Bioaffinity Sensor to Anti-DNA Antibodies Using DNA Modified Au Electrode

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Calf thymus DNA was modified on Au electrode as chemoreceptive ligand. This sensor responded selectively to anti-DNA antibody which recognizes DNA molecule. This sensing system is potentially worthy for the development of biosensor of DNA binding proteins.

Recently, electrochemical assay of DNA and related proteins are one of the most attractive targets for current biological analysis with dramatic progression. In fact, many types of DNA modified electrode have been reported as an electrochemical approach to molecular biology.[1] However, such sensors typically possess single stranded DNA and direct toward the detection of target DNA that has complementary sequence to the immobilized DNA on the electrodes.[2] On the other hand, DNA molecule can be regarded as ligand to DNA binding molecules. We previously reported gold electrode which immobilized double stranded DNA for the detection of DNA binding small molecules including intercalators.[3] In this paper, we tried extending this approach to DNA binding protein assay using anti-DNA antibody as a target. Anti-DNA antibody has been used as marker molecule of Systemic Lupus Erythematosus (SLE) which is a severe autoimmune disease.[4] Thus, the electrochemical detection method reported here is interesting from the standpoint of diagnosis of SLE.

We used double stranded calf thymus DNA (CT-DNA) as an antigen after the DNA was sonicated and dialyzed. Disulfied was introduced at 5' end of the DNA as phosphate ester with 2 hydroxyethyl -disulfide using 1-cyclohexyl-3-morpholinoethyl-carbodiimide according to the method described by Rickwood.[5] The disulfide introduction to DNA was evaluated as the concentration of sulfhydryl moiety in a certain amount of DNA using dithiothreitol (DTT) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB).[6] Disulfide introduction was thus estimated to be 68.4% (strand basis) assuming the mean DNA size of 500 base pair which was determined by gel electrophoresis. Then the disulfide-introduced CT-DNA was immobilized on Au electrode with chemisorption as described before.[3] A polished Au disk electrode (1.6 mm diameter, Bioanalytical Systems) was immersed in disulfide modified CT-DNA (50 mM in base pair concentration) 50 mM Tris-HCl (pH 7.4, containing 50 mM KCl) solution for 24 h at 5℃. Cyclic voltammetric measurements were performed in a similar manner to that reported previously by using a Bioanalytical System Co. Model CV-50W potentiostat.[2] A Pt plate (10 x 10 mm) and a standard Ag/AgCl (saturated KCl) electrode were used as counter and reference electrodes, respectively.

Cyclic voltammetric measurements showed that the reversible electrode reaction of ferrocyanide/ferricyanide redox couple was suppressed to some extent by the treatment with the modified CT-DNA (Figure 1, a → b). This is due to the electrostatic repulsion between anionic charges of the immobilized DNA and redox marker ions causing the decrease of the concentration of the marker ions near the electrode surface as discussed previously.[3]

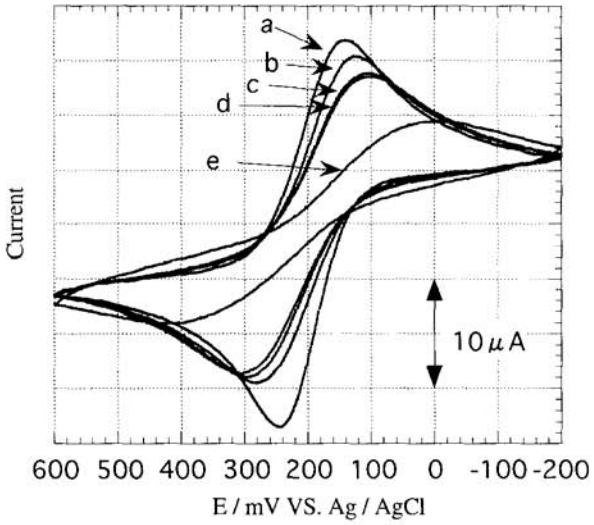

Figure 1. Cyclic voltammograms of Au electrode modified with CT-DNA in the presence of IgM antibodies at 25℃. Scan rate, 25mVs^{-1}; [K_4[Fe(CN)$_6$]]= [K_3[Fe(CN)$_6$]] = 10 mM, [KCl] = 100 mM a) bare electrode, b) electrode modified with CT-DNA, c) after treatment with 2-mercaptoethanol in b in order for blocking nonspecific adsorption of proteins, d) in the presence of anti-mouse IgM antibody (200 nM) in c, e) in the presence of anti-DNA IgM (200 nM) in c.

Figure 1 showed the electrochemical responses of anti-mouse IgM and anti-DNA IgM. Addition of anti-mouse IgM, which does not interact with DNA, did not affect the redox current (Figure 1, c → d). In contrast to this, anti-DNA antibody, which binds to both single and double stranded DNA unselectively, suppressed the redox currents of marker ions dramatically (Figure 1, c → e). These results suggest that the anchored DNA successfully acts as receptive element for anti-DNA antibody. Furthermore, decrease of the anodic redox peak currents (pia) with anti-DNA IgM was in concentration dependent manner (Figure 2). This is most likely caused by a steric hindrance of the huge protein (anti-DNA IgM), which binds to DNA strands on the electrode surface, to mainly reduce an effective area of the electrode. Electrostatic repulsive effect may also contribute to the phenomenon by blocking the electrochemical reaction of the anionic marker ions, since isoelectric point of IgM (mouse) is in the range of 4.5 to 7.0. Peak separation tends to increase with increasing concentration of the antibody, indicating that binding of the protein to the DNA diminished the electrochemical reaction rate of the marker ions. Using this electrode, anti-DNA IgM can be determined with the detection limit of 1 nM.

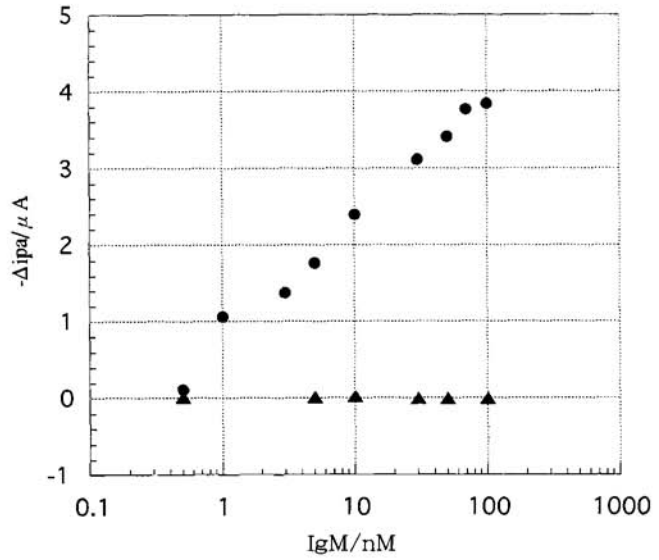

Figure 2. Effect of antibodies (IgM) on redox peak currents of ferrocyanide/ferricyanide couple on DNA modified Au electrode at 25 °C. ●, anti-DNA antibody, ▲, anti-mouse antibody. Experimental conditions are the same as those in Figure 1.

In all the experiments, the electrode surface was treated with 2-mercaptoethanol after the modification with DNA (Figure 1, b → c). If an Au electrode simply modified with CT-DNA was used, other serum proteins (e.g. anti-mouse IgM, anti-mouse IgG and bovine serum albumin), which would not interact with DNA, decreased the redox currents in a similar manner (data not shown). We tried suppressing the nonspecific response by covering the electrode surface with a blocking agent and finally found a 2-mercaptoethanol as the best one to reduce the adsorption of proteins on the electrode. After this blocking procedure, an addition of anti-mouse IgM, which does not recognize DNA, did not affect the reversible redox reaction of marker ions. This means that nonspecific adsorption of IgM proteins on the electrode is prevented with the blocking step.

Here we reported the possible electrochemical detection of anti-DNA IgM using CT-DNA as chemoreceptive component although further examinations should be needed prior to the practical use, e.g. more quantitative treatments on DNA immobilization or protein binding. For the electrochemical detection of anti-DNA antibody, there have been a few reports.[7] The methods were performed on the concept of enzyme immunoassay (EIA), which is a common way of biological molecules detection. However, EIA requires another enzyme which makes the system complicated and time consuming. The method reported here used much simpler system which relies on the concept of ion channel sensor.[8] This concept may be extended to other DNA-binding protein analyses, if the immobilized DNA has a specific sequence to the target protein.

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