhydrazines, and 2 mg/L for hydrazine (5 min reaction), also correspond to the extent of these interactions.

The interaction of hydrazine with the DNA surface layer results in a reproducible response. For example, a series of six repetitive measurements of 1.5 μg/L methylhydrazine, 3 μg/L dimethylhydrazine and 30 μg/L diphenylhydrazine yielded mean peak diminutions of 24.2, 29.2, and 30.1% and relative standard deviations of 3.9, 5.4, and 5.7%, respectively (2 min reaction time; other conditions as in Figure 1).

Discrimination among hydrazine class compounds is often a difficult analytical task and commonly requires their prior separation. The significant higher sensitivity of the new DNA sensor toward hydrazine derivatives, compared to the parent (hydrazine) compound, offers a new dimension of selectivity. Figure 4 illustrates such selectivity for measurements of methylhydrazine in the presence of a large excess of hydrazine. The sensor displays a well-defined response to 1.5 μg/L methylhydrazine (compare a and b). This response is not affected by successive additions of 50 μg/L hydrazine (c−e). Hence, a 100-fold excess of hydrazine has a negligible effect upon the methylhydrazine signal (compare b and e). Similar improvements are expected for measurements of other hydrazine derivatives in the presence of hydrazine. Even though the sensor is not able to distinguish between such derivatives, it may be used as a quick "alarm" for a sudden hydrazine contamination or in connection to a proper separation technique.

Figure 5 demonstrates the suitability of the new DNA probe for assays of relevant natural water samples. The DNA sensor displays a well-defined guanine peak for both untreated groundwater and river water samples (traces a in A and B, respectively). The peak is similar to that observed for "synthetic" samples (e.g., traces a in Figures 1 and 4). Successive additions of 5 mg/L and 1 or 2 μg/L levels of hydrazine and dimethylhydrazine, respectively, and a short (2 min) reaction time resulted in a gradual decrease of the guanine peak that allows convenient quantitation of the hydrazine compounds. It should be pointed out that the gradual decline in the presence of hydrazine differs from the sharper one observed in pure buffer solutions, indicating potential matrix effects. The sensor should thus be calibrated in the relevant sample matrix. The stability of the DNA coating in the

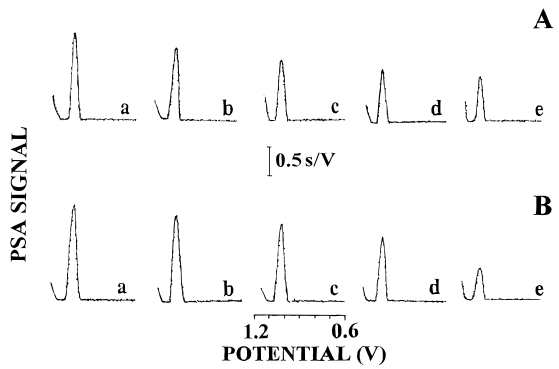

Figure 5. Assays of untreated natural water samples. (A) Potentiograms for the groundwater sample (a), spiked with 5 (b), 10 (c), 15 (d), and 20 (e) mg/L hydrazine. (B) Potentiograms for the river water sample (a), spiked with 1 (b), 3 (c), 5 (d), and 7 (e) μg/L dimethylhydrazine. Interaction time, 2 min; other concentration as in Figure 1.

natural water media was examined by immersing it in a stirred groundwater solution for 15 min while holding it at +0.2 V (i.e., the potential used during the interaction with hydrazine). The guanine peak, used as a measure of the probe stability, remained the same over the first 12 min and decreased ~8% at 15 min. In view of the short (1−5 min) times used for the hydrazine reaction, such a loss is not of major concern. The present carbon paste DNA immobilization scheme is fast (3 min), reproducible, and integrated in the assay protocol. Yet other (particularly covalent) DNA immobilization schemes may offer an even higher stability, as desired for routine sensing applications.

In conclusion, the experiments described above have demonstrated that changes in the intrinsic electrochemical response of DNA-coated carbon transducers can offer a highly sensitive biosensing of hydrazine compounds. Compared to a recent biocatalytic (enzyme inhibition) biosensor for hydrazines,[12] the DNA biosensor offers significantly lower detection limits and new dimensions of specificity. Electrocatalytic modified electrodes for hydrazines[13] do not rely on the biological action of these compounds, commonly require an alkaline media and chromatographic separation, and suffer from a gradual decrease in the response. The use of pollutant−DNA interactions for producing concentration-dependent electrochemical signals holds great promise for rapid monitoring of damaging agents and for elucidating structural changes induced by these associations. While the use of nucleic acid recognition layers is still in the embryonic stage, many exciting applications of DNA biosensors are anticipated in connection with different modes of interaction and signal transduction.

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(12) Wang, J.; Chen, L. *Anal. Chem.* **1995**, *67*, 3824.
(13) Korfahnge, K.; Ravichandran, K.; Baldwin, R. P. *Anal. Chem.* **1984**, *56*, 1517.

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