DNA-Modified Electrode: Electrode Modification with DNA/Redox-Molecule Bilayer Structure and Ion Sensor Applications for Class A Metals

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Metal ions play a central role in many biological processes, *e.g.*, the ionic homeostasis and the intercellular signalling of cells. This also applies to the biosynthesis of nucleic acids; all enzyme-catalyzed reactions with 5'-ATP, including those involving DNA and RNA polymerases, need divalent ions. We have been investigating a biosensor which comprises double-stranded (ds) DNAs as molecular recognition elements. As one of the key technologies, we have developed a new immobilization method for ds DNAs which is taking advantage of self-assembling chemistry by an organosulfur functional group on gold surfaces. By adopting the redox-molecule mediated, artificial ion-channel principle, we have successfully developed an electrochemical sensor for DNA-binding molecules and ions.

That is, ds DNAs which are immobilized on an gold electrode surface block the access of the redox-active ion (marker ion) to the vicinity of the electrode surface while the affinity reaction between the ds DNA and the DNA-binding substrate defuses that effect leading to the enhancement of current density of the electrode reaction of the marker ion. The sensory principle used here is featured by a high sensitivity of detection, however, it seems to be too sophisticated in practical uses. In the present study, we have developed a new immobilization method which enables the indicator-free detection of the molecular recognition event.

First, the gold electrode surface was modified by the self-assembling monolayer (SAM) form 11-mercaptoaminoundecane, then, 1,1'-ferrocenedicarboxylic acid (Fc) was reacted with the amino groups in the SAM in the presence of water-soluble, carbodiimide reagent. Finally, oligonucleotide (dT12) with 5'-amino function was immobilized by the coupling reaction with the carboxy group of Fc. The present immobilization chemistry was characterized by *ir* spectroscopy and the formation of DNA/Fc surface structure was confirmed. Electrochemical characterization of the modified electrodes were then made and we found that the redox activity of the modified electrode with DNA/Fc structure was reduced to *ca.* 50 % by the attachment of DNA. When we treated the DNA-modified electrode with the complementary strand, the redox activity was almost diminished. We think this observation is important from the viewpoint of gene sensor applications.

We have also started to explore biosensor applications of the modified electrode system. We studied the electrode response to anti-DNA antibody and found that the redox currents reappeared in the presence of the antibody. The peak currents were increased with the increasing concentration of the antibody in the range of 1 nM — 10 nM. The anti-DNA antibody is well-known to be a marker molecule for systemic Lupus Erythematosus (SLE) which is a severe autoimmune disease. Thus, the present system is important from the standpoint of diagnosis of SLE.