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A novel label-free electrochemical miRNA biosensor using methylene blue as redox indicator: Application to breast cancer biomarker miRNA-21

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Abstract

Small noncoding microRNAs (miRNAs) have emerged as ideal noninvasive biomarkers for early-phase cancer detection. In this report, a label-free and simple electrochemical miRNA biosensor is developed based on employing methylene blue (MB) as a redox indicator. The successfully immobilization of the single strand DNA (ss-DNA) probe and hybridization with the target miRNA sequence were confirmed by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) methods. Differential pulse voltammetry (DPV) technique was used to record the oxidation peak current of MB under optimal condition and an increase in the peak current was observed after hybridization. By employing this strategy, miRNA can detect in a range from 0.1 to 500.0 pM with a relatively low detection limit of 84.3 fM. The electrochemical response of MB on ss-DNA and duplex of miRNA/DNA was characterized by CV and chronocoulometry method. The linear relation between the redox peak currents (I_p) and scan rate (v) indicates that the electron transfer (ET) between MB and the electrode surface was mediated by the miRNA/DNA π -stacked duplex. The value of surface coverage (Γ) was calculated that indicated increase amount of MB on the surface of modified electrode after hybridization event and revealed the adsorption of MB at modified electrode is monolayer. Also, the electron transfer rate constants (k_s) of MB were estimated. The results of kinetic analysis were confirmed by chronocoulometry method. The discrimination ability of miRNA biosensor even against a noncomplementary target was also studied. Consequently, this strategy will be valuable for sensitive, selective and label-free detection of miRNA.

Keywords: MicroRNA-21, Cancer detection, Electrochemical biosensor, Methylene blue, Label-free

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