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Peptide Nanotube-Modified Electrodes for Enzyme−Biosensor Applications

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The fabrication and notably improved performance of composite electrodes based on modified self-assembled diphenylalanine peptide nanotubes is described. Peptide nanotubes were attached to gold electrodes, and we studied the resulting electrochemical behavior using cyclic voltammetry and chronoamperometry. The peptide nanotube-based electrodes demonstrated a direct and unmediated response to hydrogen peroxide and NADH at a potential of +0.4 V (vs SCE). This biosensor enables a sensitive determination of glucose by monitoring the hydrogen peroxide produced by an enzymatic reaction between the glucose oxidase attached to the peptide nanotubes and glucose. In addition, the marked electrocatalytic activity toward NADH enabled a sensitive detection of ethanol using ethanol dehydrogenase and NAD^+. The peptide nanotube-based amperometric biosensor provides a potential new tool for sensitive biosensors and biomolecular diagnostics.

Interest is increasing in exploring the unique properties and potential technological applications of various nanostructures.[1−3] Potential applications include targeted drug-delivery systems, tissue-engineering scaffolds, computational uses, and biosensing devices.[4−6] A fundamental challenge in nanotechnology concerns the use of the distinctive functional properties of nanoscale structures and the ability to manipulate such processes at the nanometer scale.[7]

Depending on their size, shape, and internal structure, nanoparticles frequently display unique physical and chemical properties.[8] Catalysis is one of the most frequently studied applications of nanoscale assemblies. Nanoparticles also facilitate electron transfer[9] and can be easily modified using a wide range of biomolecules and chemical ligands.

Since their discovery in 1991,[10] carbon nanotubes (CNTs) have been extensively studied for their properties and applications.[11,12] The unique electrical properties of CNTs have generated a huge amount of research in nanoelectronic devices and nanosensors.[13−15] Kong et al.[16] were the first to build a CNT-based chemical sensor for detecting NH_2 and NH_3 gas. Chen et al.[17] immobilized proteins on the sidewall of CNTs through a linking molecule, and Besteman et al.,[18] Lin et al.[19] and Wang and Musameh[9] demonstrated the use of CNTs as biological sensors for detecting glucose. Unique electric properties together with significant surface enlargement make the carbon nanotubes an important component in sensing applications.

We recently reported on the potential application of discrete and well-ordered self-assembled peptide nanotubes, formed by the diphenylalanine peptide, for electrochemical monitoring.[20] These tubular structures were discovered during the search for the minimal amyloidogenic self-assembled fragment of the β-amyloid polypeptide that related to Alzheimer's disease. It was found that the diphenylalanine core recognition of this polypeptides can self-assemble into discrete and well-ordered tubular structures. It was suggested that geometrically restricted aromatic interactions contribute order and directionality and mediate the formation of these well-ordered nanostructures. The diphenylalanine-based peptide nanoassemblies have many attractive properties for various nanotechnological applications as they are readily self-assembled in soluble nanostructures, are biobiocompatible, can be easily be

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modified with biological and chemical elements,[21−23] and show a notable similarity to carbon nanotubes in their morphology and aspect ratio.

The aim of the present work was to design and build a highly sensitive amperometric enzyme biosensor based on immobilized self-assembled peptide nanotubes attached to a gold electrode surface. Glucose oxidase (GOx) and ethanol dehydrogenase (ADH) were used as model enzyme systems to demonstrate the advantages of this sensor.

MATERIALS AND METHODS

Materials. Hydrogen peroxide solution, 30%, KCl, K_2HPO_4, and KH_2PO_4 were obtained from Merck. Purified GOx from *Aspergillus niger*, β-D-glucose, β-nicotinamide adenine dinucleotide, reduced form (NADH), NAD^+, ADH from baker's yeast, and polyethyleneimine (PEI) were purchased from Sigma. Phe-Phe peptides were purchased from Bachem. Glutaraldehyde (GA) solution was obtained from Fluka. All solutions were prepared with double-distilled water.

Preparation of Peptide Nanotubes. Fresh stock solutions were prepared by dissolving the lyophilized form of the peptides in 1,1,1,3,3,3-hexafluoro-2-propanol at a concentration of 100 mg/mL.[12] To avoid any preaggregation, we prepared fresh stock solutions for each experiment. The assembly of the peptide nanotubes was performed at an optimal concentration of 2 mg/mL.[20]

Thiol Modification. To connect the nanotubes onto the electrode gold surface, thiol groups was incorporated into the nanotubes by using Traut's reagent (2-iminothiolane hydrochloride, Sigma). The reagent was dissolved in HFP and 2% *N*,*N*-diisopropylethylamine to a final concentration of 100 mg/mL. Then 10 μL of the reagent solution was added immediately to the self-assembled nanotube solution. Following this reaction, the thioleted nanotubes were applied onto the gold electrode.

Electrochemical Cell. We used a 15-mL glass electrochemical cell containing three electrodes: (1) a gold disk working electrode (1 mm in diameter) embedded in Teflon, (2) a platinum wire counter electrode, and (3) a saturated calomel electrode (SCE) as the reference electrode. Before use, the gold electrodes were polished with 0.5-μm alumina, washed with double-distilled water, and then immersed for 20 min in a sonicator bath, followed by washing in double-distilled water.

Instrumentation and Measurements. We used an EG & G PAR M270 potentiostat interfaced to a personal computer. Chronoamperometric experiments with hydrogen peroxide and NADH were conducted at a constant applied potential of +0.4 V in KCl, 0.1 M solution. Measurements of glucose and ethanol were conducted at a constant potential of +0.6 V in a phosphate buffer solution (pH 7.5) that was stirred during the experiment at a constant speed of 100 rpm using a magnetic stirrer. All experiments were carried out at room temperature.

Peptide Nanotube-Modified Electrode Preparation. For the detection of hydrogen peroxide, NADH, and ethanol, an aliquot (2 μL) of the thiol-modified peptide nanotube solution was deposited on the surface of the working electrode and allowed to dry for 90 min at room temperature. For the detection of glucose, 2 μL of the thiol-modified peptide nanotubes was mixed with 1 μM GOx in the presence of 0.25% GA and 0.05% PEI. The use of GA and PEI as a matrix for enzyme immobilization on electrodes was described before.[24] The resulting enzyme-coated peptide nanotubes were deposited on the gold electrode surface and dried for 90 min at room temperature.[25]

Scanning Electron Microscopy (SEM). The modified electrodes were coated with gold. Scanning electron microscopy images were made using a JSM JEOL 6300 SEM operating at 5 kV.

Table 1. Direct and Unmediated Detection of Hydrogen Peroxide by the Peptide Nanotube-Based Gold Electrode[a]

	current response (nA)		
applied potential	0.15 V	0.4 V	0.6 V
peptide nanotube-based electrode	0	83.3 ± 15.2	340 ± 73.9
control electrode	0	5 ± 0.01	102.5 ± 30.9

[a] Current response of the peptide nanotube-based and control electrodes to the addition of 10 mM hydrogen peroxide at various applied potentials. $n = 4$.

RESULTS AND DISCUSSION

We investigated the electrochemistry of the peptide nanotube-based sensor in the context of hydrogen peroxide and NADH detection as these compounds are involved in a wide range of biosensing applications.

Direct Detection of Hydrogen Peroxide. In the first step, we compared the responses to hydrogen peroxide of peptide nanotube-based electrodes and bare gold electrodes. Table 1 depicts the average response of the modified and unmodified electrodes at three applied potentials. The anodic response current of the peptide nanotube-based electrode to the addition of 10 mM hydrogen peroxide at +0.6 V versus SCE was 3.5 times higher than that of the unmodified electrode at the same applied potential. At lower potential (+0.4 V vs SCE), a response of 100 nA was obtained for the modified electrode, whereas no significant response was obtained for the bare electrode. At 0.15 V versus SCE, no activity was observed. It is evident that the modified electrodes show much higher response currents than the bare electrodes.

The amperometric response at 0.4 V of the gold working electrodes to successive additions of hydrogen peroxide and the resulting calibration plots is presented in Figure 1. As expected, the bare electrode did not respond to hydrogen peroxide addition, whereas the peptide nanotube-based electrode responded very rapidly, producing steady-state signals within less than 5 s. The high electrocatalytic activity of peptide nanotube-modified electrodes can be explained by a direct electron transfer between the spatially aligned aromatic systems that contribute to the electronic conductivity of the assemblies.[20]

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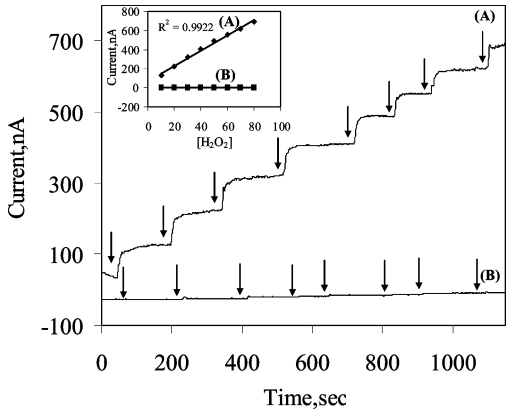

Figure 1. Amperometric response to successive additions of 10 mM H_2O_2 at +0.4 V vs SCE: (A) peptide nanotube-based electrode; (B) bare electrode. Arrows indicate additions of increasing concentrations of hydrogen peroxide. Inset: a calibration plot illustrating the linear electrode response to hydrogen peroxide addition.

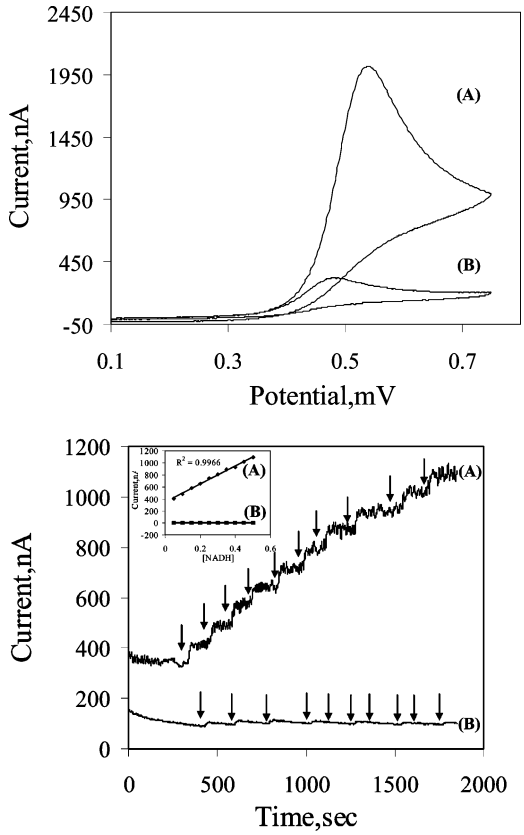

Figure 2. Direct measurement of NADH on the peptide nanotube-based electrode. (Top) Cyclic voltammetry of (A) peptide nanotube-based electrode; (B) unmodified electrode measured in a solution containing 50 mM NADH. Scan rate, 50 mV/s. (Bottom) Amperometric response to successive additions of 50 μM NADH. at 0.4 V vs SCE. (A) Peptide nanotube-based electrode; (B) bare electrode. Arrows indicate the addition of increasing concentrations of NADH.

Detection of NADH. The fast and reliable detection of NADH at low potentials is particularly important because NADH is a key component in amperometric biosensors based on the dehydrogenases, such as alcohol dehydrogenase, lactate dehydrogenase, and malate dehydrogenase. Figure 2 (top) presents the cyclic voltammetry of the peptide nanotube-based electrode in a solution containing 50 mM NADH in comparison with the bare electrode. The figure clearly shows that the presence of the peptide nanotubes significantly improved the sensitivity of the electrode. Figure 2 (bottom) shows the amperometric response at +0.4 V of the peptide nanotube-based gold electrode. The bare electrode showed almost no response to successive additions of NADH, whereas the peptide nanotube-modified electrode responded significantly and rapidly to the changes in NADH concentration.

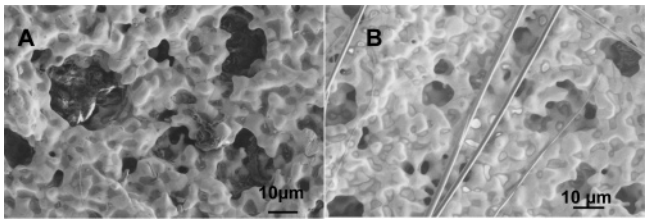

Figure 3. SEM images of (A) control gold electrode and (B) peptide nanotube-modified gold electrode.

The low-potential detection of hydrogen peroxide and NADH, along with the apparent functional surface area extension,[20] makes peptide nanotubes extremely attractive for amperometric biosensing using various enzymes.

Scanning electron microscopy provided insight into the nature of the peptide nanotube-based gold electrodes. Figure 3 shows an SEM image of the gold electrodes with modified peptide nanotubes in comparison with the bare gold electrode. A rough and jagged structure of the electrode with an array composed of elongated nanotubular structures was observed on the modified electrode (B), whereas the surface of the bare gold electrode contained no tubular structures (A).

Detection of β-D-Glucose. Figure 4 schematically depicts the procedure for using the peptide nanotube-based biosensor for the electroenzymatic detection of glucose.[26,27] Glucose detection in blood and urine is mandatory for the diagnosis of diabetes. Glucose monitoring during fermentation in the food industry is necessary because the amount of glucose greatly influences the quality of food products.[28] GOx has been widely used for constructing glucose biosensors because of the enzyme's high selectivity to glucose and high activity over a broad range of pH values. The modification of our glucose biosensor was accomplished by incorporating GOx within a three-dimensional electrode matrix containing a PEI layer with modified peptide nanotubes, connected through a thiol to the gold electrode surface. The formation of chemical bonding between the thiol group of the peptide nanotubes and the gold surface was possible through the incomplete coverage of the enzyme-modified peptide nanotubes by the PEI monolayer, which main purpose was to improve GOx performance by circumscribing an appropriate matrix for capture of intermediates created in the enzymatic reaction catalyzed by GOx.

Figure 5 shows the amperometric response to the successive addition of 0.2 mM glucose to the GOx-modified peptide nanotube-based gold electrode and to the control electrode. Immediately after the addition of glucose, the anodic current of the peptide

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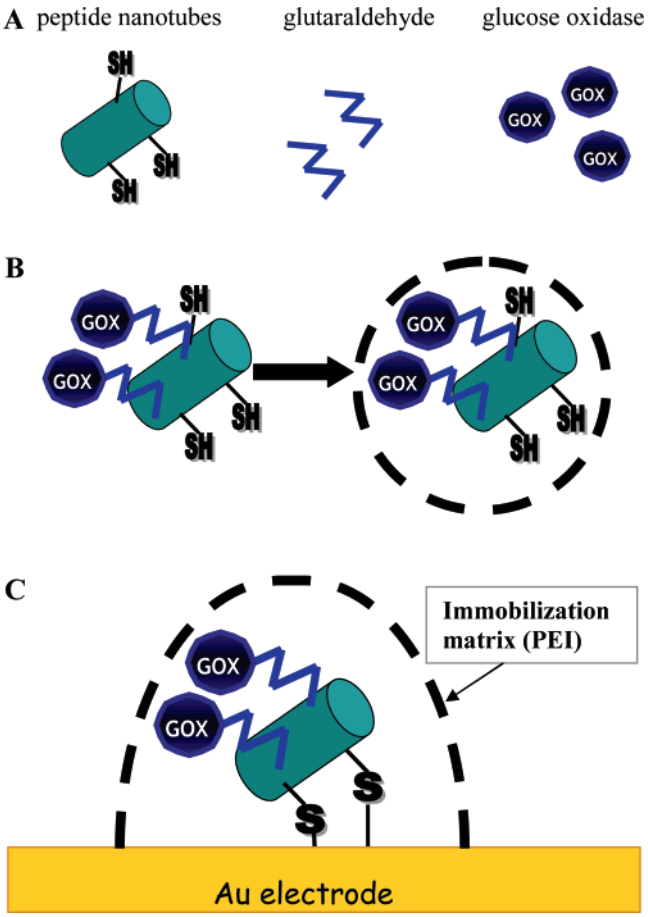

Figure 4. Schematic illustration of the procedure used for fabricating the peptide nanotube-based enzymatic electrodes. (A) Thiol-modified peptide nanotubes were mixed with 1 μM GOx in the presence of 0.25% GA. (B) 0.05% PEI was added to the solution. (C) The resulting enzyme-coated peptide nanotubes were deposited on the gold electrode surface and dried at room temperature. (Drawings are not to scale.)

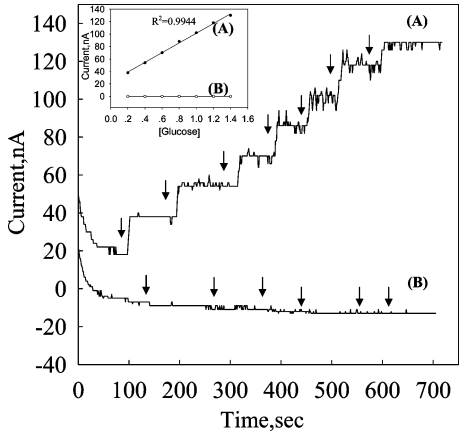

Figure 5. Amperometric response to successive additions of 0.2 mM β-D-glucose, measured at 0.6 V vs SCE. (A) Glucose oxidase and peptide nanotube-coated electrode. (B) Glucose oxidase, no nanotube electrode, 0.1 M phosphate buffer solution, 0.1 M KCl (pH 7.5). Arrows indicate the addition of increasing concentrations of glucose.

nanotube-based electrode increased, reaching a steady state in several seconds. The absence of a response to glucose addition in control experiments using the same immobilization matrix

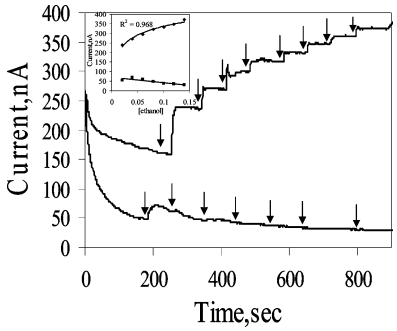

Figure 6. Amperometric response to successive additions of 20 μM ethanol to a solution containing 2 mM NAD^+ and 30 munits of ADH (One unit will convert 1.0 μmol of ethanol to acetaldehyde per min at 25 °C (pH 8.8).). (A) Peptide nanotube-modified electrode; (B) unmodified electrode, 0.1 M phosphate buffer, 0.1 M KCl (pH 8), electrode potential +0.6 V vs SCE. Arrows indicate additions of increasing concentrations of ethanol.

(GOx in PEI) but without peptide nanotubes together with the ability to detect directly hydrogen peroxide (as was shown earlier) confirmed that the current is related to hydrogen peroxide and its oxidation by the following enzyme-catalyzed reaction:

$$\text{glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2 \qquad (1)$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \qquad (2)$$

Although additional calibration tests are required before further fabrication processes, we believe that this system is highly promising for the future development of glucose sensing. It should be noted that the enzyme-modified peptide nanotube electrodes when stored at 4 °C in phosphate buffer lost 40% of their activity and then kept the same activity for at least two weeks.

Detection of Ethanol. The oxidation of ethanol by ADH proceeds as follows:

$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$$

Figure 6 shows the dynamic amperometric response of the peptide nanotube-based and control electrodes to the successive addition of 20 μM ethanol to an electrochemical cell containing 0.2 mM NAD^+ and 30 munits of alcohol dehydrogenase in phosphate buffer (pH 8). A larger current was obtained with the peptide nanotube-based electrode when compared with the insignificant current changes in control experiments.

CONCLUSIONS

A new and attractive amperometric biosensor has been presented. This biosensor is based on the immobilization of modified peptide nanotubes on an electrode surface. The resulting electrodes show improved sensitivity, as demonstrated by hydrogen peroxide and NADH detection. The modified electrodes exhibit a nonmediated electron transfer, a short detection time, a large current density, and a comparatively high stability. The modified biocomposite electrode exhibited excellent sensitivity

and reproducibility for the determination of glucose and ethanol by the electrocatalytic oxidation of enzymatically liberated hydrogen peroxide and NADH, respectively. In addition, the low cost of peptide nanotubes points to a promising future in various biosensing processes.

In summary, combining the surface attachment of peptide nanotubes and enzymatic molecular recognition properties with the molecular and electrocatalytic properties of peptide nanotubes paves the way toward the generation of unique sensing devices. More generally, the interactions between biomaterials and nanoscale structures are central to the development and fabrication of miniature biosensors.

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