Immunoassay for Environmentally Hazardous Chemicals Using Electrochemical Microscopy with Shear Force Feedback Systems

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The human exposure to environmental chemicals causes damages to biological functions and toxic effects in animals [1]. Phthalate esters, which are used for general products such as soap, shampoo and hair spray and plasticizers in polymer products in industry, have been detected in the blood. It is quite important to estimate the concentration of chemicals at local environments. Scanning electrochemical microscopy (SECM) is a powerful technique to characterize electrochemical reactions on local surfaces with microelectrodes. In particular, SECM has been found to be suitable for the quantitative analysis of the protein microarray[(2),(3)]. However, the conventional SECM has an apparent drawback of the difficulty in control of sample–probe distance, which decisively governs the sensitivity and the quality of the SECM image. We present here the detection of the dibutyl phthalate (DBP) captured on antibody microarrays with the SECM in the constant distance between the probe and substrate accurately controlled by the shear force feedback systems.

Fig.1 shows the SECM system with feedback control of sample–probe distance[(4)]. A piezoelectroric buzzer vibrated the carbon microelectrode that was attached to one of legs of a tuning fork quartz crystal. The tuning fork quartz crystal being attached to the microelectrode which was vibrated by a piezoelectric buzzer induced a voltage signal by a piezoelectric effect. The digital lock-in amplifier was used for both the drive of buzzer and detection of induced signal. When the probe approaches the substrate and the distance becomes less than 100 nm, the magnitude of the vibration decreases by the effect of shear force between the probe and substrate. To maintain a constant probe–surface distance, the amplitude of induced signal was used for the feedback control of the z-position.

A goat anti mouse IgG-Fc was immobilized via cross-linking agent, disuccinimidyl suberate (DSS), on Au band arrays modified by cystamine and was allowed to immobilize and orient a mouse anti DBP IgG. Competitive reactions of various concentrations of DBP and horseradish peroxidase (HRP) labeled hapten were performed on the resulting an antibody microarrays.

The localized HRP was detected and visualized to observe oxidized ferrocenyl methanol (FMA) produced by the enzyme catalytic reaction in a solution containing hydrogen peroxide. The tip of carbon microelectrodes was stabilized at 50 nm above the substrate with the regulation of distance for the SECM imaging which can obtain the topographic image simultaneously. Fig. 2 shows SECM images of various concentrations of DBP. Reduction currents of oxidized FMA were measured by probe held at +0.05 V vs Ag/AgCl. Responses of reduction currents decreased with increasing DBP concentration. This is caused by in the decrease of the amount of captured HRP-conjugated hapten. Responses of each line in the microarray were at the same level (within 2 %). The regulation of substrate-probe distance in very close improved the sensitivity and reproducibility of SECM sensing, because of the enhancement of both the redox cycling between the enzyme reaction and electrochemical reaction and blocking effects of the diffusion of mediators to electrode surface on glass areas.

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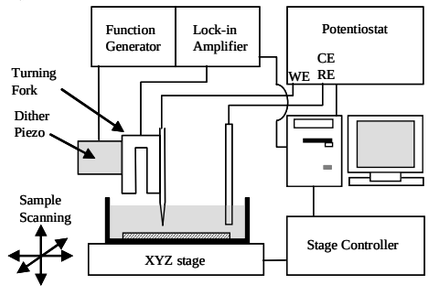

Fig.1 Schematic diagram of the SECM with shear force feedback.

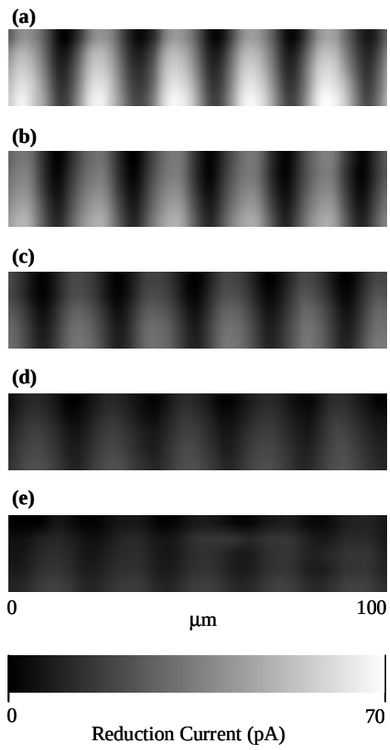

Fig.2 Competitive ELISA by using SECM. Concentrations of DBP were (a) 0, (b) 1, (c) 10, (d) 100 and (e) 1000 ng/ml, The concentration of competitive hapten-HRP was 2.0 μg/ml.