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Novel Electrochemical Biosensing Platform Using Self-Assembled Peptide Nanotubes

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ABSTRACT

Here we describe a novel electrochemical biosensing platform based on biocompatible, well-ordered, self-assembled diphenylalanine peptide nanotubes. Voltammetric and time-based amperometric techniques were applied to demonstrate the ability of the peptide nanotubes to improve the electrochemical parameters of graphite electrodes. The findings clearly show that this novel class of peptide nanotubes provides an attractive component for future electroanalytical devices.

Since their discovery in 1991,[1] carbon nanotubes (CNTs) have been extensively studied both theoretically and experimentally because of their unique physical and chemical properties. Such nanoscale tubular structures have been suggested to have potential as key functional elements in nanotechnological devices and applications.[2,3] Other studies showed that bioorganic molecules can also self-assemble into well-ordered structures at the nanometric level.[4−7] Biomolecular nanostructures are an especially intriguing group of supramolecular assemblies because they facilitate a wide range of chemical modifications. Moreover, such nanostructures enable the exploitation of the specificity of biological systems for biosensing, catalytic activity, and highly specific molecular recognition processes.[8]

We recently revealed the formation of discrete and well-ordered peptide nanotubes by the core recognition element of the Alzheimer's β-amyloid polypeptide−the diphenylalanine peptide.[9] The biocompatible and water-soluble tubes are formed under mild conditions and are inexpensive and easy to manufacture. The chemical nature of the tubes is compatible with a variety of chemical and biochemical modifications of the nanostructures. The novel peptide nanotubes show remarkable similarity to carbon nanotubes in their morphology and aspect ratio. Their assembly as individual entities rather than bundles, however, makes them appealing for use in various nanotechnological applications. Moreover, peptide nanotubes can serve as a model for the fabrication of conductive nanowires and other peptide−inorganic composites.[8,10] In addition, a stable analogue of the nanotubes composed of D-isomer amino acids is available and offers stability to proteolytic degradation.

Recently, the electrochemical properties of CNTs have been unveiled, and their application toward electrochemical sensors and biosensors has gained significant interest.[11,12] Carbon nanotubes are now under intensive investigation as potential building blocks for sensors aimed at replacing contemporary, time-consuming, sequential, and limited spectroscopy techniques. The first reported CNTs offer many advantages in sensing applications because of their small size, high aspect ratio, and conductance. The subtle electronic properties of the CNTs suggest that when used as electrode material they will have the ability to mediate electron-transfer reactions with electroactive species in solution. Over the past few years, the profound effect of exposure to humidity, oxygen, N_2O, and NH_3 on the electric properties of the CNTs has become evident.[13] Moreover, the integration of carbon nanostructures into functional sensing devices is limited by major issues related to their production, uniformity, hydrophobicity, reproducibility, and cost. The chemical nature of the tube limits their covalent modification with biological and chemical reporters. Here, we present a novel class of peptide nanotubes as an appealing alternative to CNTs in sensing applications.

In light of the similarity and possible advantages of peptide nanotubes as compared with CNTs, we extended our studies to characterize the possible use of the diphenylalanine-based peptide nanotubes in electrochemical applications.[14,15] To examine the proposed properties of the peptide nanotubes, we modified a graphite electrode by applying the peptide nanotubes on the surface of the electrode. We used screen-printed electrodes with an Ag/AgCl reference electrode and

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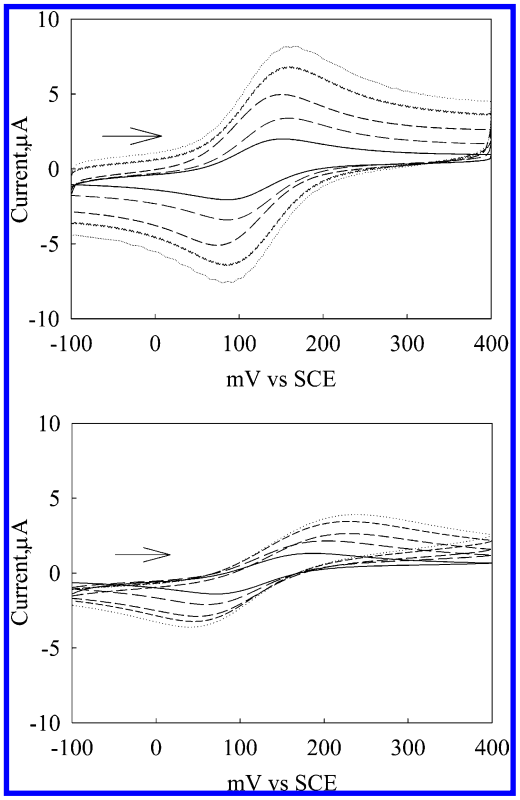

Figure 1. Comparative cyclic voltammetry of a screen-printed electrode response to 0.06 mM $K_4Fe(CN)_6$ and $K_3[Fe(CN)_6]$ with (top) and without (bottom) modification with peptide nanotubes in different scan rates (from inner to outer): 10, 25, 50, 75, 100 mV/s. The arrow indicates the initial scan direction.

graphite counter and working (1 mm in diameter) electrodes. Figure 1 depicts typical cyclic voltammograms (CVs) obtained from the peptide nanotube modified electrodes and from untreated electrodes after the addition of potassium hexacyanoferrate. The presence of well-defined, reversible anodic and cathodic peaks indicated improved electrochemical reactivity for the potassium hexacyanoferrate oxidation−reduction reaction on the peptide nanotube based electrodes. The cyclic voltammogram shows a characteristic anodic peak at $E_{pa} = 0.22$ V vs Ag/AgCl for a bare electrode and at 0.17 V for peptide nanotube modified electrode and the complementary cathodic peak at $E_{pc} = 0.08$ V for bare and 0.1 V for modified electrode. The difference between E_{pa} and E_{pc} (dE_p) decreased from 0.14 V for bare screen printed to 0.07 V in peptide nanotube modified electrode. The results demonstrate that the presence of peptide nanotubes significantly improved the electrochemical fingerprint of the electrode.

The results appear to be highly reproducible as no significant changes of the peak current were found after the potential was swept from −0.1 to +0.4 V and back at a scan rate of 50 mV/s for 50 cycles. This indicates that the peptide nanotubes are well adhered to the graphite electrode surface. Furthermore, different electrodes show very similar cyclic voltammetries, keeping the oxidation and reduction peaks at the same potential and the same peak height.

To further explore the effect of the nanotube deposition on the electrochemical process, we used chronoamperometry

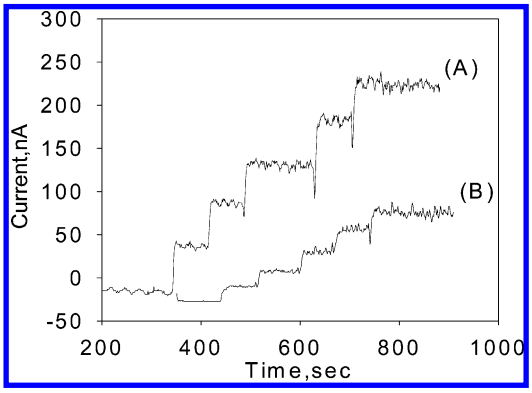

Figure 2. Representative amperometric (I to t) response of 10 μM $K_4Fe(CN)_6$ in a screen-printed electrochemical cell at 200 mV (A) modified with peptide nanotubes and (B) without any modification.

(Figure 2). The experiments were carried out in the diffusion controlled potential (200 mV vs Ag/AgCl). This potential is more positive than the oxidation peak observed in cyclic voltammetry experiments shown in Figure 1. The amperometric responses of the peptide nanotube modified electrode (Figure 2A) and the nonmodified electrode (Figure 2B) were compared under continuous stirring of the solution and successive additions of $K_4Fe(CN)_6$. It is evident from the amperometric responses, that the modified electrode (Figure 2A) had significantly higher signal (about 2.5-fold increase) as compared to the nonmodified electrode (Figure 2B).

The determination of hydrogen peroxide is becoming of practical importance in the assay of oxidoreductase substrates such as glucose, lactate, choline, and cholesterol because sensitive measurement of enzymatically formed hydrogen peroxide is necessary for the development of many enzyme electrodes for clinical and environmental applications. We explored the detection potential of peptide nanotube modified electrode properties by measuring hydrogen peroxide, using peroxidase and 4-acetaminophenol as mediator.[16,17] The current−time amperometric curve was recorded under contentious stirring of the solution, and the current was plotted for each electrode. Figure 3A presents the response of the sensor to hydrogen peroxide obtained with the peptide nanotube modified electrode. Two control experiments were performed: in the first a bare electrode was used, and in the second a peptide nanotube electrode after degradation by proteinase K. The addition of hydrogen peroxide clearly demonstrated lower responses for both control experiments (Figure 3).

Scanning electron microscopy (SEM) analysis was used to characterize the ultrastructure of the peptide nanotubes on the electrode surface (Figure 4). While no ordered structures could be observed upon SEM imaging of a control electrode (Figure 4A), an array composed of tens of elongated nanotubular structures of remarkably persistent length was observed on the deposited electrode (Figure 4B). The ultrastructural morphology of the peptide structures on the electrode surface is consistent with previously described peptide assemblies.[9,10] As these nanostrcutres were observed on the electrode after the electrochemical experiments, there is clear evidence that the tubular structures remained attached

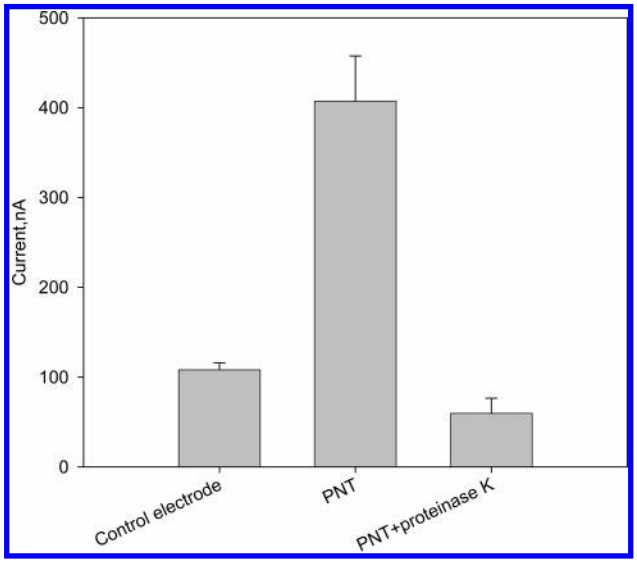

Figure 3. Amperometric response to 1 mM hydrogen peroxide addition in the presence of 1 mM 4-acetamidophenol and 0.75 μg/mL horseradish peroxidase. Each bar represents four independent experiments. The 10 mL cell contains 0.1 M phosphate buffer (pH 5.8) with the addition of 0.1 M KCl. Applied potential: −50 mV; time of detection: 15 s.

to the modified working electrode (Figure 4B). On the other hand, after the application of the proteolytic enzyme, no tubular structures were observed by SEM analysis (Figure 4C), providing a direct evidence for the efficiency of the proteolytic process. The lower electrochemical activity of this electrode in comparison with the bare electrode might be due to the remains of amino acids on the electrode that caused partial fouling of electrode surface.

Taken together, our results clearly demonstrate the effect of the peptide nanotubes on the performance of simple screen-printed electrodes. The significant enhancement in sensitivity was clearly demonstrated by both cyclic voltammetric (Figure 1) and time-based amperometric techniques (Figure 2). The effect appeared to be directly linked to the morphology of the nanotubes in the nanoscale regime (Figure 4). The remarkable effect of the nanotube deposition on the cyclic voltammetry parameters is assumed to be related to the increase of the functional electrode surface as was previously suggested for CNT.[11,12] While the exact molecular organization of the peptide nanotubes is yet unknown, we previously suggested that a parallel stacking of aromatic moieties may be the underlying mechanism for the formation of amyloid fibrils and the related peptide nanoscale assemblies.[8,9,18] Indeed, a recent solid-state NMR study provided the first indication for such structure in amyloid assemblies.[19] If indeed such molecular organization occurs in the amyloid-derived nanotubular structures, it is possible that electron transfer between spatially aligned aromatic systems could contribute to the electronic conductivity by the assemblies.

The possibility of enhancing the electrochemical activity and improving the selectivity of the peptide nanotube based electrodes offers novel perspectives for the development of sensors and biosensors having promising analytical performances. Moreover, that peptide nanotubes consist of simple

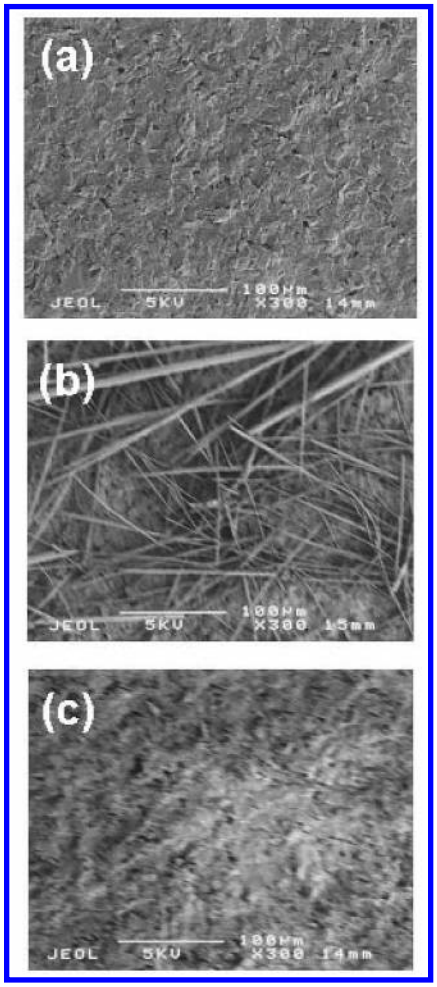

Figure 4. Scanning electron microscope images of (A) control electrode, (B) peptide nanotube modified electrode, and (C) peptide nanotubes after treatment with proteinase K electrode. Scale bar 100 μm.

dipeptides is consistent with their facile chemical modification for various applications. The chemical nature of the peptide tubes allows further modification based on the specific targeting of the amino and carboxyl moieties within the tubes. Another advantage is the solubility of the peptide nanotubes (compared with the low solubility of the highly hydrophobic CNTs[20]). Finally, the low cost and simple production of the building blocks, as compared with carbon nanotubes, makes the former a commercially viable component in mass-production settings. We are currently performing additional studies to characterize the electrochemical properties and possible applications of the peptide nanotubes.

Materials and Methods. *Materials*. Hydrogen peroxide solution 30% (H_2O_2), $K_3[Fe(CN)_6]$, KCl, K_2HPO_4, and KH_2PO_4 were obtained from Merck; 4-acetamidophenol and $K_4Fe(CN)_6$ were purchased from Fluka. Purified HRP was obtained from Sigma. Phe-Phe peptides were purchased from Bachem. All the solutions were prepared with double-distilled water.

Preparation of the Peptide Nanotubes. Fresh stock solutions were prepared by dissolving a lyophilized form of the peptide in 1,1,1,3,3,3-hexafluoro-2-propanol at a concentration of 100 mg/mL.[9] Fresh stock solutions were prepared

for each experiment to avoid pre-aggregation. The assembly of the peptide nanotubes was performed at the optimal concentration of 2 mg/mL that leads to the favorable assembly of tubular structures, as was previously described by two different groups.[9,10]

Electrodes. Screen-printed electrodes were purchased from Gwent Electronics. Each screen-printed electrode consisted of a carbon-ink working electrode, an Ag/AgCl reference electrode, and a carbon-ink counter electrode. The electrodes were printed on an underlying ceramic support.

Measurements and Apparatus. We used the EG & G potentiostat interfaced to a PC system equipped with PAR M270 software.[21] Cyclic voltammetry experiments were conducted at a 50 mV/s scan rate against the Ag/AgCl reference electrode in an unstirred solution. Chronoamperometric experiments were conducted at a constant applied potential of 0.22 V for $K_4Fe(CN)_6$ additions and −0.05 V for H_2O_2 additions. Measurements of $K_4Fe(CN)_6$ and $K_3[Fe(CN)_6]$ were conducted in a 0.1 M KCl solution and the measurements of peroxidase activity were conducted in phosphate buffer at pH 5.8. The solution was stirred during the chronoamperometric experiment at a constant speed of 100 rpm using a magnetic stirrer. All experiments were carried out at room temperature.

Peptide Nanotube Electrode Modification. An aliquot (2 μL) of peptide nanotube solution at a concentration of 2 mg/mL was deposited on the surface of the working electrode and allowed to dry at room temperature for 60 min. Scanning electron microscope images were taken before and after the experiment to verify the presence of the peptide nanotubes on the electrode.

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

Digestion of the Peptide Nanotube Structures by Proteinase K. Fresh stock solutions of the peptides were diluted to a final concentration of 2 mg/mL. One day later, the nanotubes were incubated for 1 h with a solution of proteinase K (20 μg/mL) at 37 °C and examined with the same experimental procedures.

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