Acetylcholine Esterase Antibodies on BiOI Nanoflakes/TiO2 Nanoparticles Electrode: A Case of Application for General Photoelectrochemical Enzymatic Analysis

ABSTRACT: To date, almost all the established photoelectrochemical (PEC) enzymatic biosensors require the surface-confinement procedure to immobilize enzyme as biorecognition element for probing various analytes of interest. This Letter develops a novel example without such necessity. Specifically, we first prepared a BiOI nanoflakes (NFs)/TiO2 nanoparticles (NPs) p–n heterojunction as the photo-electrode, on the basis of which acetylcholine esterase (AChE) antibody was introduced via the bridging of protein A. In such a system, enzyme could keep its optimal state in the solution if in the absence of inhibitor; otherwise, the degree of enzyme inhibition would correlate closely with the concentration of inhibitor. After immunoreaction between AChE and its antibody, the inhibitor concentration could then be determined by the biocatalytic reaction-controlled PEC response. Integrated with other enzyme-based biosystems, this simple configuration could serve as a general method for assaying enzyme inhibition or activities.

In this work, we report a photoelectrochemical (PEC) enzymatic biosensor that removes the commonly needed process of enzyme surface confinement. On the basis of the use of semiconductive materials, the newly appeared PEC bioanalysis has stimulated much recent interest as such a technique offers great promise for developing elegant and versatile miniaturized analyzers compatible with future requirements.1–23 From its very beginning, the study on enzymatic analysis has been a focus of significant research due to its relevant importance, and the basic sensing principle is that the PEC enzymatic system would convert the specific biocatalytic events into electrical responses through the interactions between the photoactive species and the biocatalyzed reaction chain. For example, addressing different analytes, several studies using various enzymes have been reported, including horseradish peroxidase,14,24 glucose oxidase,16,25–32 formaldehyde dehydrogenase,33,34 glutamate dehydrogenase,35 catalase,36 xanthine oxidase,37 and acetylcholine esterase (AChE).38–40 Recently, we also constructed an alkaline phosphatase (ALP)-based sensor using a TiO2 electrode.22 However, due to the short development time, the exploitation on this area is still in its embryonic stage. Especially, almost all the established biosensors necessitated the surface confinement of enzymes (usually via the techniques of entrapping, covalent bonding, and cross-linking as well as electrochemical polymerization) in advance, in which the procedure would inevitably injure the catalytic activity of the enzymes and also limit the uses of these protocols for simultaneously probing the enzyme activities in an aqueous environment. To date, advanced protocols for a PEC enzymatic biosensor that enables efficient and sensitive analysis are yet of high urgency.

Herein, we present an apparently general and simple PEC method for profiling enzyme activity and inhibition using AChE (a critical serine protease for acetylcholine-mediated neurotransmission) as a model enzyme. Unlike the existing analysis platforms based on the direct formation of PEC species–enzyme hybrid, the present work relies on the innovative use of enzyme antibody integrated with an ingenious photoelectrode. Specifically, as shown in Scheme 1, we first designed the BiOI nanoflakes (NFs)/TiO2 nanoparticles (NPs) p–n heterojunction on indium tin oxide (ITO) glass and used as an efficient PEC transducer (for experimental details, see Supporting Information). The subsequent immobilization of anti-AChE via protein A would result in a biosensor enabling the convenient and exquisite PEC enzymatic analysis. In such a system, increased toxin (O,O-dimethyl O-4-nitrophenyl phosphorothioate, DNP) concentration leads to the increased generation of DNP–AChE and hence aggravated enzyme inhibition, impairing the enzyme activities after immunocomplexing for catalyzing the substrate (acetylthiocholine) to generate the product (thiocholine) acting as sacrificial electron donor. The recorded variation in photocurrent responding would then be
employed to analyze the AChE inhibition or to reflect the enzyme activity. To the best of our knowledge, such a general and versatile PEC enzymatic bioanalysis strategy has never been reported.

Semiconductor \( p-n \) heterojunctions have been extensively investigated in the broad photochemistry fields such as photocatalyst or solar cells. Nevertheless, almost no efforts have been devoted to the application of such heterostructure in PEC bioanalysis. Thus, the exploitation in this work is desirable and will potentially broaden the applicability of the \( p-n \) junction in this field. Experimentally, the BiOI NFs/TiO\(_2\) NPs/ITO photoelectrode was fabricated by the sequential use of the liquid phase deposition (LPD) and successive ionic layer absorption and reaction (SILAR) techniques. Figure 1A shows the scanning electron microscopy (SEM) images of TiO\(_2\) NPs photoelectrode prior to and after BiOI loading. As shown in Figure 1A-a and -b, the LPD generated TiO\(_2\) NPs (~20 nm in diameter) film possessed nanoporous morphology with large roughness of high specific surface area, which would provide an advantageous microenvironment for the ions adsorption in the subsequent SILAR experiments. Figure 1A-c demonstrates the SEM of the as-fabricated crossed BiOI NFs layer on TiO\(_2\) NPs coating. As magnified in Figure 1A-d, the BiOI NFs possessed a distinct layered configuration with an interlaced network of high surface areas, which would permit extended light harvesting. The phase structure and the surface chemical compositions (and oxidation states) of the sample were further researched with X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS), respectively, with the results depicted and discussed in Figures S1 and S2, Supporting Information, respectively. To investigate the PEC properties of the samples, intermittent visible light irradiation of 450 nm was applied to acquire the transient photocurrent responses. As shown in Figure 1B, compared with the no response of bare ITO glass, the TiO\(_2\) NPs sample displayed weak response which could be attributed to the slight UV light leakage from the light source. However, after the SILAR procedure, the signal intensity exhibited substantial improvement, indicating not only the successful BiOI loading but also the notable coupling effect between the two semiconductors. The prompt rise of the photocurrent also indicated the rapid charge excitation, separation, and transfer in the hybrid.

Such a feature should be ascribed to the rebalanced energy levels during the formation of the \( p-n \) heterojunction as

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**Figure 1.** (A) a: SEM image of the LPD fabricated TiO\(_2\) film; b: high-magnification SEM image of the TiO\(_2\) film; c: SEM of the 3D interlaced network of BiOI layer on TiO\(_2\) NPs; d: the corresponding high-magnification SEM image. (B) PEC responses of (a) the ITO electrode after (b) TiO\(_2\) NPs modification and (c) after BiOI loading. PEC tests were performed in 0.1 M phosphate buffer (pH = 7.4) containing 0.1 M ascorbate constant potential of 0.0 V and 450 nm excitation light, under nitrogen.

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**Scheme 1.** Schematic Representation of the Novel Enzymatic Sensing Principle Using the Developed BiOI NFs/TiO\(_2\) NPs Photoelectrode
elucidated in Scheme 2. Specifically, the presence of p-type BiOI and n-type TiO$_2$ NPs would cause the ascent of Fermi level and energy band of BiOI while the decline of those of TiO$_2$ NPs until a new steady equilibrium. Upon irradiation, visible light-induced separation of electron−hole pairs could occur in BiOI NFs, and the reformed band edge in the p−n junction would then facilitate the fast transportation of these photo-generated excitons; i.e., the photoelectrons of BiOI would inject into the conduction band (CB) of TiO$_2$, and the holes of the latter would migrate to the valence band (VB) of the former. Obviously, such p−n heterojunction could contribute to the spatial charge separation as well as the enhanced carrier mobility and lifetime and, hence, the improved property for the following bioanalytical utilization.

We then applied the prepared hybrid to construct the proposed protocol for bioassay application as stated above. Figure 2A shows the development process of the biosensor recorded by transient photocurrent responses. As shown, after anchoring the protein A and anti-AChE on the BiOI NFs/TiO$_2$ NPs/ITO photoelectrode, the photocurrent displayed an obvious decrease (curves b), which could be easily attributed to the generation of hydrophobic protein layer that hindered the interfacial mass and electron transfer. Then, the as-produced electrode was incubated with the sample solution containing AChE, and the photocurrent intensity further declined slightly (curves c). However, the final enzymatic reaction in the presence of acetylthiocholine resulted in the prominent signal increase (curves d), which was due to the efficient biocatalyzed generation of thiocholine and the subsequent scavenging of the photoholes by this electron donor that contributed to the photocurrent production. The control experiment revealed that the photocurrent remained unchanged in the absence of acetylthiocholine. As shown in Figure 2A inset a, further studies found that the signal intensity was related to the concentration of acetylthiocholine. These results suggested that the immunocomplexed AChE effectively retained its bioactivities and the photocurrent variation was caused exclusively by the AChE-catalyzed hydrolysis of acetylthiocholine. Obviously, this protocol could be easily applied for evaluating enzyme activities. As shown in Figure 2A inset b, as the concentration of AChE increased, the PEC signal was enhanced, consistent with the formation of elevated amounts of thiocholine. In another case, i.e., the as-produced electrode was subjected to incubation with DNP-AChE, followed by the enzymatic reaction under the same experimental conditions; the final signal intensity (curve e of Figure 2A) was much weaker than curve d. This was because the high acute toxicity of DNP could lead to the irreversible inhibition on AChE, rendering the impaired enzymatic activity toward the substrate. All these experiments implied that the catalytic generation of the product and the corresponding PEC response in such a system are of essential relevance to the
presence of toxin. Thus, through monitoring the photocurrent variation after the specific biocatalytic reaction, which could reflect the enzyme inhibition, an exquisite PEC enzymatic biosensor could be tailored. Figure 2B depicts the photocurrent responses resulting from the photoradiation of the system in the presence of different toxin DNP concentrations. As expected, an increase of DNP concentration decreased the photocurrent, reaffirming the AChE-controlled production of thiocholine and thereby the photocurrent response. Specifically, the reason of such a trend is that the AChE-catalyzed hydrolysis of acetylthiocholine would be curbed following DNP-ALP formation, giving rise to a lower yield of electron donor and thus the less efficient holes’ neutralization. The corresponding inhibition ratio was calculated by the following expression:

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\text{inhibition (\%)} = \left[ \frac{(I_0 - I)}{I_0} \right] \times 100\%
\]

where \(I_0\) and \(I\) are the photocurrents of the AChE electrodes without and with the DNP inhibition, respectively. Figure 2B inset shows the derived calibration curve, and the detection limit was experimentally found to be 0.015 ng/mL; the leveling off at higher toxin concentration implied the near saturation of DNP-ALP formation upon enhanced DNP concentration. These preliminary results showed the feasibility of this protocol for assaying AChE inhibition; future research will address the use of this methodology for real sample analysis.

In short, this work first exploited the use of the BiOI NFs/TiO2 NPs p–n heterojunction for PEC bioanalysis application. Using AChE as a model enzyme, we further proposed a novel PEC enzymatic biosensor that eliminated the necessity of an antibody, this strategy could preserve the original catalytic activity of enzyme and facilitate the analysis of enzyme inhibition and activities. These results not only offered an ingenious transducer for future uses in the broad PEC analytical field but also presented an elaborate mechanism for the new and general enzymatic analysis providing that other enzyme antibodies are appropriately involved.

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**REFERENCES**


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