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Direct electrochemistry and electrocatalysis of catalase immobilized on a SWNT-nanocomposite film

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ABSTRACT

Investigations are reported regarding the electrochemical performance of catalase (CAT) immobilized on a film composed of chitosan-wrapped single-walled carbon nanotubes (SWNTs), and the use of such a system as a biosensor. The immobilized CAT displays a pair of well-defined and quasi-reversible redox peaks, with a formal potential ($E^{o\prime}$) of −0.476 V vs. SCE in 0.1 M, pH 7.0 phosphate buffer solution; also the electrochemical response indicates a surface-controlled electrode process. The dependence of formal potential on solution pH indicated that the direct electron transfer reaction of CAT is a one-electron transfer coupled with a one-proton transfer reaction. The heterogeneous electron transfer rate constant was measured as 118 s^{-1}, indicating electron transfer between catalase and the modified electrode surface is greatly improved over that which is typically reported in the literature. This result is likely attributed to close contacts between the electroactive centers and the SWNTs. Experimental results demonstrate that absorbed CAT exhibits remarkable electrocatalytic activities toward the reduction of oxygen, hydrogen peroxide and nitrite. Also, the modified electrode exhibited good analytical performance for the amperometric determination of hydrogen peroxide and nitrite, and might find use as a third-generation biosensor based on the direct electrochemistry of catalase.

1. Introduction

Bioelectrochemistry has contributed significantly to clarifying structure–function relationships for metalloproteins and enzymes and for construction of third-generation biosensors (i.e., biosensors not requiring redox mediators) [1–4]. However, the acquisition of reliable and reproducible electrochemical data for proteins and enzymes on an electrode surface, without the use of mediators, is often difficult, especially for species of large molecular weights and complex structures. This is the case because electroactive centers of proteins and enzymes are usually imbedded deeply within coiled polypeptide chains [5]. However, recently, systems consisting of novel nanomaterials onto which proteins and enzymes are coated have been found to overcome much of the inherent variability associated with isolation of electroactive centers, and composite systems that are quite good in promoting direct electron transfer have been identified. This latter improvement is thought to derive from the fact that the nanomaterials used have large surface area, high catalytic activity, excellent conductivity, and good biocompatibility [5–8].

Catalase (CAT, EC 1.11.1.6) is present in nearly all aerobically respiring organisms and is a heme protein belonging to the family of antioxidative enzymes. Catalase is a tetrameric enzyme, containing four identical subunits, each of 57 kDa, equipped with a high-spin ferriprotoporphyrin group [9,10]. Catalase functions in two ways: 'catalytically' decomposing hydrogen peroxide into water and oxygen, and 'peroxidatively' oxidizing alcohol, formate or nitrite using hydrogen peroxide [11]. Therefore, the direct electron transfer and electrocatalysis of catalase might represent a system for investigating the mechanisms of redox transformations in biocatalytic and metabolic processes involving electron transport in biological systems.

To date, many methods have been used to immobilize catalase on electrode surfaces, aimed at promoting direct electron transfer and bioelectrocatalytic activity. As examples, the direct electrochemistry of catalase in nanoscale islands of NiO [12], MWNTs [13,14], silk fibroin [15], silica sol–gel film [16], collagen film [17], agarose hydrogel film [18], SWNTs [19,20], polyamidoamine dendrimers [21,22], methyl cellulose film [23], polyelectrolyte [24], polyacrylamide hydrogel films [25], covalent modified glassy carbon powder [26], chitosan film [27], dimyristophosphatidycholine lipid films [28], and didodecyl-dimethylammonium bromide liquid crystal film [29] have been reported. Although direct electron transfer involving catalase was found for the aforementioned

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modified electrodes, the rates for the heterogeneous electron transfers in most deoxygenated solutions is sluggish; some studies showing large peak separations or low electrocatalytic activities.

In an effort to develop a highly sensitive catalase-based biosensor, without the need for a redox mediator, we have utilized a film consisting of chitosan-wrapped SWNTs deposited onto a glassy carbon electrode onto which catalase was immobilized. The direct electrochemistry of catalase was found to be greatly improved and the heterogeneous electron transfer rates for the Fe^{III}/Fe^{II} redox couple of catalase was found to be substantially enhanced. Voltammetric measurements are also reported, as well as kinetic parameters and the influence of pH. Additionally, the electrocatalytic activities for the reduction of oxygen, hydrogen peroxide and nitrite are reported, and it is deduced that the composite system shows promise as a biosensor; furthermore, amperometry was applied to assess the biosensor capabilities of the composite electrode for electrocatalytic reduction of hydrogen peroxide and nitrite.

2. Experimental

2.1. Chemicals and solutions

Lyophilized catalase powder (EC 232-577-1 from bovine liver) containing 1340 units/mg solid and chitosan (85% deacetylation, average molecular weight of 1×10^6 g/mol) were obtained from Sigma–Aldrich Corp., and used as received. Purified HiPco SWNTs powder was purchased from Carbon Nanotechnologies, Inc. (Houston, TX). Phosphate buffer solutions (ca. 0.1 M) of different pHs were prepared from stock solutions containing NaH_2PO_4 and Na_2HPO_4; all solutions were prepared with double-distilled water. All other chemicals were of analytical grade and used without further purification.

2.2. Film assembly on glassy carbon electrode

Chitosan-dispersed SWNTs were prepared by a method similar to that described by Yang et al. [30]. SWNTs were dispersed in chitosan solution (0.5% by weight) with the aid of ultrasonication to give a 0.5 mg mL^{-1} black suspension. A glassy carbon electrode (GCE, diameter 3 mm), prior to coating, was polished sequentially with slurries of 0.3 and 0.05 μm alumina to a mirror finish. After rinsing with doubly distilled water, the GCE was cleaned ultrasonically in water for 10 min. A 5 μL SWNT suspension was then deposited onto the surface of a GCE with a microsyringe, and allowed to dry at room temperature. The resultant electrode was immersed overnight in 10 mg mL^{-1} CAT solution at 4 °C.

2.3. Instrumentation

For all electrochemical measurements, a CHI-630B electrochemical workstation (CH Instruments, Austin, TX) was employed. A three-electrode electrochemical cell, consisting of a saturated calomel electrode (SCE), a Pt wire counter electrode, and a working electrode (the modified GCE), was employed; all potentials are reference with respect to SCE. The phosphate buffer solution (PBS) was purged with high-purity nitrogen or oxygen for 30 min prior to experiments and a nitrogen or oxygen atmosphere was maintained over the solution.

Amperometric measurements were conducted under stirred solution condition and the response current was referenced relative to the steady-state and background currents. All experiments were performed in a thermostated cell at 25 ± 1 °C.

3. Results and discussion

3.1. Direct electrochemistry of CAT at the modified GCE

Fig. 1 shows typical voltammograms (CVs) obtained with a CAT/SWNT-CHI modified electrode in pH 7.0 PBS. For comparison, voltammograms of bare and SWNT-CHI GC electrodes are also provided. No redox peaks were observed at the bare GCE (curve a) in the potential range of interest. Only a pair of weak, broad redox waves were found at ca. −0.2–0.15 V at the SWNT-CHI/GC electrode (curve b), attributable to the redox process of the functional oxygenated groups produced at the SWNTs [31]. However, a pair of well-defined and nearly symmetric redox peaks located at ca. −0.45 V and −0.50 V was obtained at the CAT/SWNT-CHI modified electrode (curve c). All the CVs were acquired at a scan rate of 100 mV s^{-1}. The ratio of the cathodic current to the anodic current was found to be close to 1, and the potential difference between the two peak currents, ΔE_p, was measured as 55 mV, which indicates that catalase undergoes a heterogeneous electron transfer process on the surface of the modified electrode. The formal potential ($E^{o\prime}$) calculated by averaging the cathodic and anodic peak potentials is −0.476 V vs. SCE, consistent with values reported for the catalase heme Fe(III)/Fe(II) redox couple [17,19–23,25,27,28].

In order to determine kinetic parameters of catalase at the modified electrode, the effect of scan rate was investigated. CV reduction and oxidation peak currents were measured (see Fig. 2A) and are found to vary linearly with potential scan rate from 0.01 to 0.1 V s^{-1} (see Fig. 2B). The linear fits for the data are $y = 0.1014 + 47.46x$, r = 0.9989 (anodic peak), and $y = -0.1238 - 47.27x$, r = 0.9996 (cathodic peak), where y is the peak current in the unit of μA and x the scan rate in the unit of V s^{-1}. These results suggest that the electrochemical reaction of catalase corresponds to a surface-confined, quasi-reversible process.

The peak-to-peak separation was found to be approximately 60 mV, at scan rates below 100 mV s^{-1}, indicating facile charge transfer kinetics over the range of sweep rates. But for scan rates above 1 V s^{-1}, ΔE_p increases with increasing scan rate. Fig. 3 shows the plots of E_p vs. the logarithm of scan rate, where it is shown (see below) that E_p depends linearly with the log of the scan rate at high scan rate. Based on the Laviron theory, the heterogeneous electron transfer rate constant (k_s) can be estimated by measuring the var-

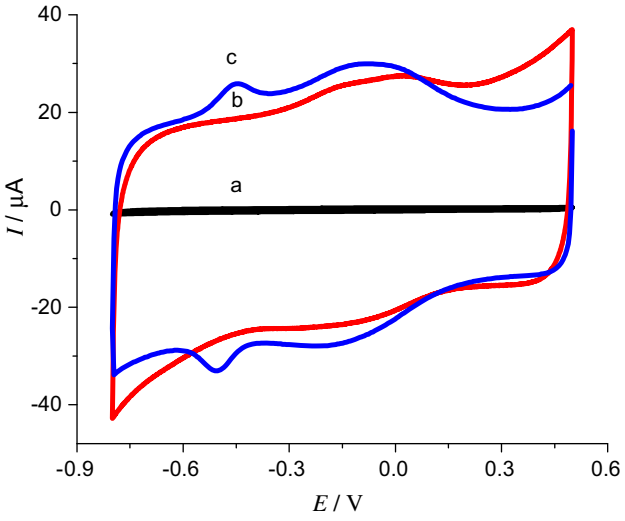

Fig. 1. CVs of (a) GC, (b) SWNT-CHI/GC, (c) CAT/SWNT-CHI/GC electrodes in an N_2-saturated 0.1 M phosphate buffer solution (pH 7.0) at a scan rate of 0.1 V s^{-1}.