A Photoelectrochemical Immunosensor for Benzo[a]pyrene Detection Amplified by Bifunctional Gold Nanoparticles

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Supplementary Information

Experiment section

Chemicals and Reagents. Titanium foil (99.8% purity, 0.127 mm thick) was purchased from Aldrich (Milwaukee, WI). Ascorbic acid, H2O2 (30%), sodium fluoride, sodium hydrogen sulfate, sodium dihydrogen phosphate and disodium hydrogen phosphate at analytical grade were purchased from commercial sources and used as received. Benzo(a)pyrene (BaP), 1-aminopyrene, naphthalene, acenaphthene, anthracene, and horseradish peroxidase (HRP) were purchased from Sigma Aldrich. Chitosan, glutaraldehyde, tween 20, bovine serum albumin (BSA) were purchased from Amresco (U.S.). PAH mouse monoclonal antibody was purchased from Santa Cruz Biotechnology, Inc. Twice distilled water was used throughout the experiment.

BSA-PAH Synthesis. BSA-PAH was synthesized by use of the diazotization reaction between 1-aminopyrene and BSA. Briefly, 21.72 mg 1-aminopyrene was dissolved in 2 mL pH 2.0, ice-cold HCl solution. 7.59 mg NaNO2 (pH 2.0, ice-cold HCl solution) was added slowly to the above solution to start the diazotizing reaction at 4 °C for 1 h. Then, 5 mL PBS buffer (pH 7.5, 0.1 M) containing 68 mg BSA was added drop by drop to the reaction solution. Mixture pH was adjusted to 11 by 1.0 M NaOH. After dialysis the reactant in 0.9 % physiological saline for 24 h.

BGNPs Synthesis and Characterization. Au nanoparticles (Au NPs) were synthesized by the reduction of tetrachloroauric acid in trisodium citrate solution according to the previously reported method by Adriano et al.1 Briefly, 100 mL of 0.01% HAuCl₄ solution was refluxed upon which 1.5 mL of 1% trisodium citrate solution was added to the solution. After the solution turned red, it was refluxed for another 30 minutes then allowed to cool. A quantity of 5 mL of the resultant solution was transferred to a small bottle, and the pH was adjusted to 9.0 by 0.1 M K₂CO₃. Surface modification was carried out by adding a mixture of 200 μL HRP (10 mg/mL) and 20 μL BSA-PAH to the above solution then stirred for 12 h at room temperature. After that, 0.8 mL 5% BSA was added and stirred for 8 h. To remove excess chemicals the solution was centrifuged at 10000 rpm for 0.5 h at 4°C. The clear supernatant was carefully removed, and the BGNPs obtained by re-suspending the precipitate in 2 mL of PBS buffer. Ultraviolet-visible (UV-vis) spectra were recorded on a Cary 300 UV-vis spectrophotometer (Varian, USA). Fourier transform infrared (FTIR) spectra were collected on Nicolet 5700 FTIR spectrometer (Thermoelectron, USA).

Immunosensor Construction. TiO₂ NTs were prepared by anodizing titanium foils at a constant potential of 15 V in an electrolyte containing 0.1 M NaF and 0.5 M NaHSO₄ for 3 h in a two-electrode configuration with a platinum cathode. Before anodization, the titanium foil was pretreated by sonication in 3% HF solution for several minutes, then washed in water. After anodization the TiO₂ NTs film was immediately washed with water and then dried in air. The anodized substrate was annealed at 450°C in oxygen for 3 h to convert the amorphous phase to crystalline anatase. A field-emission scanning electron microscope (FE-SEM) (JSM 6700F; JEOL, Tokyo, Japan) was used to characterize the topology of the substrate surface. The TiO₂ NTs/Ti substrate (0.5 cm × 2.5 cm) was coated by 20 μL chitosan solution dissolved in 1% acetic acid and dried at 50 °C for 4 h. After washing with 0.1 M NaOH and water, the substrate was dipped in 5% glutaraldehyde solution for 30 min, and then rinsed with water to remove the physically adsorbed glutaraldehyde. 10 μL of 0.04 mg/mL PAHs antibody was dropped onto the glutaraldehyde-activated substrate and incubated at 4 °C overnight. The as-prepared antibody-modified photoelectrochemical immunosensor was then rinsed with 3% (w/v) BSA overnight. The antibody-modified electrode is referenced as Ab/TiO₂ NTs.

Photoelectrochemical Measurements. Photocurrent was recorded on a CH660C Workstation (CH Instruments, Inc. USA) in a standard three-electrode system with a Pt counter electrode and a saturated Calomel electrode (SCE) reference electrode irradiated under a 300 W Xe lamp. The incident light intensity through a UV cut-filter was 100 mW cm⁻² measured by a radiometer (OPHIR, Newport, USA). As for BaP detection, 30 μL BGNPs solution mixed with different concentrations of BaP was applied on the sensor. After incubating at 39 °C for 3 h, the photocurrent was collected in a 0.1 M PBS (pH 7.0) solution containing 7.5 mM H₂O₂ and 0.1 M ascorbic acid. Ascorbic acid was used as oxidative quencher. The background was obtained with 30 μL PBS instead of the BGNPs solution. The sensor response to BGNPs in the absence of BaP was detected as the control (I₁). BaP was quantified based on the photocurrent changes with respect to I₁. The sensor responses to naphthalene, acenaphthene, and anthracene were also measured to help estimate the sensor selectivity.
Characterization section

A FE-SEM image of the top surface of the as-prepared TiO$_2$ NT arrays is shown in Fig. S1. The well-aligned TiO$_2$ NTs are vertically oriented from the Ti foil substrate providing a good sensor platform with excellent optical properties.

Fig. S1. SEM image of the annealed TiO$_2$ NTs

Optimization of experimental conditions

The effect of the H$_2$O$_2$ concentration, immunoreaction temperature and incubation time on the photocurrent responses was examined to obtain the optimum conditions. Fig. S3 shows the H$_2$O$_2$ concentration-dependent photocurrent amplitude, with the greatest value observed at 7.5 mM H$_2$O$_2$. The possible reason is that too little H$_2$O$_2$ would not cause visible amplification of the photocurrent, while too much H$_2$O$_2$ might directly react with ascorbic acid. Considering the immunoreaction, the region of 25-55°C was chosen to investigate the effect of reaction temperature, with 39°C found the optimal condition for performance (Fig. S4A). Fig. S4B exhibits the effect of incubation time, with 3 h giving the greatest response.

Fig. S2A and S2B show, respectively, UV-vis and FTIR spectra of Au NPs and BGNPs. The Au NPs exhibit an UV-vis absorption peak at 534 nm (curve a) while the BGNPs have a peak at 541 nm (curve b). As anticipated, the modification results in a red shift of 7 nm, which is consistent with the difference in between Au NPs before and after modifications.\textsuperscript{3-5} Compared with the FTIR spectra of the Au NPs, which exhibit hardly any peaks (curve c), the BGNPs have peaks at 3280 nm (\textit{\nu}(\text{NH}_2)), 1652 nm (\textit{\nu}\textsubscript{as}(C=O), \textit{\nu}\textsubscript{phenyl}(C=C)), 1531 nm (\textit{\nu}\textsubscript{phenyl}(C=C)) and 3030 nm assigned to the benzene rings (curve d), confirming the successful coating of Au NPs with HRP and BSA-PAH.

Fig. S2. UV-vis (A) and FTIR (B) spectra of Au NPs (a, c) and BGNPs (b, d).

Fig. S3. Dependence of the photocurrent intensity of the Ab/TiO$_2$ NTs working electrode on the concentration of H$_2$O$_2$ in 0.1 M PBS (pH 7.4) containing BGNPs and 0.1 M ascorbic acid. From a to h the concentration of H$_2$O$_2$ (mM) is: 0, 1.25, 3.75, 7.5, 10, 12.5, 15, 20.

Fig. S4. The effect of (A) temperature and (B) incubation time on the photocurrent intensity of Ab/TiO$_2$ NTs electrode in 0.1 M PBS (pH 7.4) containing BGNP, 0.1 M ascorbic acid, and 7.5 mM H$_2$O$_2$. 
The recovery rates

The recovery rates were investigated by analyzing water samples spiked with BaP in the concentrations of 10.5 pM, 31.5 pM and 105 pM. Recovery rate is defined as the ratio of the added amount detected to the actually added amount, i.e. $R=\frac{m_a-m_b}{m}$, where $m_a$ is the detected value after/before addition of the target, and $m$ is the added amount of target. Table S1 lists the recovery rates.

Table S1. Real sample detection and Recoveries

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*a Nd: not detected

Notes and references


