Electrocatalytic Detection of Enzyme-Linked Immunosorbent Assay

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In the field of clinical analysis and biochemical studies, a highly sensitive and high-throughput analysis technique is required to process very large amount of new information. Especially, immuno- or affinity-sensing techniques to detect biospecific interactions such as antibody-antigen, ligand-receptor and protein-protein recognition interactions, demand more sensitive and miniaturized system. But, the conventional immunoanalysis is not adaptable due to long analysis time, low sensitivity and manual assay procedures. There has been many tries to develop more efficient analytical tools in order to solve these problems [1,2]. Immunoassays have been used as a very powerful technique for detecting biochemical interaction because this method is simple, sensitive, reliable, and relatively selective. Many attempts have been made in immunosensor technology with various methods such as optical, electrochemical, and gravimetric techniques [1,2]. Among them, enzyme immunoassays with electrochemical detection have enabled the improvement of sensitivities and reduction of instrumentation costs compared with other methods.

In this study, a new method to electrochemical enzyme immunoassay for sensing antibody-antigen interaction has been developed. Partially ferrocenyl-tethered dendrimer(Fc-D) was prepared and immobilized to the electrode surface via covalently binding between amines of dendrimer and carboxylic acids of self-assembled monolayer. It is very advantageous because the unreacted amines of immobilized Fc-D are able to modify with biotin groups to associate with anti-biotin antibody and its ferrocene part acts as an electrocatalyst to enhance the electrochemical signals [3,4,5] as shown in scheme 1. The antibody-antigen recognition of alkaline phosphates conjugated antibody to anti-biotin antibody which is immobilized on the biotin-fuctionalized surface results in converting the electrochemical inactive substrates to electroactive products by enzymatic reaction.

The electroactive enzymatic products diffuse near the layer of Fc-D. When the anodic potential of ferrocene is applied, they are electrooxidezed by donating electrons to ferricinum ion as an oxidized form of ferrocene, which leads to the electrocatalytic reaction of ferrocene. As a result, the electrochemical response of enzymatic product is enhanced. And the relationship between this enhanced signal and concentration of antibody is investigated.

To characterize this mechanism, we carried out cyclic voltammetry (CV) and surface plasmon resonance (SPR) experiments.

Reference

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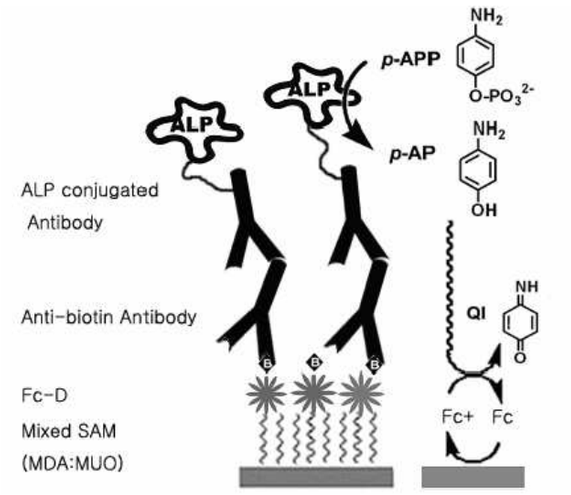

Scheme 1. Schematic illustration of enzyme-linked immunosorbent assay on Fc-dendrimer modified gold electrode.