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Target-induced diffusivity enhancement for rapid and highly sensitive homogeneous electrochemical detection of BLM in human serum

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ABSTRACT

A simple, rapid, and sensitive homogeneous electrochemical bleomycin (BLM) bioassay has been successfully developed through the target-induced specific/efficient cleavage reaction. The designed probe, denoted as MB-DNA, contains both methylene blue (MB) and target recognizable sequences, and presents relatively low electrochemical signal. Upon the addition of BLM, the recognition/cleavage reaction occurs and leads to the in-situ generation of MB tag (MB-DNA-1), leading to the reduced electrostatic repulsive force. As a result, an obvious enhancement in differential pulse voltammetry (DPV) current is determined, which is relied on the amount of BLM. Thus, a turn on homogeneous electrochemical method for BLM is really achieved, and exhibits high sensitivity of 33 pM, and the shortest response time of 20 min. Furthermore, this electrochemical bioassay presents excellent sensing performance in the analysis of BLM in real samples. Comparing with other sensing strategies for BLM, this proposed electrochemical platform is just consisted of one DNA probe alone, and affords a really rapid and sensitive strategy for BLM analysis.

1. Introduction

The development of simple, rapid, and sensitive biosensors has become a major and indispensable research focus in various fields such as disease diagnosis, cell imaging, food safety, and environmental security [1–3]. In term of this, various biosensing strategies, including colorimetry [4–7], fluorescence [8–10], chemiluminescence [11,12], electrochemiluminescence [13,14], and photoelectrochemistry [15–17], have been successfully applied as ideal tools for target analyses quantitation [18,19]. Among these protocols, the electrochemical techniques have earned them great attentions as powerful tools for targets analyzing in terms of their noticeable advantages of low cost, rapid response, and sensitivity, and have been widely applied in detection of miRNA [20–22], transcription factors [23], organophosphorus pesticide [24,25], and Hg^{2+} [26,27]. In most of the aforementioned electrochemical techniques, the target recognition/transduction/amplification reactions occur on the surface of electrodes, and the performance of the proposed biosensor relies on the surface immobilization of biosensors. Nevertheless, an inappropriate method to immobilize biorecognition molecules on the electrode surface can result in inaccurate/no signal response, subsequently leading to the false diagnosis result. Accordingly, the immobilization procedure is expensive and complicated, and it could change the geometry of the probes and reduce the configurational freedom because of the electrode's strong steric hindrance, subsequently resulting in the weak recognition efficiency toward targets. Thus, it is of theoretic importance for researchers to construct an immobilization-free electrochemical platform for the target analyte biosensing.

Recently, a large number of electrochemical platforms without immobilization procedures (homogeneous) have been constructed to probe various targets [28–36], such as pesticide, enzyme activity, and miRNA. For example, Li's group [35] reported a facile and enzyme-free strategy for the miRNA analysis based on this electrochemical method. Hsing's group [36] reported an exonuclease III-assisted amplification strategy for Hg^{2+} analysis. Compared with immobilized (heterogeneous) electrochemical platform [37,38], the homogeneous electrochemical strategies enjoy the merits of low cost, rapidness, and high sensitivity. In addition, these reported biosensors can be simply designed and developed by integrating the electrode, electroactive agent, and target analyte in the same system [29,33,34,36]. These unique properties urge it to be ideal alternative to the heterogeneous electrochemical method for various targets detection.

Bleomycin (BLM) has been intensively applied as chemotherapeutic agent for the clinical treatment of malignant lymphomas and cell carcinomas due to its advantages of low immunosuppression and myelosuppression [39,40]. However, it is reported that BLM possesses

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significant dose-limiting side effect, and unsuitable dose of BLM will cause serious renal and lung toxicity. In hopes of cancer therapy and physical security, it is of great anxiety to develop elaborately strategies for accurate diagnosis of trace level BLM in biological systems. Up to date, substantial efforts have been made to develop practicable biosensors for BLM assay, such as fluorescence [41], electrochemistry [42], and surface plasmon resonance (SPR) [18]. Among them, electrochemical biosensor has appeared as the best alternative for BLM assay. For instance, Ye's group [42] has reported a pioneering strategy for electrochemical determination of BLM via the target-induced specific cleavage reaction. Though spectacular development has been done, the fateful obstacles in these strategies are the immobilization of DNA probes, the simultaneous use of multiple DNA probes and several kinds of enzymes in one system, which usually brings about rather tedious operation procedures, increased cost, and low recognition efficiency.

Herein, we develop a simple, rapid, and sensitive homogeneous electrochemical bioassay for BLM based on the target-triggered specific cleaving reaction. In the present study, there is just one DNA probe involved in the proposed platform, offering an absolutely simple and rapid strategy for BLM detection. To realize the as-proposed strategy, the DNA probe (MB-DNA) labeled with methylene blue (MB) is ingeniously designed, and does not enjoy the ability to intensify the differential pulse voltammetry (DPV) signal for the strong repulsive force between MB-DNA and ITO electrode. Upon the addition of BLM, probe MB-DNA is effectively cut off to release abundant MB tag (MB-DNA-1) under the oxidative transformation process, and then the cleavage fragments enjoy much stronger diffusivity than that of MB-DNA because of the smaller size and less negative charge. Consequently, the significant increase in DPV current is observed and determined by the amount of BLM in the sensing system. Therefore, an ingenious strategy for the analysis of BLM is triumphantly developed based on the target-induced diffusivity enhancement.

2. Experimental

2.1. Reagents and chemicals

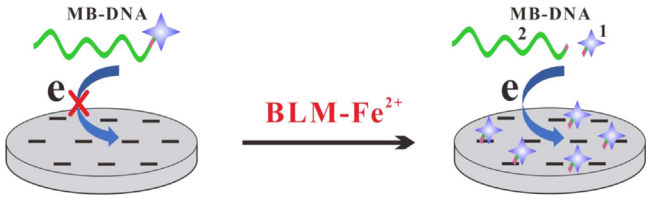
All DNA sequences including MB-DNA and P1 (listed in Table S1) were prepared and HPLC-purified by Sangon Biotech Company. Ltd. (Shanghai, China), and diluted in 20 mM PBS buffer (pH 8.0) to give the solutions of 50 μM. BLM was bought from Melong Pharmaceutical Company. Ltd. (Dalian, China) with the molecular structures exhibited in Fig. S1, and consisted of A2 and B2, the contents of which were up to 91.6%. Tryptophan, lysine, cysteine, L-threonine, ascorbate, urea, glucose, dactinomycin, daunorubicin, and mitomycin were obtained for the selectivity investigation from Sigma-Aldrich (St. Louis, MO). $MgCl_2$, $CaCl_2$, $FeCl_2$, KCl, $NaClO_4$, and other chemicals were all obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used directly. All aqueous solutions were diluted with ultrapure water (18.2 MΩ/cm, Milli-Q, Millipore).

2.2. Apparatus

Different pulse voltammetric (DPV) experiments were conducted on an Autolab electrochemical workstation (Metrohm, Netherland) with a conventional three electrode system: ITO working electrode, Ag/AgCl reference electrode, and platinum wire counter electrode. Fluorescence spectra were measured from 645 to 785 nm on an F-4600 spectrometer (Hitachi, Japan). The excitation wavelength was operated at 610 nm, and the excitation and emission slit widths were set at 10.0 nm and 10.0 nm, respectively.

2.3. Homogeneous electrochemical determination of BLM

The assay was performed in 100 μL of PBS buffer containing 1.0 μM MB-DNA and different concentrations of BLM samples for 20 min. After the cleavage reaction, DPV measurements were applied to investigate the electrochemical signal response with the potential at − 0.25 V.



Scheme 1. The principle of this homogeneous electrochemical biosensor for BLM on the basis of target-induced specific cleavage reaction.

2.4. Fluorescence detection of BLM

2.0 μM MB-DNA and 20 μg/mL GO were incubated in 50 μL of PBS buffer for 30 min to quench the fluorescence of MB-DNA. After the above operation, different amount of BLM samples was added. The solution further reacted for 20 min, and was diluted to 100 μL before fluorescence measurement.

3. Results and discussion

3.1. Design principle of the electrochemical biosensing strategy for BLM

The working principle for BLM assay is outlined in Scheme 1, and the sensing strategy is ascribed to the different diffusivity between MB-DNA and target-induced cleavage products (MB-DNA-1) toward ITO electrode. The DNA probe contains the target recognition/cleavage sequences (5′-GCT-3′) toward BLM-Fe^{2+} sample, and is labeled with MB at its 5′ terminus. It is reported that BLM-Fe^{2+} reacts with O_2 to form activated BLM (BLM-Fe^{3+}-OOH), which is capable of abstracting C4′ hydrogen atom from deoxyribose, subsequently inducing the strand scission at 5′-G/CT-3′. When BLM is not present, MB-DNA could not be recognized/cleaved, and still maintain its high negative charge density, subsequently leading to strong repulsive force between MB-DNA and ITO electrode. Owing to the diffusion effect, which is dependent on the repulsive force, weak electrochemical signal is observed for the MB-DNA probe. Nevertheless, upon the addition of BLM, owing to the specific/efficient cleavage reaction of MB-DNA by BLM, a large number of two-mer MB-lined oligonucleotide fragments (MB-DNA-1) are released, of which negatively charge density remarkably reduce compared with that of MB-DNA. As depicted in Fig. S2, the newly appeared band (lane b) was ascribed to the in-situ generation of MB-DNA-2, which successfully confirms the formation of MB-DNA-1. Thus, a distinct electrochemical signal enhancement is readily acquired, and relevant to BLM concentration, since the generation of MB-DNA-1 is related to the concentration of target.

3.2. Feasibility of the electrochemical biosensor for BLM

DPV measurements were applied to characterize the feasibility of this probing strategy under various conditions. The sensing results were manifested in Fig. 1A. It can be clear seen that DPV currents were acquired with the potential of − 0.25 V, which was ascribed to the electrochemical signal of MB. When BLM was absent, MB-DNA was not cut off, and still enjoyed the high negatively charge density, subsequently causing strong repulsive force toward ITO electrode and thus a relatively low electrochemical signal of about 5.19 nA (curve a). Nevertheless, upon the addition of BLM-Fe^{2+} complex, effective cleavage of MB-DNA occurred, and thus abundant MB-DNA-1 fragments generated. Owing to the smaller size and less negative charge density of MB-DNA-1 than that of MB-DNA, repulsive force evidently decreased, accompanied with the diffusivity enhancement. As a consequence, the DPV current increased significantly from 5.19 to 30.48 nA in the presence of 1 nM