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Mg^{2+}-Selective Electrode Comprising Double-Helical DNA as Receptive Entity

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Cyclic voltammograms of ferrocyanide/ferricyanide redox couple with a DNA-immobilized electrode gave the peak currents due to the reversible electrode reaction, which were significantly enhanced on adding Mg^{2+}. The electrode responded also to Ca^{2+} and Ba^{2+}, although the onset concentrations of the electrode response were 50-times larger than that for Mg^{2+}. The selectivity in the order of $Mg^{2+} > Ca^{2+}$, $Ba^{2+} >> Na^{+}$, K^{+} seems consistent with the binding affinity of the metal ions with double-helical DNA.

Among all the chemical sensors including electrochemical sensors and optical sensors, only few proper to Mg^{2+} have been developed.[1,2] This is due to limited availability of host compounds, either synthetic or naturally occurring, that can be bound to Mg^{2+} preferentially to other alkali and alkaline earth metal ions. On the other hand, Mg^{2+} complexes with nucleotides and nucleic acids have attracted much attention in relation to their unique roles in biological processes.[3] We describe a Mg^{2+}-selective voltammetric response of a Au electrode on which double-helical DNA is immobilized.

DNA double strands were immobilized on a Au electrode via chemisorption, *i.e.*, a coordination to Au with a sulfur-containing moiety.[4] Sonicated calf thymus DNA (30-300 bp, 10 mg) was reacted with 2-hydroxyethyl disulfide (4.6 mg) in the presence of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (Aldrich, 0.4 g) in 0.4 cm^3 of 0.04 M MES buffer (pH 6.0, M = mol dm^{-3}) for 24 h at 25 °C. The modified DNA at the terminal(s) with disulfide group(s) was purified by gel filtration using NAP-10 column (Pharmacia). In a solution of the disulfide-modified DNA (0.3 mM in base-pair concentration), a polished Au disk electrode (1.6 mm diameter, Bioanalytical Systems) was immersed for 24 h at 5 °C. The modified electrode was then washed with and stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 7.2) at 5 °C before and between uses.

Cyclic voltammetric (CV) measurements were performed similarly to the previous papers[4,5] by using a Solartron Co. Model 1286 potentiostat with a conventional design of a three-electrodes system. A Pt plate (10 × 10 mm) and a standard Ag/AgCl (saturated KCl) electrode were used as counter and reference electrodes, respectively.

Cyclic voltammograms of ferrocyanide/ferricyanide redox couple with a bare Au electrode and the modified one are shown in Fig. 1. The peak currents due to the reversible electrode reaction were significantly suppressed on the DNA-immobilized electrode as compared with the bare one, probably due to the electrostatic repulsion between the anionic redox couple ions and the immobilized layer of polyanionic DNA (Fig. 2). On

adding Mg^{2+} (as $MgCl_2$), however, the peak currents increased with increasing concentration of the ion. As illustrated in Fig. 2, the ionic binding of Mg^{2+} with the immobilized DNA should reduce the negative charge on the electrode so that the redox couple can make an electrochemical communication more effectively with the underlying Au metal. This type of voltammetric sensing, in which a redox active indicator is employed to detect a change in the net charge of a modifying layer, was first proposed by Sugawara *et al.*[6] The method was successfully applied to the present DNA-modified electrode.

Figure 3 shows the relationship between the increment of the cathodic peak current (i_{pc}) and the concentration of Mg^{2+}. The DNA-immobilized electrode thus showed highly-sensitive response towards Mg^{2+} in the concentration around 10^{-6} M. The different preparation of the modified electrode gave a difference in terms of the absolute value of i_{pc} change, but the Mg^{2+} concentration range for response remained the same (see Fig. 3, ○ and ●). For the same electrode, repeated use (3 times) within a week gave reproducible responses.

The DNA-immobilized electrode was found to be responsive also towards Ca^{2+} and Ba^{2+} as seen in Fig. 3, although the onset concentrations of the electrode response were ca. 10^{-5} M, 50-times larger than that for Mg^{2+}. As for Na^+, such a concentration-dependent change was not observed at the range of 10^{-7} — 10^{-3} M. In fact, all the CV measurements in this work were made in the presence of 10^{-2} M of KCl as a supporting electrolyte. The selectivity in the order of $Mg^{2+} > Ca^{2+}$, $Ba^{2+} >> Na^+$, K^+ seems consistent with the binding affinity of the metal ions with double-helical DNA.[7] These results suggest that the electrode responses were due to the specific interaction of the immobilized DNA with alkaline earth metal ions, among which Mg^{2+} was most strongly bound to DNA to give a discriminating response.

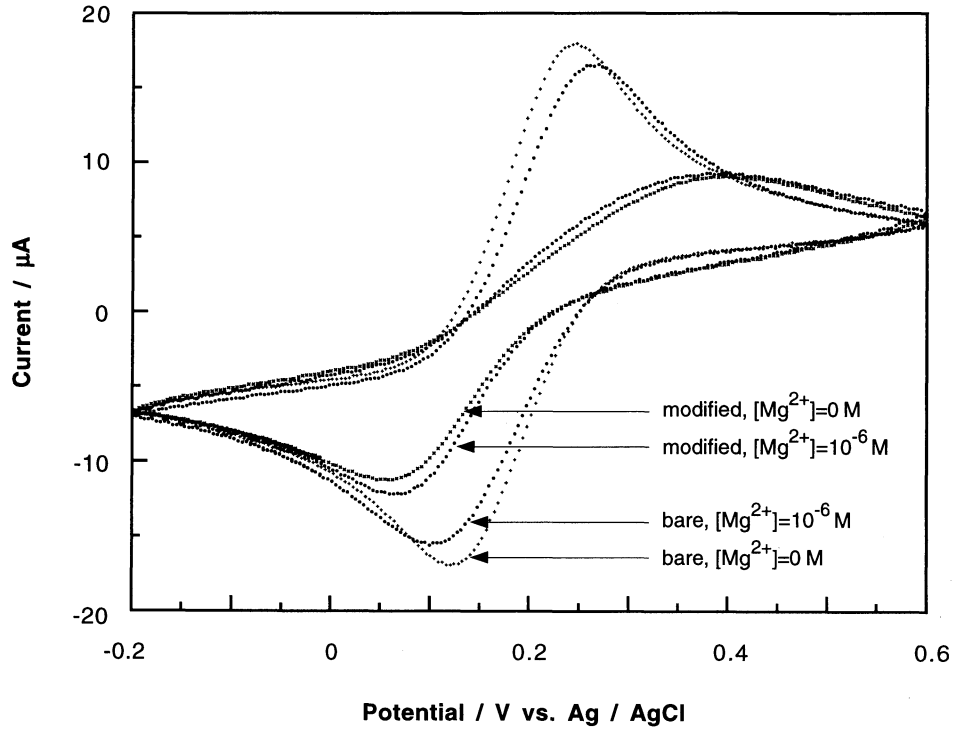

Fig. 1. The Mg^{2+}-dependent changes in cyclic voltammograms of the bare and the DNA-immobilized Au electrodes at 25 °C. Scan rate, 25 mV s^{-1}; $[K_4[Fe(CN)_6]] = [K_3[Fe(CN)_6]] = 5$ mM, [KCl] = 0.01 M.

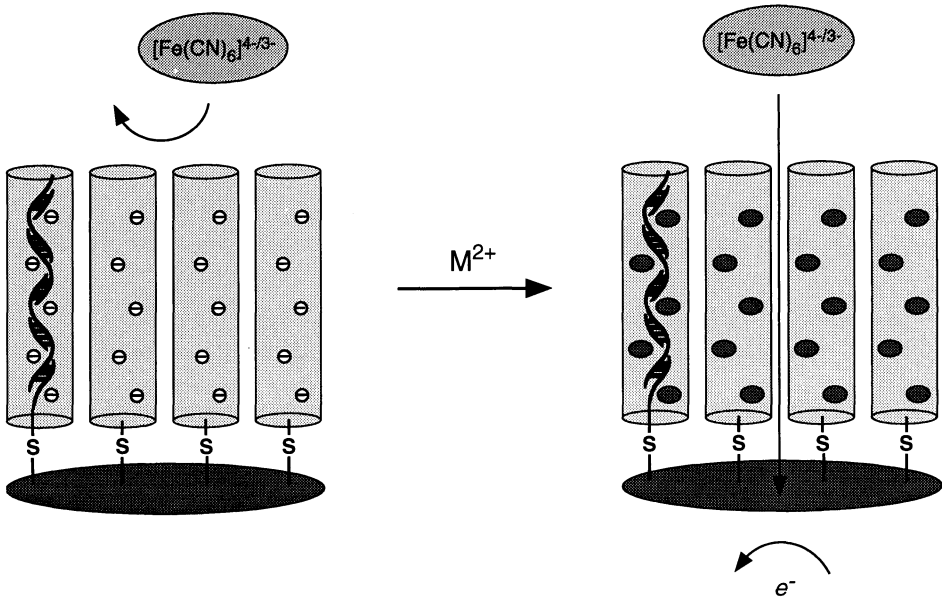

Fig. 2. Schematic illustration of the Au electrode modified with double-stranded DNA and its response to Mg^{2+} in the presence of redox active species.

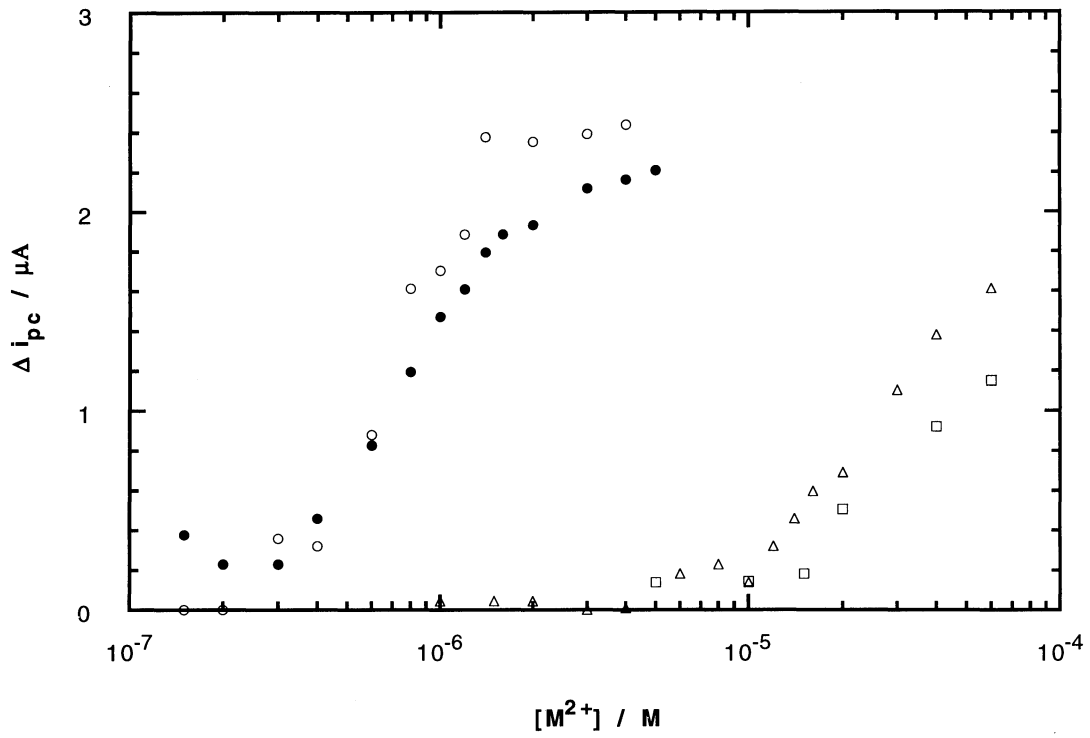

Fig. 3. The metal ion-dependent changes in the cathodic peak current (i_{pc}) on the DNA-immobilized Au electrode. Exprimental conditions are the same as those in Fig. 1. ○ and ●, Mg^{2+} (for two different electrodes); △, Ca^{2+}; □, Ba^{2+}. For open symbols, the same electrode was used.

It should be noted that Mg^{2+} and Ca^{2+} induced an appreciable change in CV profiles of ferrocyanide/ferricyanide for a bare Au electrode (see Fig. 1 for Mg^{2+}). The reason for this is not clear at present. An interaction between the metal ions and the redox couple in solution does not account for this since the effect was seen when as little as 10^{-6} M of Mg^{2+} was added to 10 mM (10^4-times excess) of the redox couple. Another explanation may be that a complex of Mg^{2+} with some surface Au oxide (or chloride) species on the Au electrode prevents access of the redox couple for electron transfer. In any event, the change was not negligible (decrease of 2.6 and 2.0 μA in i_{pc} for 10^{-6} M of Mg^{2+} and Ca^{2+}, respectively), but was opposite in direction to the effect of these ions observed at the DNA-immobilized electrode. Thus, it is possible that the increment of i_{pc} presented in the ordinate of Fig. 2 have been offset or reduced in part by this DNA-independent effect of the alkaline earth metal ions.

DNA biosensors have been developed in recent years.[8-12] Most of them were designed to detect nucleic acids. For the purpose, single-stranded DNA having a sequence complementary to the target was immobilized on the electrochemical[8,9] or piezoelectric[10,11] transducers. In contrast, our interest has concentrated on taking advantage of double-helical DNA in order to develop a sensor for DNA-binding substances such as metal ions, drugs and proteins.[12] A similar approach was employed for the detection of intercalative dye molecules by using a cast film from a 1:1 DNA-lipid complex formed on a quartz-crystal microbalance.[13] However, the immobilized DNA by such a procedure does not seem a good host for DNA-binding ions and proteins, since the DNA phosphate groups are hindered by lipid molecules through the polyion complex formation. In the present work, the sensor comprises a double-helical DNA in its intact form and was successfully demonstrated to be applicable for Mg^{2+}-selective ion sensing on the basis of some unique interaction of biological importance.

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