Electrochemical Evaluation of Interaction between Avidin and Biotin Self-assembled Using Marker Ions

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The interaction between avidin and biotin as self-assembled monolayer on a gold electrode surface was electrochemically evaluated by monitoring the changes in the electrode response of the ferrocyanide ion as redox-marker. It was clarified that the permeability changes of avidin membrane introduced onto the biotin self-assembled monolayer against ferrocyanide ion were ascribed to electrostatic interaction between avidin and marker ion.

Recently, many exciting artificial membrane systems based on the mechanisms of biomembranes have been developed. The sensing systems based on analyte-triggered changes in the permeability of membranes on electrode surface was first proposed by Sugawara et al.[1] as "ion channel sensor". Complexation between receptors immobilized on electrode surface and anionic or cationic analyte influences permeability of electroactive markers which passes through between the receptor molecules to access the electrode surface. Such permeability changes can be easily observed with cyclic voltammetry. Several types of channel mimetic sensing membranes, such as origopeptide,[2] calixarene derivatives,[3] carboxylate,[4] nucleotide,[5] has been reported.

The electrochemical evaluation of the interaction between ligand immobilized on electrode surface and protein makes possible the dynamic monitoring for the formation of the ligand-protein complex and the development of the high sensitive electrochemically biosensor. Avidin-biotin binding is well known as one of the strongest bindings between protein and ligand. Therefore, this strong interaction has been applied as avidin-biotin techniques to various fields containing enzyme-linked immunoassay[6,7] and DNA hybridization[8-10] etc. Furthermore, some studies for characterization of biotinylated self-assembled monolayer (SAM) on gold surface have performed[11-14] and indicated that specific interactions can take place between streptavidin and biotin on SAM.[13,14]

In this study, avidin-biotin and NutrAvidin-biotin were selected as a model ligand-protein. The interaction between avidin and biotin on SAM and the function as channel mimetic membranes of avidin membrane introduced onto the biotin-SAM were electrochemically evaluated by monitoring the change in the electrode response of the marker ion.

The polished gold electrode (1.6 mm diameter, Bioanalytical Systems (BAS)) was immersed for overnight at 4 ℃ in 0.1 M phosphate buffer (pH 7.0) including 2.5 mM Sulfo-NHS-SS-Biotin (Pierce). In this time, biotinylated thiol species and mercaptopropionic acid were modified on gold electrode surface because the N-hydroxysulfosuccinimide portion in the NHS-SS-Biotin is very easy to hydrolysis by water. The avidin-linked self-assembled monolayer (avidin-SAM) was prepared on the biotin-SAM electrode surface by incubation in 0.3 mg/ml avidin aqueous solution at least 20 min. Cyclic voltammetric measurements were carried out by a CV-50 W analyzer (BAS). A platinum wire was used as a counter electrode, and a Ag|AgCl electrode as a reference electrode.

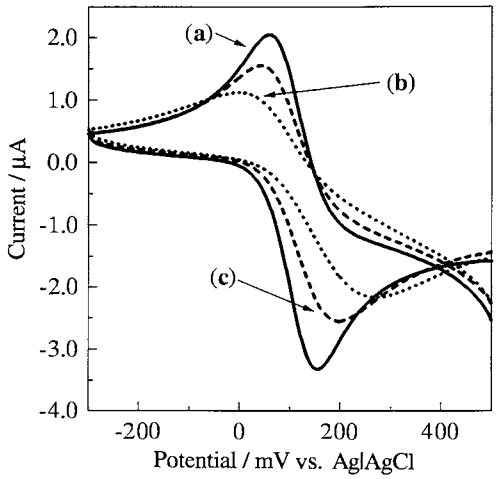

Figure 1. Cyclic voltammograms of 1.0 mM potassium ferrocyanide at: a) bare Au electrode. b) biotin-SAM electrode. c) avidin-SAM electrode in 0.1 M phosphate buffer (pH 5.5). Scan rate 50 mV/s.

Figure 1 shows the cyclic voltammograms of 1.0 mM potassium ferrocyanide in 0.1 M phosphate buffer (pH 5.5) obtained from a bare gold electrode, the biotin-SAM electrode and the avidin-SAM electrode. The peak currents of the ferrocyanide ion obtained at the biotin-SAM electrode decreased (curve b), compared to that of a bare electrode (curve a). This decrease of peak currents is attributed to the steric hindrance of biotin-SAM against the electrode reaction of marker ion. That is, the redox reaction of the ferrocyanide ion is suppressed by the decrease in the electrode active area or an increase of distance between marker ion and electrode active surface. On the other hand, the electrode response of ferrocyanide ion was restored by the introduction of the avidin membrane onto the biotin-SAM (curve c). Although the reason is not clear, it may be caused by following reasons. The steric hindrance of the biotin-SAM against marker ion was canceled by entrapment due to the electrostatic effect of the avidin membrane, or a decrease of distance between marker ion and gold electrode surface, because of the biotin-SAM was pushed down on an electrode surface due to avidin bound strongly with biotin. While using dopamine and Cu(II) as a cationic marker, the electrode response of redox-marker then decreased by the introduction of the avidin membrane.

In order to confirm that the specific avidin-biotin binding provides the difference in electrode response of marker ion between the avidin-SAM electrode and the biotin-SAM electrode, the relation between the peak current and incubation time in avidin solution was investigated. The peak currents of ferrocyanide ion increased with the increase in incubation time and became a

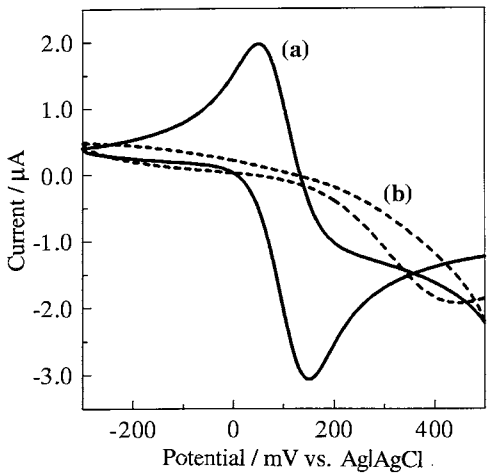

Figure 2. Cyclic voltammograms of 1.0 mM potassium ferrocyanide at NutrAvidin-SAM electrode in: a) 0.1 M acetate buffer (pH 4.0). b) 0.1 M phosphate buffer (pH 7.0). Scan rate 50 mV/s.

constant value over 5 min. However, a constant peak current could not be obtained from a gold electrode in which the avidin nonspecific adsorbed. Therefore, this fast adsorption of avidin to the biotin-SAM arose from the specific binding between avidin and biotin but not by the adsorption of the protein on the electrode surface.

To examine the electrostatic effect of the avidin membrane against marker ion, the same experiments were carried out by using NutrAvidin which is deglycosylated avidin with isoelectric point 6.3. It is possible to easily change the electric charge of avidin membrane introduced onto the electrode, because the isoelectric point of the NutrAvidin is lower than avidin (p*I*=10-10.5). The cyclic voltammograms of 1.0 mM potassium ferrocyanide obtained at the NutrAvidin-SAM electrode were shown in Figure 2. At lower pH than the isoelectric point, the electrode response of marker ion was the same as one of a bare gold electrode (curve a). On the other hand, the electrode response of marker ion more decreased than that of the biotin-SAM electrode at higher pH (curve b). Thus, it has been clarified that the changes in electrode response of ferrocyanide ion between the avidin-SAM electrode and the biotin-SAM electrode has been ascribed to electrostatic interaction between avidin and marker ion. When using dopamine as a cationic marker, the electrode response was decreased than that of the biotin-SAM electrode at pH 4.0. This means that the permeability of avidin membrane for the marker ion can be controlled by only pH of solution. On the other hand, the peak currents for the reduction of ferrocyanide ion obtained from the biotin-SAM electrode and avidin-SAM electrode in phosphate buffer (pH 5.5) are proportional to the square root of the scan rates. Therefore, the electrode process of ferrocyanide ion is controlled by diffusion although the avidin was introduced into the biotin-SAM. It shows that ferrocyanide ion permeates the avidin membrane freely.

In order to develop a ligand-protein assay using this mechanism, we are now studying a quantitative evaluation of the prepared biotin-SAM and the avidin-SAM.

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