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Electrochemical measurement of phenothiazine-interacted DNA

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Abstract

The amount of DNA was measured by using thioridazine, which would be attached to the DNA, as an electrochemical indicator. An indicator (thioridazine) solution, a test solution (DNA solution), and a poly-L-lysine solution were successively placed on a glassy carbon electrode, and the electrode was allowed to dry; DNA was immobilized on an electrode surface by the electrostatic binding between DNA and poly-L-lysine. The electrode was immersed into a buffer solution for 15 min, and then differential pulse voltammetry (DPV) was carried out: the oxidation current peak of thioridazine was observed, and its magnitude depended on the amount of DNA in the solution which was used for preparing the electrode. It could be estimated between 0.2 μg DNA (corresponds to 630 pmol nucleotides) to 20 μg DNA (63 nmol nucleotides) from the oxidation peak current of DPV.

Keywords: Thioridazine; DNA; Polyion complex; Poly-L-lysine; Electrochemical measurement

1. Introduction

Much attention has been paid for the preparation of biosensing devices by immobilizing biomolecules through the electrostatic interaction between polycationic and polyanionic molecules [1–8]. We have reported several methods for immobilizing enzymes onto the electrode with the polycation–polyanion interaction [1–3,8]. DNA, another important biomolecule, can easily be immobilized with polycation [7]; polyanaionic DNA and polycation will make a water-insoluble polyion complex membrane.

Recently there has been growing interests for detecting DNA electrochemically [9–12]. Many electrochemical approaches have been developed for the detection of DNA [13–18]. In these researches, stripping analysis or direct oxidation of DNA could open the ways of DNA determination with simply and easily [16–18]. However, cathodic stripping method used mercury electrode [16], and the oxidation current of DNA was observed at relatively oxidative potential in the other methods [17,18]; in such a high potential, electroactive species in the samples would be oxidized to cause an error. To avoid the problem, we adopt electroactive compounds, phenothiazine derivatives as an electrochemical indicator at low oxidative potential; phenothiazines are known as the compounds which interact with DNA [13]. In this paper, we prepared a polycation-DNA membrane with a phenothiazine compound, thioridazine, on a glassy carbon electrode, and the electrode system was applied to the determination of the amount of DNA attached on the surface.

2. Experimental

Preparation of polycation-DNA membrane with thioridazine on a glassy carbon electrode.

Certain concentration of DNA (Salmon testes; Sigma, St. Louis, MO, USA) solution (20 μl), thioridazine solution (Sigma, 1 mM, 20 μl) and poly-L-lysine solution (Average MW, 70,000, Sigma, 0.5 mg/ml, 20 μl) were successively placed on a glassy carbon electrode (3 mm in diameter, Bioanalytical Systems, West Lafayette, IN, USA). Then the electrode was allowed to dry for 4 h. The electrode was rinsed with water and the electrochemical measurement was performed.

3. Electrochemical measurement

The electrode was immersed into a 0.1 M phosphate buffer (pH 5.0) solution (15 ml). Platinum wire and Ag/AgCl electrodes were used as an auxiliary and reference

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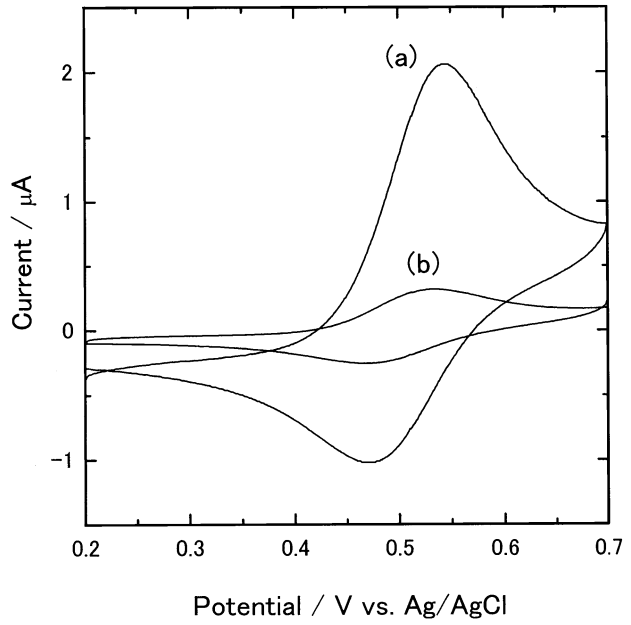

Fig. 1. Cyclic voltammograms (CV) on the electrode, which immobilized thioridazine-DNA. For preparing the electrode, 1 mg/ml DNA (20 μl), 1 mM thioridazine (20 μl) and 0.5 mg/ml poly-L-lysine (20 μl) were used. Scan rate of CV was 10 mV/s. (a) CV recorded immediately after immersing the electrode into the buffer solution. (b) CV recorded 10 min after soaking into the buffer solution.

electrodes. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed by electrochemical analyzer (Bioanalytical Systems, Model 100-B). For DPV, the electrode was immersed into a buffer solution for 15 min before the measurement, and following parameters were used for DPV measurement; pulse amplitude was 50 mV, pulse interval, 1 s, and pulse width, 50 ms.

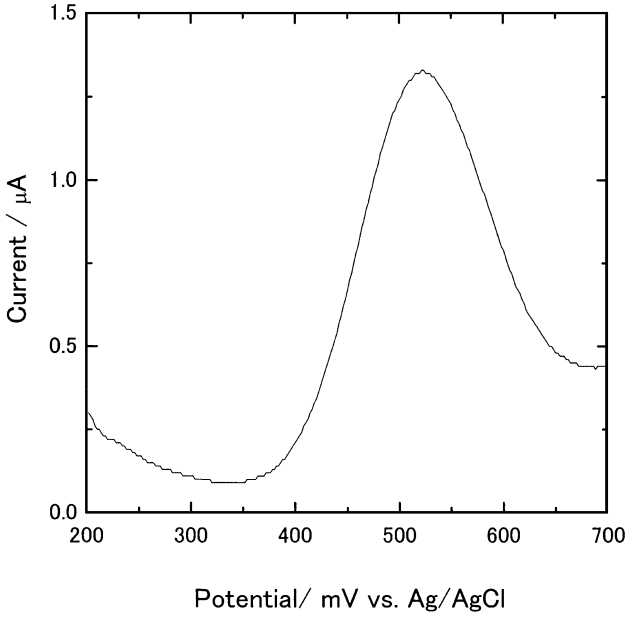

Fig. 2. Oxidative direction of the differential pulse voltammograms (DPV) on the electrode, which immobilized thioridazine-DNA. For preparing the electrode, 1 mg/ml DNA (20 μl), 1 mM thioridazine (20 μl) and 0.5 mg/ml poly-L-lysine (20 μl) were used. DPV was measured after soaking the electrode into the buffer solution.

4. Results and discussion

Phenotiazine compounds are used in this research because it is known that the interaction of the compounds to DNA occur in the short time [15]. Then the CV characteristics of a series of phenothiazine compounds (e.g., thioridazine, promethazine and chlorpromethazine) were examined, and thioridazine showed the highest reversibility in CVs (data not shown). Hence thioridazine has been selected to use for the following experiments.

The thioridazine solution was mixed with DNA and poly-L-lysine on the glassy carbon electrode to form thioridazine-interacted DNA (thioridazine-DNA), and the electrode was allowed to dry. After drying, the electrode was immersed into a buffer solution, and the electrochemical measurement was performed. Fig. 1a shows the CV on the electrode (0.5 mg/ml DNA solution was used for preparation) recorded immediately after immersing the electrode into the buffer solution. Oxidation and reduction current peaks were observed clearly. The current peak heights were decreased by immersing the electrode into the buffer for 10 min and then reached to give steady-state values (Fig. 1b). These phenomena, i.e., the redox currents decreasing and reaching to be the steady-state value, were observed for electrodes prepared by using different amount of DNA. Moreover the redox peak heights depended on the amount of DNA used; lager peak heights were obtained when a larger amount of DNA was employed. The above results suggested that the redox current in Fig. 1b

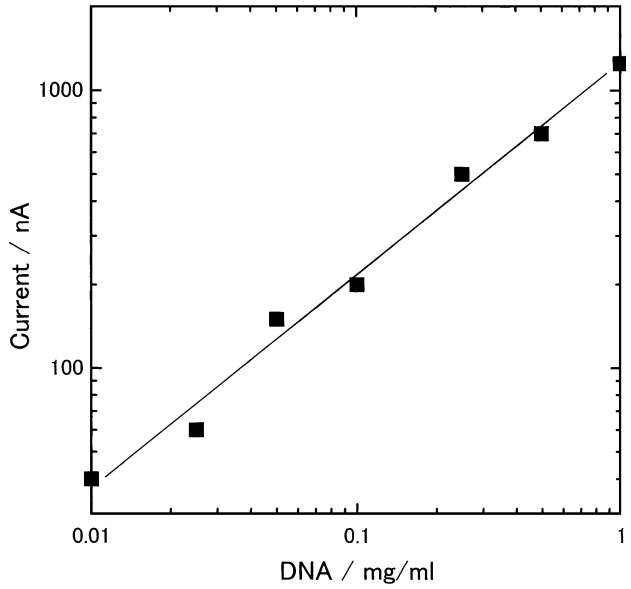

Fig. 3. Dependence of the oxidation peak current in DPV on the amount of DNA used for immobilization. For immobilizing 20 μl of DNA, 1 mM thioridazine (20 μl) and 0.5 mg/ml poly-L-lysine (20 μl) were used.

would be caused by the interacted thioridazine. After the preparation of the polyion complex-based electrode, a part of thioridazine molecules forms DNA complex and the excess thioridazine is physically attached near the electrode surface. The thioridazine-DNA complex would be water-insoluble, since the DNA complex forms a water insoluble polyion complex with poly-L-lysine. Hence the steady-state electrode response depended on the amount of DNA. Previous report also showed that interacted phenothiazine compounds were electroactive, and the magnitude of the current was dependent to the amount of interacted phenothiazine [15]. The amount of DNA would be estimated from the redox current.

For the precise determination of thioridazine-DNA on the glassy carbon electrode, the DPV measurement was carried out after immersing the electrode into a 0.1 M phosphate buffer (pH 5.0) solution for 15 min, which was enough to remove the excess (uninteracted) thioridazine from the electrode surface. As the sensitive and selective electrochemical measurement, DPV was employed. Fig. 2 shows typical voltammogram of DPV on the electrode (oxidative direction of DPV was measured because the oxidation current was larger than the reduction one). A peak for the oxidation of interacted thioridazine was observed on the voltammogram. The peak current for the oxidation was plotted against the amount of DNA used (Fig. 3). The height of the peak current increased with the increasing in the amount of DNA used for preparing the electrode, as expected. Under the present measurement condition (20 μl sample DNA solution was used for immobilizing), DNA in the range from 0.2 μg DNA (corresponds to 0.01 mg/ml DNA; ~ 630 pmol nucleotides) to 20 μg DNA (1 mg/ml DNA; ~ 63 nmol nucleotides) can be determined from the DPV measurement. Adhesion of the polycation-DNA membrane is influenced by the surface condition; suitable pretreatment of the electrode surface would improve the sensitivity of the DNA determination. Studies for increasing the sensitivity are now in progress.

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