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Letter

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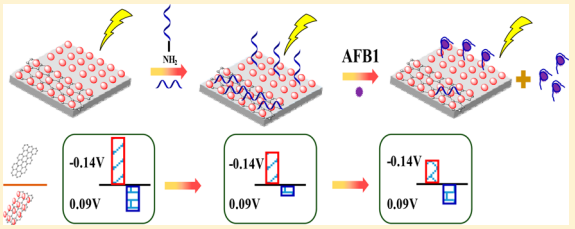
Design of a Dual Channel Self-Reference Photoelectrochemical Biosensor

Nan Hao, Ying Zhang, Hui Zhong, Zhou Zhou, Rong Hua, Jing Qian, Qian Liu, Henan Li, and Kun Wang*

Key Laboratory of Modern Agriculture Equipment and Technology, School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013, PR China

Supporting Information

ABSTRACT: Photoelectrochemical (PEC) biosensors are usually based on the single photocurrent change caused by biorecognition events between analytes and probes. However, the photocurrent may be influenced by other factors besides target analytes and bring a false result. To improve the accuracy and reliability of PEC detection, here we proposed the design of a dual channel self-reference PEC biosensors. CdTe and CdTe-graphene oxide (GO) were chosen as the two PEC active material and modified onto two adjacent areas on the ITO electrode. Then they were functionalized with Aflatoxin B1 (AFB1) aptamer through covalent binding or physical adsorption, respectively. The cathodic current from CdTe-GO and anodic current from CdTe can be well distinguished by adjusting the bias voltage. With the simultaneous application of "signal on" and "signal off" model, dual concentration information may be obtained in one detection and serve as a reference for each other. By comparing these two results, this sensor can clearly distinguish whether the signal change was caused by AFB1 or other interference factors. Compared to traditional PEC biosensors, this design can provide a better accuracy and reliability, which is promising in the future development of PEC detection.



In recent years, photoelectrochemical (PEC) biosensors have attracted great attention.This technique has a good sensitivity and does not require expensive equipment, which is promising in the development of biological analysis.[1,2] To fabricate a typical PEC system, photoactive materials and biological receptors are two necessary elements.[3] Served as the photon-to-electricity energy conversion layer, charge separation and transfer of photoactive materials would occur under excitation light illumination and generate photocurrent as the detection signal. Biological receptors, such as antibodies, enzymes, and DNA probes, can specially capture target analytes through biological recognitions, which would change the interface state of photoactive materials and lead to the corresponding variation of photocurrents.[4] The signal change may come from the introduction of photoactive reporter or other materials influencing PEC reaction. It also may directly originate from the target that can affect charge transfer through various mechanisms.[5]

Generally speaking, these PEC strategies can be classified into two types, "signal on" and "signal off",[6−10] depending on the presence of target analytes causes the enhancement or decrement of the photocurrent before and after biorecognition events. For example, the binding of photoactive threading bis-intercalator on the duplex formed by target ssDNA hybridized with capture probes can significantly enhance the photocurrent, allowing the ultrasensitive PCR-free DNA detection.[11] Also on the basis of the DNA-hybridization, DNA detection may be achieved by target induced conformational change of the hairpin probe. The open of stem-loop structure increased the distance between the tagged photosensitizer and the electrode change and decreased the photocurrent.[12] Obviously, no matter "signal on" and "signal off", the quantification of targets relies on the single output signal change. However, the photocurrent may be influenced by other factors besides target analytes. The modified electrodes fabricated with nonstandard processes may show different PEC performances. The improper storage of electrodes would affect the analysis reproducibility. Also, in the detection process, the distance change between electrodes and the light source, the long-term light stability, and the applied buffer solutions with slight differences, all can result in the photocurrent change. Furthermore, it is difficult to distinguish whether the signal change is caused by targets or other factors and a false positive or negative error may occur. Because of this, the anti-interference ability and sensitivity of PEC biosensors were inevitably limited, especially for trace analysis in complex matrix. To improve the performance, more signal channels are needed for one analyte detection as the internal reference approach. For fluorescence sensors, two or more emission intensity changes at different wavelengths can be applied.[13−15] For electrochemical or electrochemiluminescence (ECL) sensors, the simultaneous use of two active materials with different excitation potentials have been well studied.[16−18]

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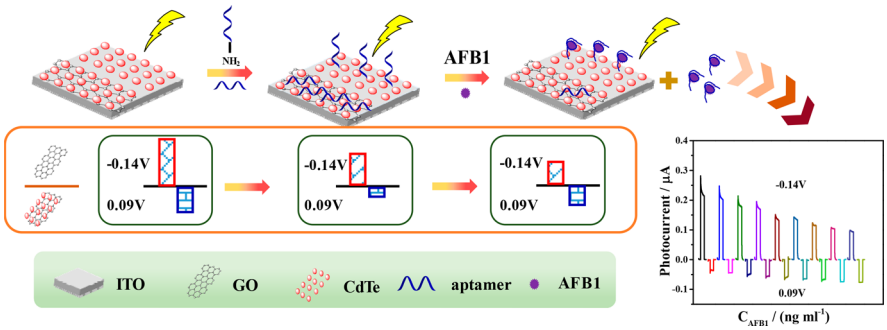
Scheme 1. Progress of Constructing Self-Reference PEC Biosensor for the Detection of AFB1



However, similar studies in PEC sensors are few because it is more difficult to eliminate each other's interruptions from different PEC active materials. So it is meaningful to explore the design of PEC biosensors with two output signals that can effectively increase detection reliability.

In this work, we fabricated a dual channel self-reference PEC biosensor for the first time. Two detection models, "signal on" and "signal off", were simultaneously applied by choosing two appropriate PEC active materials and two independent detection results may be obtained for one sample. Aflatoxin B1 (AFB1) is a prevalent and toxic mycotoxin that seriously affects food safety and human health worldwide.[19−21] In a proof of concept experiment, the PEC properties of CdTe quantum dots (QDs) and CdTe-graphene oxide (GO) nanocomposite were investigated and applied in the construction of the self-reference PEC AFB1 biosensor. By comparing these two results, this sensor can clearly distinguish whether the output signal change was caused by AFB1 or other interference factors and provide a more reliable detection.

The processes of the dual channel self-reference PEC biosensor are illustrated in Scheme 1. First, CdTe and CdTe-GO were prepared and modified onto two adjacent areas on the ITO electrode. Then these two materials were functionalized with AFB1 aptamer through covalent bonding or physical absorption, respectively. This functionalization caused the decrease of cathodic photocurrent at 0.09 V and anodic photocurrent at −0.14 V due to the increased steric hindrance. Next, target AFB1 was added on the ITO electrode and lead to the conformation change of aptamers. The conformation change caused different signal changes in two areas.[22] For CdTe-GO, the aptamer would be released from the electrode surface and the cathodic photocurrent recovered. For CdTe, AFB1 was captured on the surface and caused the further decrease of anodic photocurrent. So the quantification of AFB1 can be achieved based on the increase of cathodic photocurrent and the decline of anodic photocurrent independently. As discussed above, the fabrication, storage, or other factors may influence the photocurrent. However with these influences, the two AFB1 concentrations obtained by cathodic photocurrent and anodic photocurrent would have a significant difference. Also, we can easily judge whether the detection is reliable by comparing the results from "signal on" and "signal off" channels, so we named it as "self-reference" biosensors. Therefore, a universal dual channel self-reference photoelectrochemical biosensors was constructed for sensitive and precise detection.

Detailed synthetic steps of CdTe QDs and CdTe-GO were placed in the Supporting Information.[23−25] Fluorescence (Figure S1A) and UV−vis spectra (Figure S1B) showed the successful preparation of CdTe QDs and CdTe-GO. The photocurrent responses of CdTe-GO at 0 V, −0.1 V, −0.13 V, −0.14 V, and −0.15 V and CdTe at 0 V, 0.05 V, 0.07 V, 0.09 V, and 0.1 V were shown in Figure 1A,B, which clearly stated the

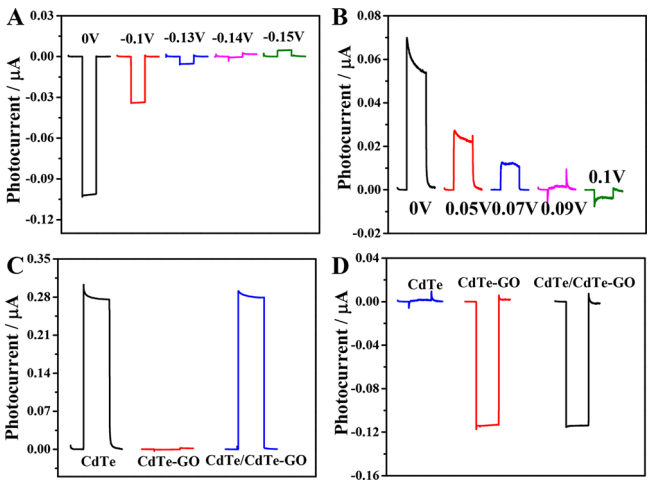


Figure 1. Photocurrents of CdTe-GO (A) and CdTe (B) at different bias voltage; photocurrents of CdTe, CdTe-GO, CdTe/CdTe-GO at −0.14 V (C) and 0.09 V (D).

critical voltages of CdTe-GO and CdTe were −0.14 and 0.09 V, respectively. In Figure 1C and 1D, it was obvious that two materials modified on one ITO electrode would not influence the photocurrent value of each other. This CdTe/CdTe-GO modified electrode has the same anodic photocurrents as that of CdTe modified electrode at −0.14 V (Figure 1C). Likewise, the cathodic photocurrent was the same as that of CdTe-GO at 0.09 V. This phenomenon provided the foundation for dual channel self-reference PEC biosensor.

In order to obtain the optimum experimental conditions, a series of conditional optimizations including the concentration of aptamer, the modification time, binding time between aptamer and target, and the temperature (Figure S2) were carried out as shown in Figure S2. The analytical ability of this PEC AFB1 biosensor was shown in Figure 2. The anodic and cathodic photocurrent responses of CdTe/CdTe-GO electrode in the presence of 0, 0.01, 0.1, 0.5, 2, 10, 20, 50, and 100 ng mL^{-1} AFB1 were exhibited in Figure 2A. The anodic photocurrent decreased gradually with increasing the AFB1 concentration due to the enhanced steric hindrance after aptamer binding with AFB1. On the contrary, the cathodic photocurrent increased with the increased concentration of AFB1 because the aptamer would be released from the

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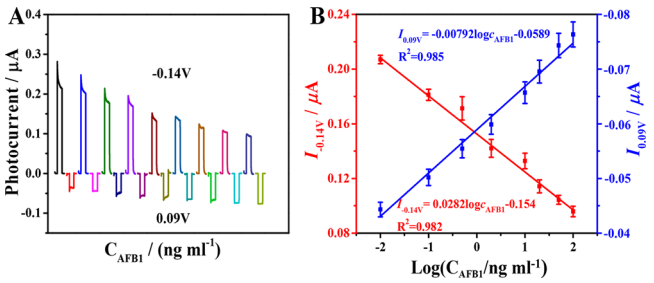

Figure 2. Photocurrents of CdTe/CdTe-GO electrode at −0.14 and 0.09 V in the existence of 0.01, 0.1, 0.5, 2, 10, 20, 50, 100 ng mL^{-1}AFB1 (A). The linear calibration curve between $I_{-0.14\ V}$ (anodic photocurrent), $I_{0.09\ V}$ (cathodic photocurrent), and the logarithmic concentration of AFB1 (B).

electrode after the specific binding. Figure 2B manifested the linear relationship between the anodic and cathodic photocurrent responses and the logarithmic values of AFB1 concentrations from 10 pg mL^{-1} to 100 ng mL^{-1}. At the same time, the selectivity of the PEC biosensor was presented in Figure S3A by comparing the photocurrent at −0.14 and 0.09 V for AFB1, ochratoxin A (OTA), and fumonisin B1 (FB1). This biosensor showed a good selectivity because of the specific binding between the aptamer and AFB1 molecules. The stability was shown in Figure S3B, and it was kept invariable with 15 repeated switching on and off of the lamp and produced a satisfying stability.

Here we chose two possible interference factor that cause the increase or decrease of photocurrent to better illustrate the advantages of the self-reference biosensors with the presence of 50 ng mL^{-1} AFB1. One example is the influence of the light intensity, the light intensity may fluctuate somewhat in the long term operation. As shown in Figure 3A, when the power of light source increased, the value of anodic and cathodic photocurrent increased correspondingly. Another example is the widely applied ITO electrode. The electrochemical performance of ITO electrode may vary due to storage conditions and pretreatment processes, etc. Figure 3B showed

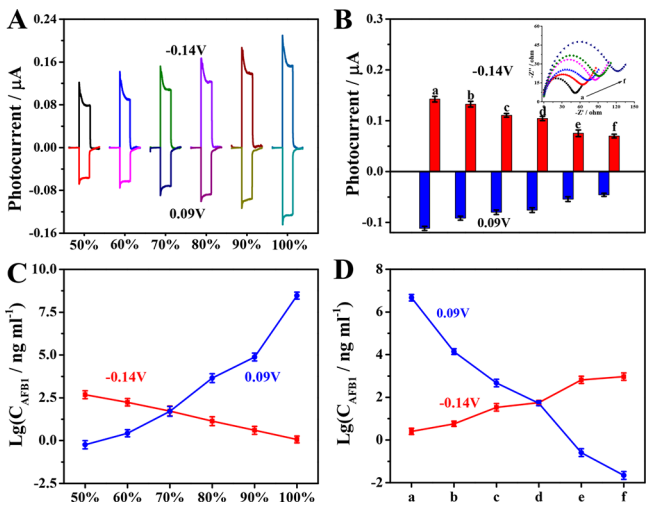

Figure 3. Photocurrents of CdTe/CdTe-GO modified one ITO electrode at different light intensity (A) and modified different ITO electrodes (B) at −0.14 and 0.09 V. The logarithm of AFB1 concentration at different light intensity (C) and modified different ITO electrodes (D).

the anodic and cathodic photocurrents from a series of ITO electrodes with different impedances. When the impedances increased gradually (shown in the inset figure), the photocurrent value decreased from a to f. From these photocurrents in Figure 3A,B, different concentrations may be calculated according to the linear calibration curves (Figure 3C,D). The concentrations from two channels were close to the actual value 50 ng mL^{-1} when the light intensity (70%) and electrochemical performance of ITO electrode (d) were similar to the experiment conditions of linear calibration curves. When the light intensity or impedances changed, the concentrations from two channels deviated from the actual value and move toward different directions. One is higher than the actual value while the other one is lower. So we can easily distinguish whether the result is reliable by comparing the two results from "signal on" and "signal off" channels. When the two results have a significant difference, it indicates some interference factors existed and the data was unreliable. So the detection accuracy and reliability is improved because unreliable data can be recognized and eliminated.

In conclusion, a novel dual channel self-reference photoelectrochemical biosensors has been successfully developed. CdTe and CdTe-GO were chosen as the two PEC active material and functionalized with AFB1 aptamer through covalent binding or physical adsorption, respectively. The cathodic current from CdTe-GO and anodic current from CdTe can be well distinguished by adjusting the bias voltage. With the simultaneous application of the "signal on" and "signal off" models, dual concentration information may be obtained in one detection and serve as a reference for each other. Compared to traditional PEC biosensors, this design can provide a better accuracy and reliability, which is promising in the future development of PEC detection.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b03132.

Experimental section, synthesis procedures, fluorescent and UV−vis absorption spectra of CdTe QDs and CdTe-GO NCs, fabrication of PEC biosensor, optimization of experimental conditions, and selectivity and stability study of PEC biosensor (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +86 511 88791800. E-mail: wangkun@ujs.edu.cn.

ORCID

Kun Wang: 0000-0001-6764-8686

Notes

The authors declare no competing financial interest.

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Anal. Chem. XXXX, XXX, XXX−XXX

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Anal. Chem. XXXX, XXX, XXX−XXX