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Microfabricated Electrochemical Sensor for the Detection of Radiation-Induced DNA Damage

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An electrochemical biosensor protocol for the detection of radiation-induced DNA damage is described. The procedure employs a dsDNA-coated screen-printed electrode and relies on changes in the guanine−DNA oxidation signal upon exposure to ultraviolet radiation. The decreased signal is ascribed primarily to conformational changes in the DNA and to the photoconversion of the guanine−DNA moiety to a nonelectroactive monomeric base product. Factors influencing the response of these microfabricated DNA sensors, such as irradiation time, wavelength, and distance, are explored, and future prospects are discussed. Similar results are given for the use of bare strip electrodes in connection with irradiated DNA solutions.

Damage to DNA in cells leads to a serious disturbance of the cell functions.[1,2] Such a process usually involves minor variations in the DNA structure or conformation, and hence its detection requires a highly sensitive analytical technique. Current procedures for measuring DNA damage rely on lengthy and insufficiently sensitive chromatographic or electrophoretic separation assays.[2] In addition, such techniques cannot follow the dynamics of processes occurring in an exposure of DNA to physical or chemical damaging agents. Previous studies by Vorlickova and Palecek[3] and Nurnberg et al.[4] illustrated that polarography can detect DNA damage induced by exposure to ultraviolet or γ radiation. However, since this strategy relies on mercury drop electrodes, it is not suitable for widespread sensing of DNA radiation damage.

This note describes a solid-state electrochemical sensor, based on a DNA-modified screen-printed electrode, for the rapid detection of radiation-induced DNA damage. The screen-printing (thick-film) microfabrication technology offers large-scale mass production of extremely inexpensive and yet highly reproducible electrode transducers. Recent work in this laboratory has illustrated the utility of nucleic acid-coated thick-film carbon electrodes for detecting ultratrace levels of DNA and RNA or specific DNA sequences.[5,6] Transfer of these DNA-modified microfabricated strips to a blank solution yields a well-defined and stable chronopotentiometric anodic peak, associated with oxidation of the guanine−DNA residue. In the following sections, we employ changes in this intrinsic DNA oxidation response induced by exposure to ultraviolet (UV) radiation for the sensing of DNA damage.

EXPERIMENTAL SECTION

Apparatus. Potentiometric stripping analysis (PSA) was performed in a three-electrode electrochemical cell (Kimble Glass Inc., Vineland, NJ), using a TraceLab potentiometric stripping unit (PSU 20, Radiometer, Copenhagen, Denmark) interfaced with an IBM PS/2 55SX computer. The three-electrode system consisted of a screen-printed electrode (SPE), a Ag/AgCl reference electrode (Model RE-1, BAS Inc., West Lafayette, IN), and a platinum wire auxiliary electrode. The three electrodes entered the cell through a Teflon cover. According to the TraceLab protocol, the potentials were sampled at a frequency of 30 kHz, and the derivative PSA signal (dt/dE) vs potential (E) was recorded (as the s/V vs V plot) after filtering and baseline fitting. The area of the guanine−DNA oxidation peak (at about +1.0 V) served as the analytical signal.

An ultraviolet lamp (Model UVSL·25, Ultra-violet Products, Inc., San Gabriel, CA) was used for all UV radiation measurements. The lamp output was measured using a Research Radiometer dosimeter (Model IL 1700, International Light Inc., Newburyport, MA) connected to an SED 240 detector. Radiometric irradiance values of 6.0 and 100 μW/cm^2 were thus obtained for the 254 nm source using sample−UV lamp distances of 15 and 1 cm, respectively. All radiation doses mentioned below were also measured with this dosimeter. The experimental setup for the irradiation experiments is shown in Figure 1.

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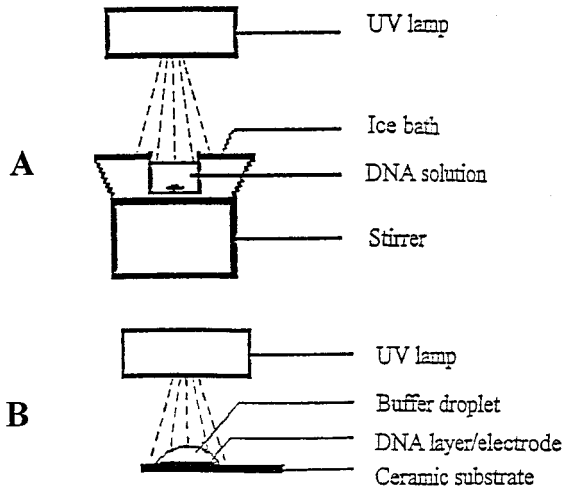


Figure 1. Schematic diagrams of the experimental setups for the irradiation experiments used with the solution-phase (A) and surface-confined (B) dsDNA.

A thermocouple thermometer (Barrant 115 Model 6002810, Barrant, Barrington, IL) was used for measuring the temperature in the solution droplet covering the strip sensor.

Chemicals and Solutions. Double-stranded calf thymus DNA (dsDNA, activated and lyophilized, Catalog No. 4522), single-stranded (ss) DNA, and poly(G) were purchased from Sigma (St. Louis, MO). Monosodium phosphate (Catalog No. S3139) and sodium acetate buffer (3 M, pH 5.2 ± 0.1 at 25 °C, Catalog No. S7899) were also received from Sigma and were certified free of DNase and RNase.

DNA stock solutions (nominally 1000 μg/mL) were prepared with the TE buffer (1× concentrate, 10 mM Tris-HCl, 1 mM EDTA, pH 8). Sterile distilled water was used for preparing all the solutions. A diode array spectrophotometer (Model 8452A, Hewlett Packard) was employed to determine the exact concentration of nucleic acids by measuring absorbance at 260 nm.

All glassware, containers, pipet tips, and the cell (with the exception of the electrodes) were autoclaved for 30 min. Sterilized water was used to rinse the electrodes prior to use.

Electrode Preparation. The SPEs were prepared using a semiautomatic screen printer (Model TF-100, MPM Inc., Franklin, MA). Commercial carbon ink (Product No. C10903D14, Gwent Electric Materials Ltd., Gwent, UK) was printed onto alumina ceramic plates (33.5 mm × 101.5 mm, Coors Ceramic Co., Golden, CO) through a patterned stencil to give a group of 10 printed carbon electrodes, with each carbon strip being 1.5 mm × 30 mm. The electrodes were subsequently cured for 1 h (at 180 °C) and then allowed to cool. A layer of insulator (celluose nitrate-based nail varnish) was then placed onto part of the printed carbon strip, leaving a nominal 1.5 mm × 5 mm working area.

Procedure. *Solution-Phase DNA Damage.* Two milliliters of the DNA solution were tested before and after UV radiation by using PSA. The solution was kept in an ice bath while being exposed to either 254 or 310 nm radiation (Figure 1A). The SPE was pretreated by applying a potential of +1.8 V for 1 min, followed by adsorptive accumulation for 2 min at +0.2 V in the stirred DNA solution (0.2 M sodium acetate buffer, pH 5.2, containing 5 μg/mL of dsDNA). The chronopotentiometric measurement of the accumulated nucleic acid was carried out in the same solution with an initial potential of +0.2 V and a constant current of +4 μA.

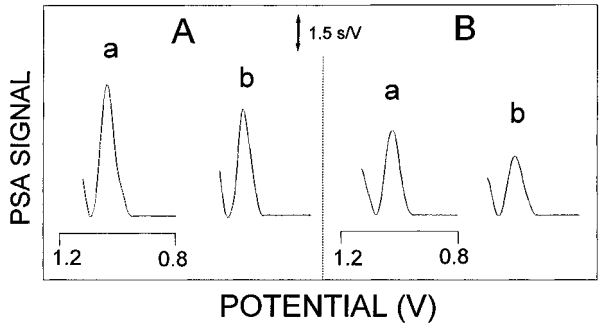


Figure 2. Chronopotentiograms for solution-phase (A) and surface-confined (B) calf thymus dsDNA before (a) and after (b) exposure to UV radiation. (A) Sample solution 5 μg/mL dsDNA in 0.2 M sodium acetate buffer (pH 5.2); irradiation time 15 min; sample−UV lamp distance 2.5 cm; wavelength 254 nm; adsorptive stripping potentiometry at the SPEs after 1 min pretreatment at +1.8 V and 2 min adsorptive accumulation at +0.2 V; stripping current +4 μA. (B) dsDNA-coated SPEs covered with a 50 μL droplet of 0.02 M sodium phosphate buffer (pH 7.0); dsDNA was immobilized from a sodium acetate buffer (0.2 M, pH 5.2) containing 5 μg/mL dsDNA by applying +1.8 V for 1 min, followed by +0.2 V for 2 min; irradiation time 5 min; sample−UV lamp distance 1.0 cm; chronopotentiometric transduction was performed in a blank sodium acetate buffer at a stripping current of +4 μA.

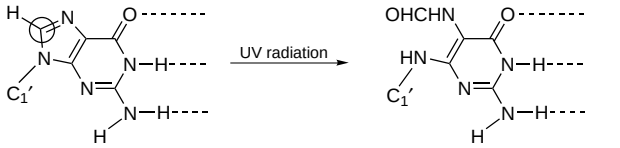
Surface-Confined DNA Damage. The DNA was first immobilized onto the SPE by dipping it into the DNA solution (0.2 M sodium acetate buffer, pH 5.2, containing 5 μg/mL of dsDNA) and applying +1.8 V for 1 min, followed by +0.2 V for 2 min. After being gently rinsed with water (3 s), the DNA-coated electrode was covered with 50 μL of 20 mM sodium phosphate buffer solution (pH 7.0) and placed under the UV lamp for a given period (Figure 1B). The electrode was then rinsed with water for 3 s. The irradiated DNA layer was examined using chronopotentiometry with an initial potential of +0.2 V and a constant current of +4 μA in a 0.2 M sodium acetate buffer. Control experiments were performed using the same protocol, except that the lamp was off during the selected period. Such experiments were used for calculating the relative signals (irradiated/nonirradiated) reported in the next section.

All data points (both the irradiated and nonirradiated ones) were obtained at a new SPE. Unless stated otherwise, all operations were carried out at room temperature (22.0 ± 0.5 °C).

RESULTS AND DISCUSSION

Our previous studies[5,6] have shown that screen-printed carbon strip electrodes can be modified with a DNA layer and that this layer remains stable upon transferring the coated strips to a blank electrolyte solution. A 2 min adsorption time from a 5 μg/L dsDNA solution is sufficient for complete coverage of the surface. Similar to the DNA response of mercury drop electrodes,[3,4] the response of screen-printed carbon strips is strongly influenced by small changes in the immobilized probe resulting from exposure to ultraviolet radiation. Figure 2 displays chronopotentiograms for solution-phase (A) and surface-confined (B) dsDNA at thick-film carbon electrodes before (a) and after (b) short exposure (15 and 5 min, respectively) to 254 nm radiation. As expected from our previous studies,[5,6] such constant-current chronopotentiometric operation results in a well-defined guanine−DNA oxidation peak (despite its extreme potential of +1.02 V). Distinctly smaller chronopotentiometric peaks are observed following the ultraviolet irradiation. For example, peak areas for

Scheme 1. Photoreaction of the Guanine Moiety in DNA (○, Oxidation Site)



Guanine moiety

2,6-Diamino-4-hydroxy-5-formamidopyrimidine moiety

the solution-phase dsDNA at the nonirradiated and irradiated solution correspond to 329 and 250 ms, respectively (a vs b, A). A slightly greater peak diminution is observed when the dsDNA-coated strip electrode is used (216 (a) vs 159 (b) ms, B). There is no apparent change in the position of the peak upon irradiation.

Many radiation-induced damage processes have been identified. Those involving the formation of localized strand breaks[3,4] would be expected to lead to an increased electrochemical signal and cannot be responsible for the behavior observed in Figure 2 (and throughout this study). Conformational changes related to the formation of thymine and other dimers may influence negatively the surface accessibility of the guanine oxidation site. Such a hypothesis is certainly consistent with our results (although the lack of electroactivity of thymine prevents a direct assessment of this). As expected for the presence of the double-helical structure, we do find that native DNA is more sensitive to radiation damage than denatured DNA. Under the conditions where dsDNA's guanine signal decreases to 75% of its original value, that of ssDNA decreases only to 92%.

Another hypothesis was examined. A recent report[7] suggested that the guanine moiety might be converted to the corresponding 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua; Scheme 1).

Suppression of the electrochemical response of the guanine−DNA is expected in the absence of the N^7 oxidation site in the FapyGua product. One might, therefore, expect to be able to demonstrate this rather easily with a poly(G) homopolymer. Yet, with poly(G) we find no significant decrease of the signal. Poly(G) is, of course, single-stranded, and its insensitivity to radiation damage may be related to this fact. Apparently, more than guanine residues are required for the photoreaction to proceed. We cannot, therefore, exclude the hypothesis that the observed response is related to this photoreaction. In view of the complexity of DNA damage processes.[1] we cannot rule out the involvement of other radiation-induced changes in the observed electrochemical behavior.

Figure 3A demonstrates the effect of the strip−lamp distance on the anodic response of the DNA-coated electrode following 5 min of radiation. As expected, the guanine response decreases as the distance shortens. For example, while a relative signal of 81% is observed for a 3 cm distance, only 71% and 40% relative signals are obtained at 1.0 and 0.4 cm, respectively. Under these conditions, the radiation dose increases from ~160 J/m^2 at 3.0 cm to 300 and 600 J/m^2 at 1.0 and 0.4 cm, respectively. Similarly, Figure 3B displays the influence of the irradiation time on the response of the dsDNA screen-printed coated electrode (using a fixed distance of 1.0 cm). The longer the time, the larger is the diminution of the guanine−DNA signal. For example, while a

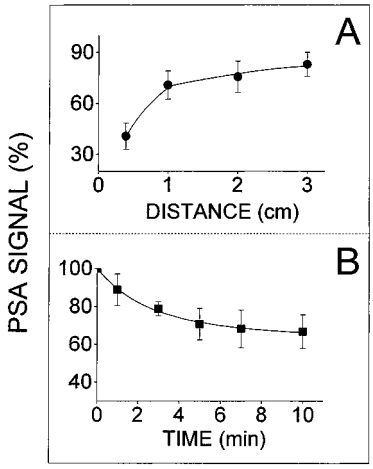


Figure 3. Effect of (A) strip−UV lamp distance and (B) irradiation time on the oxidation response of the surface-confined DNA. Chronopotentiometric signal obtained before the UV radiation was taken as 100%. (A) Irradiation time 5 min; (B) sample−lamp distance 1 cm. Other conditions as in Figure 2B.

9% decrease is observed following a 1 min irradiation, 21% and 32% peak diminutions are observed for the 3 and 7 min periods, respectively. The radiation dose increases from ~60 J/m^2 for 1 min to 300 and 600 J/m^2 at 5 and 10 min, respectively.

Each data point in Figure 3 represents the mean of six different measurements (with different strip electrodes). The relative standard deviation for each series is around 10%; hence, the error bars (±SD) are considerably smaller than the reported changes in the guanine peak. Similar deviations were reported for the GC/MS identification of monomeric base damage products.[7] Series of repetitive measurements of the guanine−DNA response of 20 different nonirradiated and 254-nm-irradiated coated strips (from different printing batches) yielded relative standard deviations of 9% and 11%, respectively, with an average peak diminution of 23% (5 min radiation at 1.0 cm lamp−strip distance). Temperature changes resulting from the irradiation have a negligible effect on the response under the experimental conditions of Figure 3. For example, the temperature of the solution droplet (covering the coated strip) rose to only 27 and 34 °C following 5 min irradiation at 1.0 and 0.4 cm distances, respectively. Irradiation at 310 nm resulted in a negligible decrease in the response of the dsDNA-coated electrodes. Such behavior is in agreement with the greatly reduced extent of the guanine photoreactions at 310 nm (as compared to the 254 nm processes).[7] UV irradiation had no effect on the electrochemical reactivity of the SPE surface toward the dsDNA or on the adsorption properties of the nucleic acid. This was indicated from control experiments with irradiated bare strip electrodes that were exposed to the dsDNA. Additional control experiments using the nonirradiated dsDNA-coated strips, and measurement of the guanine signal, confirmed the stabilty of the surface-confined nucleic acid layer for the irradiation times used throughout this study. (Note that all data are presented as the relative signal based on these control experiments.)

The sensitivity of the DNA damage sensor is influenced by the immobilization conditions as well as other experimental variables. For example, the best results (largest radiation-induced peak diminution) were obtained upon immobilization from a 5 mg/L dsDNA solution. Attempts to irradiate the dsDNA-coated electrode in the absence of a solution droplet yielded irreproduc-

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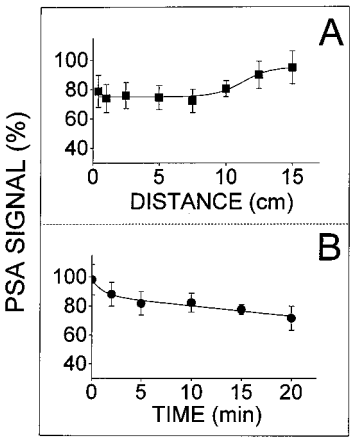

Figure 4. Effect of (A) sample−UV lamp distance and (B) irradiation time on the guanine chronopotentiometric signal of the solution-phase dsDNA. The signal obtained before the UV radiation was taken as 100%. (A) Irradiation time 15 min; (B) sample−lamp distance 0.5 cm. Other conditions as in Figure 2A.

ible results. Coverage with a 0.02 M phosphate buffer solution (50 μL) offered the best performance.

In addition to the use of biosensors based on a surface-confined dsDNA layer, we also assessed the utility of adsorptive PSA measurements[6] at bare carbon strip electrodes for detecting radiation damage in solution-phase dsDNA. Typical stripping potentiograms for the nonirradiated and irradiated DNA solutions were shown earlier in Figure 1A. Figure 4A illustrates the effect of the lamp−solution distance on the potentiometric stripping response for the irradiated dsDNA solution following a 15 min irradiation. The relative response remains about the same (~75%) upon increasing the distance between 0.4 and 7.5 cm and increases slowly to 95% at 15 cm. Under these conditions, the radiation dose increases from 50 J/m^2 at 15 cm to 1700 J/m^2 at 0.4 cm. Figure 4B displays the influence of the irradiation time on the potentiometric stripping response when a distance of 2.5 cm is used. The relative response decreases rapidly to 88% at 2 min (i.e., 70 J/m^2) and then more slowly down to 72% at 20 min (720 J/m^2).

In conclusion, the experiments described above have illustrated that changes in the anodic response of DNA-modified carbon strip electrodes can lead to a new sensing strategy for detecting DNA radiation damage. The decreased guanine−DNA signal is ascribed primarily to conformational changes in the double helix; photoconversion of the guanine−DNA moiety to a nonelectroactive monomeric base product may also contribute to the observed behavior. Further work is required to correlate the changes in the electrochemical behavior with the radiation-induced structural and conformational changes. Such correlations may offer a better understanding of photoinduced DNA modifications. Unlike commonly used separation assays, the new electrochemical sensing strategy should allow monitoring the dynamics of DNA damage processes. Even though the concept is presented in the context of ultraviolet radiation damage, it could be extended to the detection of damage induced by other types of radiation (e.g., ionizing radiation). The coupling of these "one-shot" microfabricated sensor strips with new hand-held chronopotentiometric analyzers[8] should facilitate the on-site electrochemical detection of radiation-induced DNA damage.

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