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A proximity-dependent surface hybridization strategy for constructing an efficient signal-on electrochemical DNAzyme sensing system†

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A proximity-dependent surface hybridization strategy is employed for designing a "signal-on" electrochemical DNAzyme biosensor. By taking advantage of the high sensitivity of the PDSH strategy, and by realizing the enzymatic hydrolysis reaction in a homogenous system with a unimolecular design, the proposed biosensor shows a very high sensitivity to target molecules.

DNAzymes are a class of synthetic DNA oligonucleotides which show catalytic activities toward specific substrates in the presence of certain cofactors.[1,2] Moreover, DNAzymes possess several unique features, such as simple synthesis, easy labeling, good stability, and design flexibility, which make DNAzymes particularly attractive as a platform for designing biosensors for cofactors such as metal ions and neutral molecules.[3,4] As a result, several DNAzymes that are highly specific for a number of cofactors such as Pb^{2+},[5] Cu^{2+},[6] Zn^{2+},[7] Co^{2+},[8] UO_2^{2+},[9] and L-histidine[10] have been isolated, and converted into highly sensitive and selective biosensors.[11,12] Most of these sensors are based on fluorescence[11] or colorimetry signal transduction mechanisms.[12]

Electrochemical sensors have received increasing attention due to their remarkable features such as high sensitivity, simple instrumentation, low production cost and promising response speed.[13] Although many electrochemical biosensors based on DNA and aptamer as recognition elements have been well developed for various target molecules, only a few DNAzyme-based electrochemical biosensors have been reported.[14–16] Unfortunately, for most of these DNAzyme biosensors, the enzymatic hydrolysis reaction of the substrate strand (might be the rate limiting step) is performed on a solid surface,[14,15] which is expected to exhibit limited cleavage efficiency than those carried out in a homogenous system. Moreover, some of them exhibit "signal-off" electrochemical responses toward targets,[14,15] which show theoretically poorer sensitivity than those showing target-triggered "signal-on" responses. Therefore, design of DNAzyme biosensors with the enzymatic hydrolysis reaction completed in a homogenous system, and providing a "signal-on" electrochemical response following target stimuli, is a significant challenge and an active field for the current electroanalytical chemistry.

Fredriksson and co-workers have proposed an aptamer-based proximity ligation assay for amplified detection of proteins.[17] Inspired by this pioneering work, the Yu group have proposed a proximity-dependent surface hybridization (PDSH) strategy for constructing aptamer-based electrochemical biosensors for single-step and sensitive detection of proteins.[18] Such a PDSH electrochemical assay depends on simultaneous recognition of a target molecule by a pair of affinity probes followed by the binding of the ferrocene (Fc)-labeled proximate affinity probes with electrode surface-tethered oligonucleotide strands, which could provide a highly selective, sensitive and signal-on electrochemical response toward target molecules.[18,19] Since some DNAzymes show high specificity for various metal ions or neutral molecules, we envisioned that the combination of DNAzymes with the PDSH strategy might result in efficient signal-on electrochemical biosensors for various cofactors. In this work, by taking advantage of the high specificity toward targets of DNAzymes and the high sensitivity and selectivity of PDSH assay, and by realizing the enzymatic hydrolysis reaction in a homogenous system, we report a universal amplified strategy for constructing electrochemical biosensors for highly sensitive and "signal-on" detection of target cofactors.

To demonstrate the feasibility of the proposed strategy, an L-histidine-dependent DNAzyme was chosen as a model to construct the PDSH-based electrochemical DNAzyme biosensor for neutral molecules.[10] L-Histidine plays a significant role in the growth and repair of tissues, as well as in controlling the transmission of metal elements in biological bases.[20] The design of the electrochemical DNAzyme biosensor is shown in Scheme 1. It is composed of three elements: a unimolecule (uDNAzyme) with the DNAzyme strand linked with the substrate strand through 10 T bases to form a molecular beacon structure was designed as the catalytic recognition unit; a 3′-thiol-modified oligonucleotide was selected as a capture probe; and a 5′-Fc-modified oligonucleotide was chosen as a reporter probe. The hydrolysis product (oligonucleotide A in Scheme 1) of uDNAzyme was designed with its 3′-terminal partially complementary to the capture probe sequence close to the 5′-terminal, and its 5′-terminal partially complementary to the signal probe sequence close to the 3′-terminal, while 3′-terminal of the capture probe was partially complementary to the signal probe sequence close to

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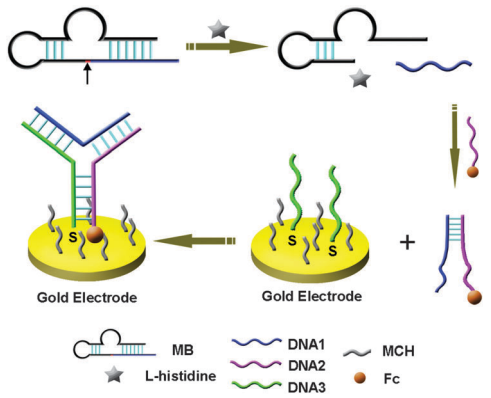

Scheme 1 Schematic illustration of the electrochemical DNAzyme sensing system based on the PDSH strategy.

the 5′-terminal. To construct the sensing interface, the capture probe is covalently attached to a gold electrode *via* Au–thiol chemisorption then treated with 6-mercaptohexanol (MCH) to block the nonspecific sites on the surface. When the uDNAzyme was incubated with L-histidine, the DNAzyme was activated and cleaved the substrate section to release oligonucleotide A. Oligonucleotide A together with the signal probe will trigger the PDSH process, and hybridize with the capture probe to form a Y-shaped junction structure at the electrochemical sensing interface, which was confirmed by agarose gel electrophoresis (see Fig. S1 in the ESI†). In such case, the Fc label on the signal probe will be brought close to the electrode surface, and result in a great increase of electrochemical signal. In the absence of L-histidine, the whole catalytic recognition unit cannot trigger the PDSH process due to the high melting temperature of intermolecular hybridization of the MB stem, and the signal probe alone cannot hybridize with the capture probe at room temperature due to a predesigned low melting temperature ($\sim$17 °C), ensuring a low background signal.

To study the controlled factor of the electrochemical process on the electrode surface, the effect of scan rate on the peak current was investigated by cyclic voltammetry. The reduction peak currents increased in linear correlation with the scan rate ranging from 30 to 200 mV s^{-1} (see Fig. S2 in the ESI†), which confirmed that the process is controlled by the surface reaction, and the ferrocene-conjugated probes are confined to the electrode surface. In other words, in the presence of L-histidine, signal enhancement is indeed caused by the formation of the ternary "Y" junction structure. The interface properties of the electrode upon different modification were also characterized by electrochemical impedance spectroscopy (see Fig. S3 in the ESI†).

Agarose gel electrophoresis was used to further confirm the L-histidine-dependent catalyzing capability for substrate-hydrolysis of DNAzyme (Fig. 1). For this study, the concentration of the uDNAzyme was fixed at 5 μM with addition of different concentrations of L-histidine (0, 50, 200 and 5000 μM, respectively) or mixed other amino acids. From Fig. 1 one could find that the unimolecular DNAzyme was not cleaved in the absence of L-histidine, or in the presence of mixed other amino acids (lanes 1 and 2). The introduction of L-histidine, however, could trigger the hydrolysis of the uDNAzyme to give two short oligonucleotides with molecular weight smaller than the uDNAzyme itself, and the cleavage ratio is increased with the increasing concentration of L-histidine (lanes 3–5). All these results indicated that the

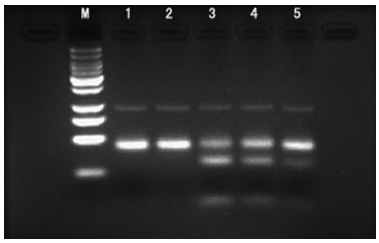

Fig. 1 Agarose gel electrophoresis of the sensing system under different conditions: lane M, DNA marker; lane 1, uDNAzyme only; lane 2, uDNAzyme incubated with 10 mM mixed other amino acids (cysteine/lysine/phenylalanine/arginine) for 0.5 h; lanes 3–5: uDNAzyme incubated with 5 mM, 200 μM and 50 μM L-histidine for 0.5 h, respectively.

DNAzyme does show L-histidine-specific and concentration-dependent catalyzing capability towards the substrate in our designed unimolecular form. Kinetic fluorescence experiment showed that the catalytic cleavage could be finished in 10 min with introduction of 5 mM L-histidine (see Fig. S4 in the ESI†).

Fig. 2A shows the DPV response of the sensor to target L-histidine at different concentrations under optimal experimental conditions. The peak current increased significantly with the increase in target concentration, a signal to background ratio of about 670% was achieved upon triggering with 5 mM L-histidine (see Fig. S5 in the ESI†). Fig. 2B depicts the relationship between the response current and the increasing L-histidine concentration ranging from 1 μM to 5 mM. L-Histidine could be quantified perfectly over a concentration variation from 1 μM to 1 mM, and a detection limit of 300 nM L-histidine could be estimated using the 3σ rule. The low detection limit for L-histidine is comparable to that of a fluorescent DNAzyme biosensor employing an

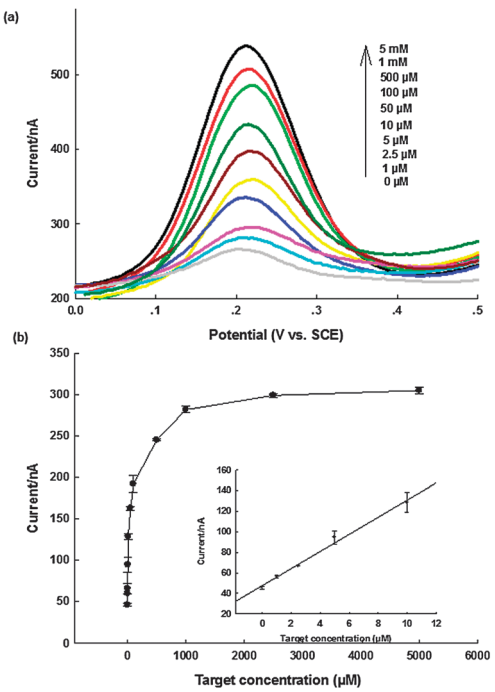

Fig. 2 (a) Typical DPV curves of the sensor in response to L-histidine of varying concentrations; (b) DPV peak currents for different L-histidine concentrations. Inset shows the responses of a sensing system to L-histidine at low concentration. The error bars indicate the standard deviations of three independent experiments.

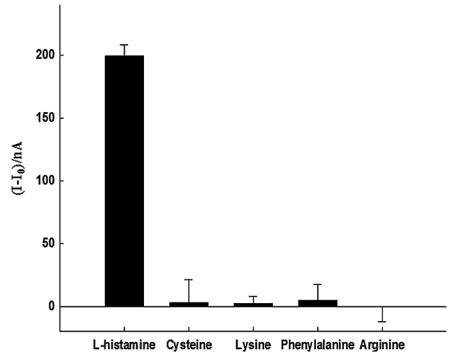

Fig. 3 Specificity investigation: the concentration of L-histidine was 500 μM, while the concentrations of other amino acids were 5 mM, respectively. I and I_0 represented the DPV peak current in the presence and absence of amino acid.

endonuclease-based signal amplified strategy,[21] indicating a high sensitivity of our electrochemical DNAzyme biosensor.

Besides sensitivity, selectivity is another important factor to assess the performance of a sensor. Particularly, for a biosensor with potential application in bio-samples, a highly selective response to the target over other potentially competing species is a necessity. Therefore, the selectivity experiments for the proposed electrochemical biosensor were extended to various amino acids. As shown in Fig. 3, little peak current changes of the biosensor were observed for all other amino acids, even existing at 10-fold higher concentrations than those of L-histidine, only slight current changes were detected. These results indicate that the proposed electrochemical biosensor is highly selective for L-histidine, and has great potential for practical biomedical applications. Reusability is also an important issue of an electrochemical biosensor with potential practical applications. In the present study, the sensing interface could be easily regenerated by immersing the electrode into water at 85 °C for 10 min to remove the surface-adsorbed oligonucleotide molecules. It was found that the sensor could be reused over six times without significant loss of its original current sensing efficiency (see Fig. S6 in the ESI†), indicating good reusability of our biosensor.

To test the practicality of this biosensor in complex biological samples, we carried out detection of L-histidine in cellular homogenate, one of the most challenging media containing a variety of proteins and other potential interferents. Cellular homogenate samples containing different concentrations of L-histidine were first prepared by spiking concentrated L-histidine into the diluted (100-fold) cellular homogenate samples (5000 cells mL^{-1}), and then the peak current changes of the electrochemical biosensor induced by these samples were recorded. It was found that the peak current enhancement obtained in cellular homogenate was similar to that measured in buffered solution, although the signal to background ratio showed a slight decrease (see Fig. S7 in the ESI†). These results revealed that the proposed biosensor was applicable for practical L-histidine detection in real biological samples with other potential interferents co-existing.

In summary, we have developed a "signal-on" electrochemical DNAzyme biosensor for neutral molecules using a PDSH strategy. By combining the highly sensitive PDSH strategy with the improving enzymatic cleavage efficiency *via* performing the enzymatic hydrolysis reaction in a homogenous system, the proposed amplified aptasensor shows a high sensitivity to L-histidine with a detection limit of 300 nM, which is comparable to that of a fluorescent DNAzyme biosensor even employing an endonuclease-based signal amplified strategy. This novel electrochemical biosensor is simple in design and can be easily used by simple mixing and incubation. It could still work well in cellular homogenate, demonstrating its value in the practical applications in biological samples. Since numerous DNAzymes have been selected for various metal ions or neutral molecules, our strategy provides a new general platform for constructing highly sensitive "signal-on" electrochemical biosensors for various targets, and holds promising potential in biomedical applications.

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