초청강연1

Development of a Detection System Based on Electrochemiluminescence and Magnetic Microbeads

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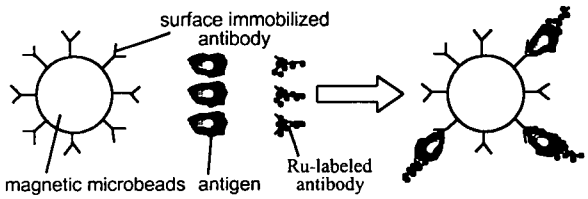
In the last two decades, various new analytical techniques have been applied to clinical and biomedical analysis in seeking for higher sensitivity and simplicity of diagnosis. Recently, in immunoassay, a technique based on luminescent labels has attracted much attention because of its high sensitivity. Among luminescence immunoassays, electrochemiluminescence(ECL)-based immunoassay (ECLIA) has several advantages: First of all, since an ECL reaction occurs through a redox reaction at electrode surface, detection of an analyte based on ECL is readily controlled through potential or current application to electrodes. In addition, by positioning a detector just in front of an electrode where ECL reactions take place, photons form ECL can be collected efficiently, leading to higher sensitivity. In this study, we develop a highly sensitive ECLIA system using magnetic microbeads, the surface of which serves as an immunoassay support with immobilized antibodies. In a detection scheme, following coupling reactions shown in Scheme 1, microbeads are collected at electrode surface with a magnet, which corresponds to separation and concentration of an analyte as well as ECL label. ECL labels are oxidized or reduced at the electrode surface to induce an ECL reaction, the magnitude of which depends on concentration of the analyte.

A magnetic microbead used in this study was superparamagnetic, polystyrene-coated microsphere (d=4.5mm). To examine ECL reactions from microbeads, we immobilized a derivative of tris(2,2-bipyridyl) ruthenium(II) which was covalently attached to immunoglobulin G, yielding a luminescent microbead. Measurements of ECL response were carried out with a home-built photon detection system equipped with an electrolytic cell and magnet in a dark box and a photomultiplier module on the lid of the box. A CCD video camera was used for in situ observation of microbeads on electrode surface.

The luminescent magnetic microbeads were successfully collected on Pt electrode surface by positioning a magnet beneath the electrode and thus the Ru-labels were highly concentrated on the electrode surface. Figure 1 shows typical sets of electrochemical (current) and ECL (light emission) response curves recorded during a potential cycle between 0 and 1.2V vs. Ag/AgCl. The Ru-labeled, luminescent microbead alone did not give any current and ECL responses (I). On the other hand, in the presence of tripropylamine (TPA), intense light emission from the Ru complex was observed. The light emission occurs via an electron-transfer reaction between Ru(III) complex and TPA radical which are formed by concurrent oxidations of the Ru(II) complex and TPA, respectively, at the electrode surface. Intensity of ECL response of the luminescent microbeads was dependent on concentration of TPA and pH of solutions. The ECL intensity was also function of surface population of microbeads. CCD images of microbeads on Pt electrode surface showed that the microbeads highly aggregated when a magnet was located beneath the electrode. The extent of the aggregation seemed to increase with increasing population of the microbeads. Furthermore, it was found that the aggregation reduced the ECL intensity, i.e., the more independently microbeads are scattered on electrode surface, the larger the ECL intensity is.



Scheme 1 A reaction scheme for detection of an antigen with a magnetic microbead and an ECL-labeled antibody.

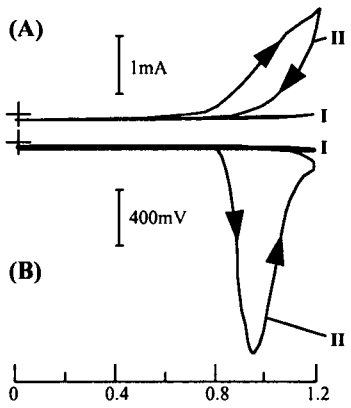


Figure 1 (A) Current- and (B) light emission-potential curves of Ru-labeled magnetic microbeads (I) in the presence and (II) absence of 0.1M TPA.