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Acetylcholine Esterase-Labeled CdS Nanoparticles on Electrodes: Photoelectrochemical Sensing of the Enzyme Inhibitors

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The unique electronic and photonic properties of semiconductor quantum dots have been used in a range of optoelectronic applications.[1] Specifically, the photophysical features of semiconductor nanoparticles are employed to develop sensor[2] and biosensor systems,[3] light-emitting diodes,[4] and lasers.[5] Protein-functionalized quantum-size semiconductor particles or antibody-modified nanoparticles were suggested as luminescent labels for biorecognition events.[6] Similarly, nucleic acid-modified semiconductor nanoparticles were reported to act as luminescent probes for DNA hybridization.[3c,7] Recently, oligonucleotide-derivatized quantum dots were used as building blocks to form extended networks of DNA cross-linked nanoparticles, and the photoelectrochemical features of the arrays were examined.[8] Here we wish to report on an acetylcholine esterase (AChE)/CdS nanoparticle hybrid system used for the photoelectrochemical detection of AChE inhibitors. The enzyme/CdS nanoparticle system may be employed as a versatile photoelectrochemical label for biosensing events. Acetylcholine is a central neurotransmitter that activates the synapse and the neural response. The neurotransmitter, after activating the neural system, is rapidly hydrolyzed by the serine protease AChE to restore the resting potential of the synaptic membrane. Different reagents, such as the nerve gas diisopropyl fluorophosphate (Sarin) or toxins (e.g., cobratoxin) act as inhibitors of AChE. Blocking of the enzyme-stimulated nerve conduction leads to rapid paralysis of vital functions of living systems. Thus, the assembly described here may be further developed to a biomaterial−semiconductor hybrid system for biosensing of biological warfare.

CdS nanoparticles (diameter 3 nm) were capped with a protecting monolayer of cysteamine and mercaptoethane sulfonic acid (see the Supporting Information).[8] XPS analysis indicate that the ca. 84% of the Cd^{2+} surface groups are linked to the thiolated molecules and that the ratio between the cysteamine and thiol sulfonate units is ca. 1:10, respectively. The capped CdS nanoparticles were covalently linked to an Au-electrode functionalized with a *N*-hydroxysuccinimide active ester cysteic acid, Scheme 1. Microgravimetric quartz crystal microbalance (QCM) measurements for the analogous association of the CdS nanoparticles on an Au-quartz crystal indicate that the binding of the CdS nanoparticles to the surface involves a change of $\Delta f = 140$ Hz that corresponds to a surface coverage of 5.7×10^{12} particles·cm^{-2}. The AChE (EC 3.1.1.7, Type VI-S from electric eel) was then covalently linked to the CdS nanoparticles using glutaric dialdehyde as the bridging unit. Parallel microgravimetric QCM measurements indicate that the surface coverage of AChE is 3.9×10^{-12} mol·cm^{-2}. Thus, ca. 2.4 nanoparticles are associated with each AChE unit.

The CdS nanoparticle/AChE hybrid system is photoelectrochemically active in the presence of acetylthiocholine, (**1**), as substrate. Figure 1 depicts the photocurrent action spectra resulting from the photoirradiation of the system in the presence of different concentrations of acetylthiocholine. The photocurrent spectra overlap the absorption spectrum of the CdS nanoparticles, implying

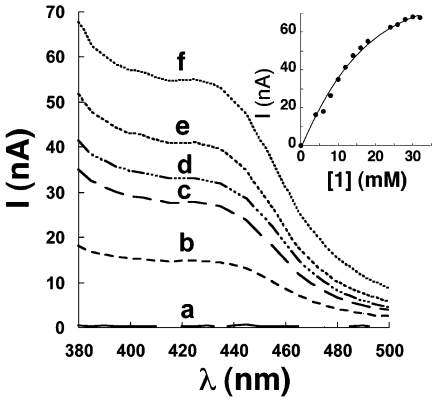
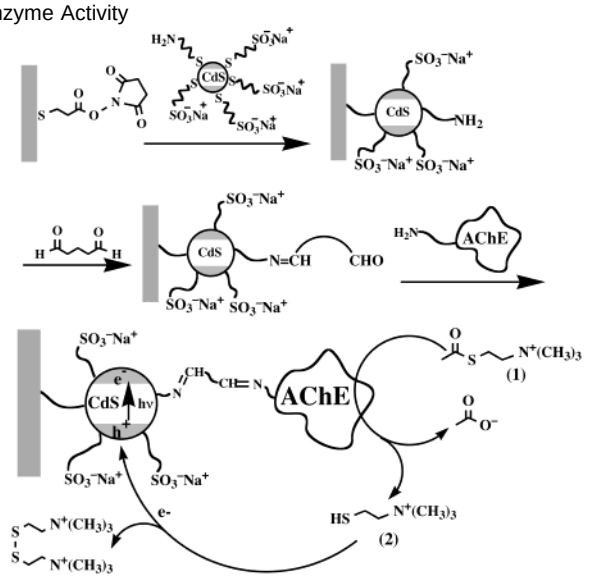


Figure 1. Photocurrent action spectra observed in the presence of acetylthiocholine (**1**): (a) 0 mM, (b) 6 mM, (c) 10 mM, (d) 12 mM, (e) 16 mM, (f) 30 mM. (Inset) Calibration curve corresponding to the photocurrent at $\lambda = 380$ nm at variable concentrations of **1**. Spectra were recorded in 0.1 M phosphate buffer, pH = 8.1, under argon.

Scheme 1: Assembly of the CdS nanoparticle/AChE Hybrid System Used for the Photoelectrochemical Detection of the Enzyme Activity



that the photocurrent originates from the excitation of the semiconductor nanoparticles.

Control experiments reveal that no photocurrent is generated in the system in the absence of acetylthiocholine. Also, irradiation of the CdS nanoparticle monolayer that lacks AChE in the presence of acetylthiocholine or the irradiation of an electrode on which AChE is directly linked in the presence of **1** does not yield any photocurrent. Thus, the photocurrent generation in the system is attributed to the AChE-catalyzed hydrolysis of acetylthiocholine, (**1**), to acetate and thiocholine, (**2**). The latter product acts as donor

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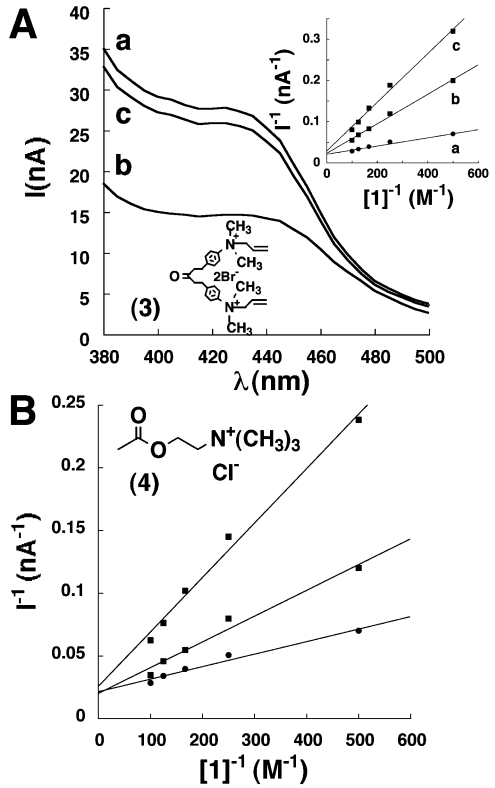

Figure 2. (A) Photocurrent spectra corresponding to the CdS/AChE system in the presence of **1**, 10 mM, (a) without the inhibitor, (b) upon addition of (**3**) 10 μM, (c) after rinsing the system and excluding of the inhibitor. (Inset) Lineweaver−Burke plots corresponding to the photocurrent at variable concentrations of **1**, in the presence of (a) 0 μM of **3**, (b) 10 μM of **3**, (c) 20 μM of **3**. Data were recorded in 0.1 M phosphate buffer, pH=8.1, under argon. (B) Lineweaver−Burke plots corresponding to the photocurrent at variable concentrations of (**1**), in the presence of (a) 0 mM of **4**, (b) 1 mM of **4**, (c) 2 mM of **4**. Data were recorded in 0.1 M phosphate buffer, pH = 8.1, under argon.

for the holes generated in the valence band upon excitation of the CdS nanoparticles. Thus, oxidation of thiocholine by the holes eliminates the electron−hole recombination, and a steady-state photocurrent is generated.[9] The photocurrents generated by the system are stable for at least 1 h. As the concentration of **1** is elevated, the concentration of **2** at the particle surface is higher, and the photocurrent is enhanced, Figure 1 (Inset). In further control experiments we find that the photocurrents generated by the AChE-functionalized CdS monolayer in the presence of different concentrations of the related electron donor cysteamine are similar to the photocurrents generated by the analogous concentrations of acetylthiocholine (**1**). These results suggest that all of the substrate **1** at the CdS nanoparticle interface is transformed to **2** by the biocatalyzed process, and that the oxidation of **2** by the valence-band holes is efficient and prevents the diffusion of **2** to the bulk solution.

Figure 2A shows the photocurrent action spectra of the AChE-functionalized CdS nanoparticle electrode in the presence of **1**, 10 mM, curve (a), and upon the addition of the inhibitor[10] 1,5-bis(4-allyldimethylammoniumphenyl)pentane-3-one dibromide (**3**), curve (b). Increase of the concentration of **3** decreases the photocurrent, Figure 2A, inset. Washing off the inhibitor from the cell and addition of **1**, 10 mM almost restores the initial photocurrent, Figure 2A, curve (c). Figure 2A inset, shows the Lineweaver−Burk plots that correspond to the inhibition of the photocurrents in the presence of different concentrations of **3**. From these plots we conclude that **3** acts as competitive inhibitor $K_I = 7$ μM. The K_M value of the AChE linked to the CdS nanoparticles toward acetylthiocholine (**1**), is $K_M = 5$ mM. This value is higher than the $K_M = 0.13$ mM of AChE and **1** in solution.[11] The higher K_M value for the nanoparticle-immobilized AChE may be attributed to slight deactivation and structural perturbation of the biocatalyst as a result of surface linkage. The decrease in the observed photocurrent in the presence of the inhibitor is attributed to the lower yields for the biocatalyzed formation of thiocholine, and thus less efficient removal of the valence-band holes. Related results are observed upon analyzing the photocurrents generated by the AChE/CdS nanoparticle/**1** system in the presence of different concentrations of acetylcholine (**4**), Figure 2B. Acetylcholine (**4**), competes with acetylthiocholine for the active sites. As a result, increase of acetylcholine results in a decrease in the observed photocurrent.

In conclusion, the present study has addressed a novel concept of tailoring an AChE/CdS nanoparticle hybrid system for the generation of photocurrents controlled by the concentration of acetylthiocholine. The driving force for the formation of the photocurrent is the biocatalyzed formation of thiocholine that scavenges the photogenerated valence-band holes. We demonstrated that enzyme inhibitors decrease the photocurrents, and thus the nanoparticle/AChE system acts as a biosensor for the respective inhibitor. The CdS nanoparticle/AChE/acetylthiocholine system may be a versatile photoelectrochemical label for different biosensors, and the system may be further developed for the biosensing of biological warfare. As far as we are aware, this is the first example of a coupled semiconductor nanoparticle/enzyme hybrid system for photocurrent generation and biosensor applications.

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Supporting Information Available: Details of the nanoparticle synthesis and the electrode modification (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The capping of the CdS nanoparticles by the mixed monolayer is essential to generate water-soluble nanoparticles.
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