Electrochemical Enzyme Immunoassay for Phtalate Esters Using Antibody Immobilized Microelectrodes

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Phthalate esters are generally used as plasticizers in polymer products to give a flexibility and resilience. Animal studies and in vitro tests have elucidated that phthalate esters have the antiandrogenic properties, showing developmental toxicity[1)]. The enzyme-linked immunosorbent assay (ELISA) have been widely used for detective of biologically important species. Recently, the electrochemical detection systems for ELISA have received much attention due to miniaturization of whole installations and an easy handling, although the optical methods of the fluorescence and the luminescence have been mainly used for the detection of antibody microarrays. In this study, we present the electrochemical immunoassay system with antibody immobilized arrays electrode. We choose the dibutyl phthalate (DBP), major phthalate ester for plasticizer, as a target molecule to estimate the performance of this system.

An Au microelectrode (ϕ = 300 um) was immersed in a 2-aminoethanthiol solution for introduction of the amino-group to the surface. A mouse anti DBP antibody which was immobilized on the surface introduced the amino-group via cross-linking agent (glutaraldehyde). Competitive reactions of various concentrations of DBP and horseradish peroxidase (HRP) labeled hapten were performed on the resulting antibody immobilized electrodes.

HRP molecules on the electrode surface catalyze the oxidation of ferrocenemethanol (FMA) by H_2O_2 to yield FMA^+, which can be detected by the electrode[2), 3)]. Amperograms of competitive reaction for DBP on antibody immobilized electrode is shown in Fig.2. The substrate was immersed in 0.1 M KCl + 0.1 M phosphate buffer (pH 7.0) containing 0.5 mM H_2O_2 and 0.5 mM FMA, and the potential of electrodes were held at 0.05 V vs Ag/AgCl. Responses for the enzymatic reaction decreased with the competitive reaction. This is responsible for the decrease of the amount of HRP captured on the electrode. The current responses various concentrations for DBP, containing 2 ug/ml HRP-labeled antigen, on the antibody immobilized electrodes is shown in Fig.3. The reduction current decreased with increasing DBP concentration. The current response is very sensitive, indicatory that the system with the array electrode can be applied to detect phthalate esters in environmental samples.

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References:

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2) H. Shiku, et al., *Anal. Chem*., **68**, 1276 (1996)
3) S. Kasai et al., *Anal. Chem*., **72**, 5761(2000)

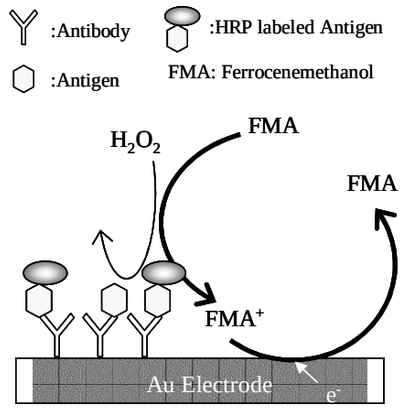

Fig.1 Schematic diagram of electrochemical enzyme immunoassay with antibody immobilized electrode

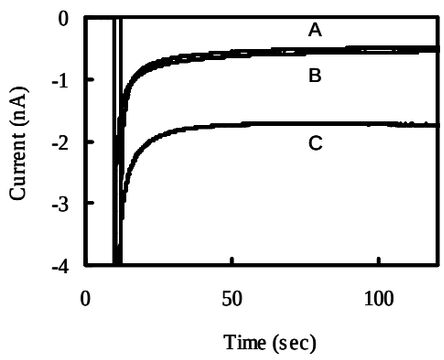

Fig.2 Current responses with anti DBP antibody immobilized electrode using competitive reaction.
A: Negative control (antigen 0 g/ml, HRP-antigen 0 g/ml)
B: Competitive reaction response (antigen 100 ug/ml, HRP-antigen 10 ug/ml)
C: Positive control (antigen 0g/ml, HRP-antigen 10 ug/ml)

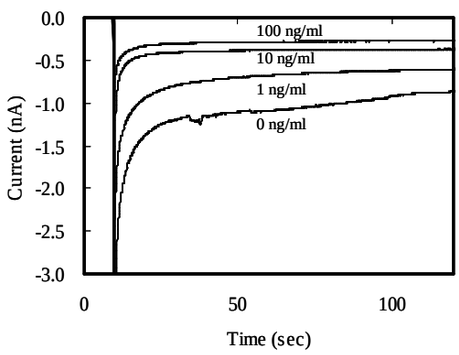

Fig.3 Current responses using DBP competitive immunoassay for 0, 1, 10, 100 ng/ml