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Folding-based photoelectrochemical biosensor: binding-induced conformation change of a quantum dot-tagged DNA probe for mercury(II) detection†

Zheng-Yuan Ma, Jian-Bin Pan, Cai-Yu Lu, Wei-Wei Zhao, Jing-Juan Xu* and Hong-Yuan Chen*

Using CdS QD-tagged mercury-specific oligonucleotides, a novel folding-based photoelectrochemical sensor has been successfully fabricated for reagentless and highly sensitive Hg^{2+} detection.

As a new yet actively developing technique, photoelectrochemical (PEC) bioanalysis has attracted more and more research interest.[1] Coupling photoirradiation with electrochemical detection, PEC bioanalysis possesses the advantages of both optical methods and electrochemical sensors, which lower the instrumentation cost remarkably. Since this new technique offers great promise for developing elegant and versatile miniaturized devices compatible with future requirements in biosensing, enormous efforts have been devoted to its exploitation.[2] To date, many strategies for PEC bioanalysis have been proposed and significant progress has been achieved for probing various biorecognition events. However, since the research on PEC detection is presently still in its embryonic stage, most previous efforts were directed toward the usual areas of small molecules,[3] DNA (damage) analysis,[4] immunoassays and enzymatic sensing.[5] As yet, only a rather limited amount of research has explored the application of the PEC method in ion detection.[6]

Because of the persistence of mercury species in the environment, which leads to bioaccumulation and high toxicity, they have been listed as a priority pollutant by many countries and international agencies.[7] Once introduced into the aqueous environment, they can also be converted to methyl mercury, which is known to cause brain damage and other chronic diseases, and accumulate through the food chain.[8] On account of this, efforts towards the sensitive detection of mercury ions have never died away. Among these endeavors, using the thymine (T)-rich, mercury-specific oligonucleotides (MSOs) is a smart strategy since Hg^{2+} ions can specifically interact with thymine to form stable thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) complexes. As a matter of fact, many techniques such as fluorescence,[9] electrochemical,[10] colorimetric,[11] electrochemiluminescence[12] and PEC methods[6b,c] were exploited using MSOs for Hg^{2+} detection. Although these ideas demonstrated good performances with high sensitivities and good selectivities, most of them involved laborious procedures associated with the methodology[6c,10,11] and were based on the use of specially functionalized probe molecules[9] or organic semiconductors,[6b,12] which limited their universality for portable detection. Undeniably, it is still imperative to develop novel and facile Hg^{2+} ion sensors, especially ones which induce a prompt response directly evoked by the analyte.

Owing to its single-step, reagentless facility in bioanalytical applications, the study of binding-induced DNA folding has become a current research hotspot.[13] Herein, we report the first folding-based PEC biosensor that is capable of rapid, sensitive and reagentless Hg^{2+} detection. Based on the conformational change of the MSO probe, the PEC signaling could be turned on in the presence of Hg^{2+}. As shown in Scheme 1, the MSO probes were initially tethered onto the Au electrode before subsequent functionalization with CdS QDs on their aminated ends. In the presence of Hg^{2+}, the MSO probes form folding configurations owing to the effects of the T–Hg^{2+}–T interactions, rendering the CdS QDs in direct contact with the electrode surface. Upon stimulation with exogenous light, interfacial electron transfer could be facilely achieved. Specifically, as illustrated, photoexcitation of the CdS QDs yields electron–hole pairs and constant photocurrent is monitored with ejection of the conduction-band electrons into the electrode. Furthermore, the valence-band holes are sacrificed by concomitant scavenging from the soluble ascorbic acid donor. *Via* combining the robust features of CdS QDs and the MSO probe configuration strategy, this work can offer a new perspective for expedient, low-cost and high-sensitive detection of Hg^{2+} ions.

Experimentally, water-soluble and thioglycolic acid (TGA)-stabilized CdS QDs were first synthesized according to the procedure described in our previous literature.[5a] The sequence of the MSO probe used here is 5′-SHCTTGTTTCTCCCCCCTGTT TCTTG-NH_2-3′, which is separated by the mute spacer in the middle into two complementary moieties with C and G bases

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China. E-mail: xujj@nju.edu.cn, hychen@nju.edu.cn; Fax: +86-25-83594862; Tel: +86-25-83597294

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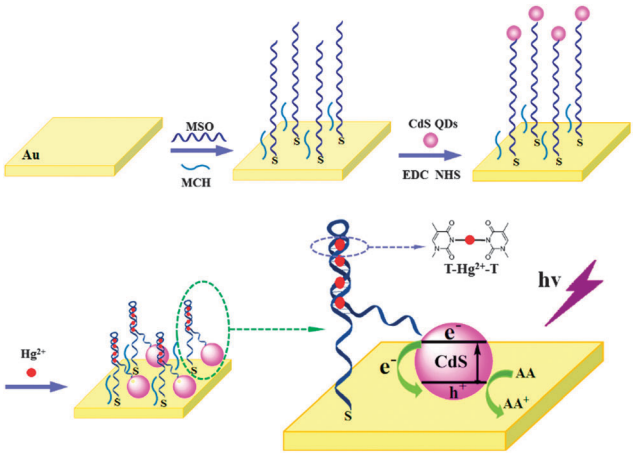

Scheme 1 Schematic illustration for fabricating the folding-based Hg^{2+} biosensor and PEC analysis of Hg^{2+} through the T–Hg^{2+}–T complex.

distributed correspondingly. Through the classic thiol–gold interaction, the Au electrode surface was covered by the MSO molecules, which have amino groups on the other end by prior decoration. Subsequently, the CdS QDs can be anchored onto the electrode *via* a classic *N*-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC) coupling reaction between the COOH groups on the surface of the CdS QDs and the NH_2 groups of the MSO probe. Owing to the doubly-functionalized MSO molecules, the Au electrode surface and the CdS QDs can be bridged together, the complex of which acts not only as a specific recognition device for Hg^{2+} but also as a photosensitizer. Once mercury ions are trapped on the probe, a photoelectric switch is also driven to its turn-on mode. Moreover, since the intensities of the resulting photocurrents are controlled by the amount of T–Hg^{2+}–T and CdS QD complexes associated with the electrode, higher conversion efficiency is expected to be observed as the ion concentration increases (for experimental details see ESI†).

Fig. 1A and B (inset) show the transmission electron microscopy (TEM) image and UV-vis absorption of the as-prepared TGA-capped CdS QDs, respectively. As indicated by TEM measurements, the size of the CdS QDs corresponds to *ca.* 5 ± 1 nm. The absorption spectrum shows that the CdS QDs have a broad absorption in the visible region, which is suitable for their employment in a biological system. We inspected the photocurrent response of the biosensor under irradiation from 400 nm to 600 nm wavelength. According to Fig. 1B and the inset, the photocurrent action spectrum follows the absorbance features of the CdS QDs and has a noticeable photoresponsive signal from 400 to 480 nm, indicating that the photocurrents originate entirely from the excitation of the semiconductor QDs.

The entire PEC conversion proposal is also corroborated by Fig. 2, which reveals the developing process of the PEC Hg^{2+} detection through stepwise measurements of the photocurrent. After anchoring the MSO probes, no obvious photocurrent was monitored due to the lack of photoelectrically active material, corresponding to curve a. The successful assembly of the CdS QDs was next established by obvious photoelectric response in curve b. Since single-strand DNA usually appears in a tanglesome morphology on the electrode surface, the distance of CdS QDs to the electrode is unpredictable in the absence of Hg^{2+}, which means that the initial photocurrent stays at a low level owing to the failure of efficient electron transmission. Since Hg^{2+} ions can specifically interact with thymine bases to form strong and stable thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) complexes, which are even more stable than the Watson–Crick A–T pair,[14] the emergence of mercury ions can firmly lock the MSO chain, inducing a folding structure distortion. In addition, with an increase in Hg^{2+} concentration leading to a greater proportion of the folding configuration, higher electron transfer efficiency will be realized and rising photocurrent can be monitored consistently. As a consequence, the photocurrent increased remarkably with increased Hg^{2+} concentration (curves c to d), which convincingly demonstrated the formation of T–Hg^{2+}–T complexes and completely coincided with our prediction. Based on this proportional amplification effect, highly sensitive detection for Hg^{2+} ions was accomplished successfully. Control experiments, which were performed with the CdS QD-modified photoelectrode incubated in pure water free of Hg^{2+} at 37 °C for 60 min, manifested no change in the photocurrent intensity, further implying that the photocurrent increase was due to the mercury-induced folding.

It is worth noting that the MSO probe anchoring and following MCH (6-mercapto-1-hexanol) blocking failed to show any photoelectric variation because of the lack of a photoactive element, which can be traced using electrochemical impedance spectroscopy (EIS) (see Fig. S1, ESI†). Also, the XPS elemental analysis could further confirm the CdS QD tethering onto the MSO linker-modified Au

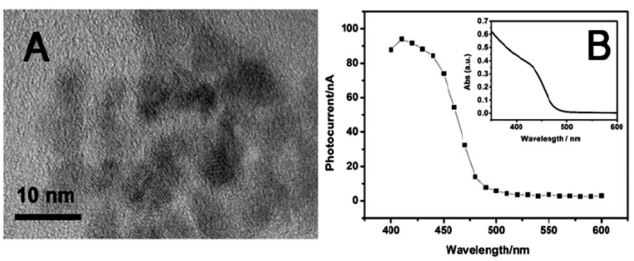

Fig. 1 (A) TEM images of the synthesized CdS QDs. (B) Photocurrent action spectrum of the CdS QD-modified electrode under irradiation from 400 nm to 600 nm wavelength. The inset is the corresponding UV-vis absorptions of CdS QDs.

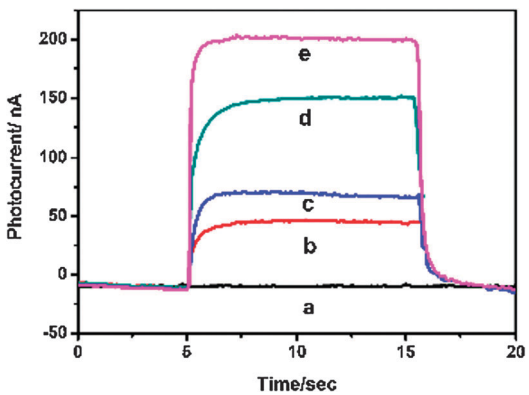

Fig. 2 Photocurrent response of the modified electrode (a) before and (b) after CdS QD anchoring, (c–e) after Hg^{2+} incubation (5, 100, 500 pM). The PEC tests were performed in a 0.10 M PBS solution containing 0.10 M AA with 0.0 V applied voltage and 410 nm excitation light.

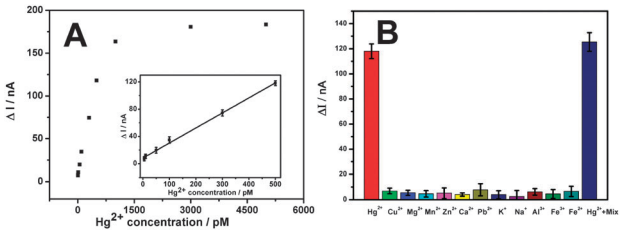

Fig. 3 (A) Effect of different Hg^{2+} concentrations on the differential photocurrent responses. $\Delta I = I - I_0$, where I_0 stands for the photocurrent of the modified electrode before Hg^{2+} capturing and I is the final photocurrent after incubation with elevated concentrations of Hg^{2+} corresponding to 5, 10, 50, 100, 300, 500, 1000, 3000 and 5000 pM, respectively. The inset is the corresponding derived calibration curve. Error bars represent the standard deviation of triplicates. (B) Effects of other metal ions on the detection of 0.5 nM Hg^{2+} ions in a 0.1 mol L^{-1} PBS solution (pH 7.0) at the bias voltage of 0 V following visible light irradiation (λ = 410 nm). The bars represent 0.5 nM Hg^{2+} ions, 100 nM of other metal ions, and a mixture of 0.5 nM Hg^{2+} ions and 100 nM of each of the other eleven metal ions, respectively. Error bars represent the standard deviation of three replicates.

surface. As Table S1† suggests, obvious changes appeared for the elemental composition of the electrode, with a Cd content appearing, rising from 0 to 0.17%, implying that CdS QDs were coupled onto the MSO probe successfully.

After optimization of MSO probe and CdS QD concentrations for sensor fabrication, an exquisite PEC Hg^{2+} transducer could be tailored through tracking the increase in the photocurrent. Fig. 3A displays the resulting photocurrents after incubation with Hg^{2+} of variable concentrations and the inset figure shows the corresponding derived calibration curve. Although the extent of final photocurrent enlargement closely correlates with the Hg^{2+} concentration, the exactly linear dependence was observed only in the range of 5 to 500 pM Hg^{2+}, with a saturation platform at 1 nM. Benefiting from the use of ascorbic acid as the electron donor to amplify the photocurrent, a calculated detection limit of 1 pM was obtained, which is among the most sensitive sensors reported to date[6b,c] and well below the maximum level of 10 nM for Hg^{2+} ions in drinking water permitted by the U.S. Environmental Protection Agency.

The selectivity of this system for Hg^{2+} ions at a 0.5 nM concentration was evaluated by comparing the response of the assay to other common metal ions, such as K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Fe^{2+}, Al^{3+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Cu^{2+}, each at a concentration of 100 nM. As shown in Fig. 3B, only the solution containing Hg^{2+} ions was clearly "signal on", even with 200 times excess of other metal ions. This excellent selectivity could be attributed to the highly specific interaction of the T–Hg^{2+}–T coordination chemistry. Since stability is an important parameter for the performance of a PEC sensor, the process of photoexcitation was repeated tens of times over 400 s, as demonstrated in Fig. S2 in the ESI.† There was no obvious change in the stimulation process, illustrating remarkable robustness.

In summary, a highly sensitive and selective PEC biosensor for Hg^{2+} based on binding-induced DNA folding was developed. Integrating the sensitivity, substantial background suppression and operational convenience of a folding-based biosensor, we employ photoelectrochemistry to monitor binding-induced changes in the rigidity of a CdS QD-tagged MSO probe. Through the specific T–Hg^{2+}–T interaction and the broad absorption of the CdS light center, an adjustable distance of the electron transport route was obtained and the exquisite PEC conversion switch was turned on directly by the analyte, which promised high selectivity. In addition, integrating the traditional PEC method with the dynamic conversion protocol, the cost is reduced remarkably by use of cheap light-emitting LED lamps and simple electronics, and high sensitivity and robustness are achieved in the meantime. Since the whole process requires a very low sample volume (25 μL), it can be configured readily into a portable device for routine on-site monitoring of Hg^{2+}. This work underlies a new and general PEC biosensor format that could be extended for probing other metal ions and biomolecules with similar target-base pairs, and might open a new perspective for PEC conversion research as well as artificial smart materials.

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