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学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Development of Microdevice-Based Aptamer Sensors for Mycotoxin Analysis
(マイコトキシン分析のためのマイクロデバイスベースのアプタマーセンサーの開発)

Since the systematic evolution of ligands by exponential enrichment (SELEX) method was developed, aptamers have made significant contributions as bio-recognition sensors. The aptamer that affinity and specificity parallel those of antibody is expected to have a future impact on a biosensor. In addition, aptamers have an unleashed potential to circumvent limitations associated with antibodies and could be utilized in practical settings where their performance could be compared directly to that of antibodies. In this research, we have developed aptamer sensor on the microdevice system for detection of ochratoxin A (OTA), a carcinogenic toxin that produced by secondary metabolism of *Penicillium* and *Aspergillus* fungal strains. Microdevice systems allow for low reagent consumption, high-throughput of samples, and disposability. Due to these advantages, there has been an increasing demand to develop microdevice-based aptasensor for analytical technique application. Highly sensitive detection techniques, such as electrochemical and optical detection, have been integrated into lab-on-a-chip aptasensor devices towards the goal of establishing point-of-care diagnoses for target OTA analyses. In this context, the general introduction including present perspective are described in chapter 1.

In chapter 2, microdevice based aptamer sensor for the simple, portable, and low-cost detection system to quantitatively measure the ochratoxin A in real sample have been developed. First, a miniaturized electrochemical aptamer-based sensor was developed for the label-free with signal-off detection or TID (target induced dissociation) method. For the construction of the sensor, a gold thin-film three-electrode system was fabricated using standard microfabrication techniques on a polystyrene substrate (25 mm × 25 mm). Subsequently, the thiol-modified linker, 6-mercaptohexanol, DNA aptamer, and methylene blue (MB) were sequentially applied to the working electrode to construct a sensing layer. MB served as a redox indicator that interacted with the aptamer via the guanine bases and phosphate backbone to form complexes. The addition of OTA to the sensor induced the folding of the aptamer, which was accompanied by the release of the aptamer-MB-OTA complex from the sensor. Thus, the amount of MB decreased with increasing concentration of OTA. Differential pulse voltammetry (DPV) was used for monitoring the highly sensitive detection. The standard curve for OTA exhibited a wide linearity ranging from 0.1 to 300 ng mL⁻¹ with a detection limit of 78.3 pg mL⁻¹ (S/N = 3). The selectivity test confirmed that the aptamer had high affinity only for the target. The OTA recoveries with the proposed sensor in commercial samples of coffee and beer were 86.4 – 107%.

Chapter 3 proposed the miniaturized electrochemical aptasensor by using a dithiol-modified aptamer which had a high stability on the gold electrode because of their two anchors. The sensor based on structure-switching of the aptamer upon the interaction with OTA, which produced the signal current. The non-covalent interaction of methylene blue (MB) with the aptamer was also used as an electrochemical indicator for the simple sensor fabrication. In this study, the performance of the sensor was characterized, including the dissociation constant of the aptamer-OTA complex. The proposed sensor exhibited high reproducibility and enough sensitivity to detect the minimum amount of OTA required for the analysis of real food samples with a limit of detection of 113 pM (45 pg mL⁻¹). The sensor holds great promise for a simple inexpensive technique with low-reagent consumption for on-site detection of OTA.

In chapter 4, describes competitive fluorescence polarization (FP) assay based on fluorescence polarization immunoassay (FPIA) and fluorescence polarization aptamer assay (FPAA). The FP method is a homogenous assay which not required separation and washing step. In addition, integration of FP detection in the miniaturized device give more advantages such as low reagent consumption and portability. The optimized FPIA detection had a wide detection range from 1 to 1000 ng mL⁻¹ with detection limit 0.522 ng mL⁻¹ and 0.403 ng mL⁻¹ for conventional and miniaturized FP analyzer, respectively. In FPAA, we have designed biotinylated-modified aptamer that subjected to bind with streptavidin as a signal amplifier. Determination of sensitivity and signal strength of FPAA still on progress, therefore all results will be described later.

The final chapter is the summary of the results and findings during the research. In addition, future prospect and research for aptamer-based fluorescence polarization are described in this chapter.