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An acetylcholinesterase enzyme electrode stabilized by an electrodeposited gold nanoparticle layer

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Abstract

Electrodeposition of colloidal gold nanoparticles onto a planar gold electrode was used to create a more favorable surface for the attachment of the enzyme acetylcholinesterase. Atomic force microscopy demonstrated that the gold nanoparticles roughened the surface consequently enhancing the interaction of the enzyme with the gold electrode. The enzyme-modified electrode sensor was utilized for the sensitive electrochemical detection of thiocholine at the gold surface after hydrolysis of acetylthiocholine by the immobilized enzyme. In the absence of the nanoparticle layer, the sensor response to acetylthiocholine was significantly reduced and the utility of the electrode was limited. The ability of the nanoparticle-based sensor to reliably measure concentrations of the organophosphate pesticide carbofuran at nM concentrations was demonstrated by monitoring the inhibition of the hydrolysis of acetylthiocholine. This relatively straightforward strategy is potentially valuable for the development of new devices for the sensitive detection of potentially dangerous and deadly neurotoxins.

Keywords: Acetylcholinesterase; Biosensor; Carbofuran; Enzyme electrode; Gold nanoparticles

1. Introduction

Acetylcholinesterase (AChE) is a serine esterase that is attached to postsynaptic membranes. AChE rapidly converts the neurotransmitter acetylcholine (ACh) to choline (Ch) and acetate after transmission of a nerve impulse. Ch is then captured and recycled by the presynaptic terminal through the high-affinity choline uptake transporter protein. As part of normal cholinergic neurotransmission, proper functioning of AChE is a critical step [1,2]. However, organophosphorus compounds used as pesticides and nerve gas agents (e.g., sarin) are known to inhibit AChE. In some cases, these agents are highly neurotoxic, leading to cholinergic dysfunction and death [2]. Simple and sensitive strategies for detecting such organophosphorus compounds are therefore critically important.

Electrochemical strategies for the detection of organophosphorus compounds have received much attention [3–24]. A common approach involves the measurement of the oxidation of thiocholine produced from the enzymatic hydrolysis of acetylthiocholine (ASCh) by AChE [11]. In the presence of an organophosphorus compound, the enzyme function is inhibited limiting the production of thiocholine. The degree of inhibition is therefore correlated to the reduction in thiocholine oxidative current relative to the current measured in the absence of an inhibitor. Amperometric sensing schemes based on thiocholine detection typically use two approaches. In one case an unmodified electrode is used to measure thiocholine after addition of AChE to the sample and ASCh [10,11], while the second approach focuses on immobilization of AChE on an electrode surface to create an enzyme-based electrochemical sensor [12–23]. A major challenge for the latter approach is maintaining the enzyme performance upon immobilization, especially on metal surfaces [24]. Therefore, new strat-

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egies have been aimed at improving the performance of AChE electrode sensors for the direct monitoring of AChE inhibitors [25–27].

Metal nanoparticles have been used to aid the immobilization of enzymes such as xanthine oxidase [28], glucose oxidase [29,30], horseradish peroxidase [31–33] and cholesterol oxidase [34] in the preparation of sensors for detection of the corresponding enzyme substrates. CdS nanoparticles [35] and a Au–CdS nanoparticle array [36] deposited on gold electrodes have also been labeled with AChE and used in a photoelectrochemical sensing scheme to detect AChE inhibitors. In addition, zirconia nanoparticles immobilized on glassy carbon [37] and electrodeposited on gold [38] electrodes have also led to the development of electrochemical sensors for organophosphate pesticides. In this communication, we demonstrate the use of colloidal gold nanoparticles (AuNP) to enhance the immobilization of functional AChE on a bulk gold electrode surface. This approach produces a simple electrochemical sensor, which can be used for sensitive monitoring of inhibitors of AChE in solution via detection of thiocholine after enzymatic hydrolysis of ASCh. This approach is depicted in Fig. 1.

2. Experimental

2.1. Materials

Acetylthiocholine chloride (99% purity) and AChE (EC 3.1.1.7, Type III from electric eel, 1100 U/mg) were obtained from Sigma (St. Louis, MO) and stored at −10 °C. Hydrogen tetrachloroaurate (99.99% purity) and carbofuran were purchased from Alfa Aesar (Ward Hill, MA) and Aldrich (Milwaukee, WI), respectively. Gold wire (99.99% purity, 0.2 mm diameter) was purchased from Goodfellow (Berwyn, PA). Aluminum oxide sanding gel was obtained from Nicsand Inc. (Cleveland, OH). Thiocholine was prepared by a procedure developed by Hart [39], where 10 mL of 20 mM ASCh was mixed with 5 μL of AChE. The mixture was stirred for 30 min allowing for complete hydrolysis of ASCh. The final concentration of thiocholine was determined by spectrophotometry, after reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Aldrich) as described by Ellman [40]. All other chemicals were reagent grade and used as received. Liquid electrical tape, manufactured by Plasti Dip International, Inc. (Circle Pines, MN), and copper wire, produced by Anchor Wire (Goodlettsville, TN) were purchased locally. All solutions were prepared with distilled, deionized water purified to a resistivity of at least 17 MΩ cm by a Barnstead B-pure water purification system (Dubuque, IA).

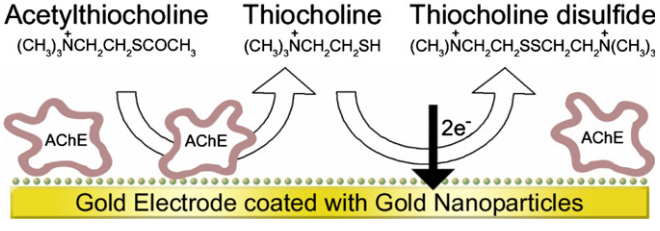

Fig. 1. Schematic diagram of the enzymatic reaction at the gold nanoparticle-coated AChE electrode.

2.2. Methods

The AChE electrode was prepared by a two-step procedure. Gold nanoparticles were first electrodeposited on the surface of a gold electrode by a modified literature procedure [41]. Prior to deposition, the gold electrode was polished with alumina (<0.1 μm) and inserted into a deoxygenated solution of 0.77 mM $HAuCl_4$ in 0.5 M H_2SO_4. The potential was cycled twice between 1100 and 0 mV vs. a Ag/AgCl reference electrode at 10 mV/s under constant stirring to deposit the gold particles. The amount of gold nanoparticles on the electrode surface was controlled by variations in the scanning rate and the number of scans. After electrodeposition, the electrode was rinsed with water and dried.

Enzyme immobilization was accomplished by immersing the gold nanoparticle electrode into a 300 μL solution of 0.1 M phosphate buffer pH 8, containing 60 μg of AChE for 24 h at 4 °C. The AChE-modified electrode was removed from the enzyme solution and soaked in phosphate buffer for 5 min prior to use. The activity of enzyme before and after immobilization on the electrode surface was determined by the application of continuous and discontinuous assays developed by Ellman et al. [42]. The continuous enzymatic assay was performed with a Lambda 1 spectrophotometer (Perkin–Elmer, Fremont, CA). The discontinuous assay used a Genesys 20 spectrophotometer (Spectronic Instruments, Rochester, NY). For continuous monitoring, the typical experiment used 940 μL of phosphate buffer, pH 8, 20 μL of DTNB, 20 μL of enzyme (2.7 mg/mL), and 20 μL of 10 mM ASCh. A plot of absorbance at 412 nm vs. time was generated and used to calculate the initial velocity. For the discontinuous assay the AuNP/AChE electrode was dipped in a solution of ASCh of a particular concentration with efficient stirring. An aliquot was taken every 2 min, mixed with appropriate amounts of phosphate buffer and DTNB, and the absorbance at 412 nm was measured. Michaelis constants (K_m) for the bulk and immobilized enzyme were determined by construction of double reciprocal plots of 1/velocity vs. 1/[S], where [S] is a mM concentration of ASCh. Each K_m value is reported as the average of three independent determinations.

All electrochemical measurements were performed in a conventional three-electrode electrochemical cell under anaerobic conditions with a Bioanalytical Systems (BAS, West Lafayette, IN) 100B/W electrochemical analyzer. Either a bare or a modified gold electrode (BAS, MF-2014, 1.6 mm in diameter) was used as the working electrode. The bare gold electrode was polished with aluminum