学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Surface plasmon resonance immunosensor using Au nanoparticle for detection of beta agonist

（βアゴニストを検出するための金ナノ粒子を用いた表面プラズモン共鳴免疫センサの開発）

Quality of life is supported by the sensor technologies. These technologies involve disease detection, drug discovery, environmental pollutant management, and food quality control. In terms of food quality control, a reliable detection and quantification of toxic agent are considered the first step to ensure the quality of human health. In this thesis, the immunosensing of β-Agonist, which is commonly used as a sports doping agent and a drug in respiratory disease, was studied. Aiming to develop a practical sensor, Surface Plasmon Resonance (SPR) immunosensor was investigated. In the past reports, the SPR immunosensing with Au nanoparticles provides a high sensitivity. Therefore, an immunoassay for detection of small molecule by using Au nanoparticle was designed based on the kinetic analysis.

In Chapter 1, the present situation of environmental problems and its adverse effects on human beings are described. Also, inadequacy of classical methodologies to overcome the environmental assessments and monitoring was mentioned. Because this thesis focused on SPR, the principle and its application were reported. In addition, the sensors using an immunoassay have been introduced to deal with environmental challenges. The scope and outline of the thesis have been briefly summarized.

In chapter 2, the instrument and their principle were described. In particular, SPR configuration and the sensor surface fabrication process were introduced. Experimental condition of electrochemical measurement, UV-Vis spectroscopy, and X-ray Photoelectron Spectroscopy is mentioned.

In chapter 3, immunoreaction using the conjugates of antibodies and Au nanoparticle (Ab-Au NP conjugate) was studied. Ab-Au NP conjugate were synthesized. It was found that the lowest limit of detection (LOD) was improved 40 times lower than that of the unlabeled antibody in indirect competitive inhibition immunoassay. Furthermore, the SPR response was also improved by 3-times for Ab-Au NP conjugate. To identify the key factors in determining the LOD of the immunoassay, the affinity constants of surface immunoreaction (K_1) and solution immunoreaction (αK_2) were evaluated. According to the modified Langmuir isotherm, the simulation plot of LOD with respect to K_1 and αK_2 was constructed. It showed that a K_1 of one order of magnitude lower than αK_2 produced a ppq-level LOD. Therefore, it was concluded that the formation of conjugate with Au NP could control the affinity constant of solution immunoreaction. As a result, K_1 and controlled αK_2 determined the LOD of indirect competitive inhibition immunoassay.

In chapter 4, the secondary immunoreaction using Ab-Au NP conjugate was investigated. The

secondary immunoreaction was employed in order to study the control of affinity constant of solution immunoreaction by using Ab-Au NP conjugate. It was found that the SPR response was enhanced by 2-fold compared to the primary immunoreaction. To compare the effect of K_1, a low affinity (K_1=2.9 x 10^8 M^{-1}) and a high affinity sensor surface (K_1=2.9 x 10^8 M^{-1}) were fabricated. It was noticed that Au NP – 2nd Ab amplified SPR response approximately 5-fold compared to the unlabeled secondary Ab for a high affinity sensor surface. In comparison of LOD, the low affinity sensor surface produced 70 ppq (fg mL^{-1}), while a 30 ppq LOD was obtained for the high affinity sensor surface. Therefore, it was concluded that the high affinity sensor surface was preferable for secondary immunoreaction.

In chapter 5, a highly sensitive immunosensing of beta agonist in a real urine sample was developed. However, the real sample may contain many kinds of inhibitors. There are challenges for real sample analysis, even though the high sensitivity was realized in an ideal condition. In this study, the indirect competitive inhibition immunoassay with secondary immunoreaction optimized in above chapters was employed for detection of beta agonist. Although the sensitivity of 30 ppq was achieved in ideal condition using PBS buffer solution, SPR did not respond to the monoclonal antibody of beta agonist in non-pretreated urine. First, in order to eliminate the pH effect, the pH was adjusted by mixing with PBS buffer solution (1:1). The pH of sample solution became always 7.4 by the buffer effect. Next, the filtration methods for urine of cows were investigated. Although very high SPR response was obtained, the sensor surface could not be regenerated again. It is suggested that the high non-specific adsorption of inhibitors was caused. Therefore, it was concluded that the physical filtration using pore-size filtration could not remove the inhibitors. Subsequently the chemical filtration method was examined. Three types of silica filter modified with functional group (octadecyl, amide and carboxyl acid) were employed for urine sample. All three filters showed the removal performances. This result implied that the inhibitors chemically interrupted the immunoreaction. It was noticed that the carboxylic acid group modified silica filter provided the best filtration among three filters. It was found that the highest SPR signal with a magnitude of 45 mdeg was obtained. Furthermore, a low limit of detection with 100 ppq was achieved by using secondary immunoreaction using Ab – Au NP conjugate. It was almost comparable sensitivity to that in PBS buffer solution. Therefore, the low limit of detection as well as short detection time would be an alternative to the conventional methods.

In chapter 6, the concept of the sensitivity determination factor was summarized. Based on the kinetic analysis, the strategy for the design of the highly-sensitive immunoresensing was discussed. Further, toward to a practical use of immunosensor, the pretreatment method for a real urine sample was proposed.