Voltammetric Behaviors of Lectin-Sugar Binding Using Au Electrode Modified with Galactosamine

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Voltammetric behaviors of binding between soybean agglutinin (SBA) having sites for galactosamine and the sugar were investigated. A self-assembled monolayer (SAM) of galactosamine was designed on an Au electrode surface, and SBA-galactosamine interaction was monitored through ferrocyanide ion as marker. Electrode responses of ferrocyanide ion decreased with increasing the concentrations of the SBA. This is because SBA selectively combines to sugar part on the electrode surface. To confirm the binding, voltammetric behaviors of several lectins not having sites for galactosamine were compared to that of SBA.

Many biological molecules have excellent properties of recognition to molecules. The biospecific binding relates to neurotransmitters, secretion of hormone and metabolisms. On the other hand, monitoring of various electron transfers in living body is important to understand their biological functions. Therefore, many studies of sugar and protein forming the living body have been reported.[1, 2]

Bioaffinity between biomacromolecule and ligand contributes to development of electrochemical biosensor. For example, sensing of a DNA-binding drug with a DNA-immobilized Au electrode has been investigated.[3] Tohda et al. has reported sensor for nucleotides based on multitopic hydrogen bonding.[4] Furthermore, monitoring of antigen using quartz crystal microbalance has been attemptted.[5] Bioaffinity sensors using electrodes modified with SAM are also useful.[6, 7] In addition, photo controlled kinetics of binding to Cocanavalin A(Con A) at sugar-modified electrode by SAM was studied.[8] In previous studies, we have proposed several electrochemical methods to evaluate avidin-biotin or lectin-sugar interaction by ligand labeled with electroactive compound.[9, 10] Although these methods are convenient and rapid, the methods have the possibility that non-specific adsorption of another organic compounds on an electrode influences on the evaluation of the protein-ligand interaction. When functional molecular unit is introduced on an electrode surface, it is expected that non-specific adsorption of organic compound is suppressed. For lectin-sugar binding, the electrode with the special sugar makes exact evaluation of the binding possible.

This paper describes voltammetric behaviors of SBA at an Au electrode modified with galactosamine-SAM. Ferrocyanide ion was used as marker to monitor an interaction between SBA and galactosamine. It is expected that evaluation of the interaction is achieved by change of permeability of the marker ion in SBA film due to the binding.

SBA, Con A and wheat germ agglutinin (WGA) were supplied from Sigma. D-Galactosamine was purchased from Wako Pure Chem. Co. (Osaka, Japan). 3,3'-Dithiobis(sulfosuccinimidyl propionate) (DTSSP) was obtained from Pierce. The modifier was prepared from mixing of DTSSP and galactosamine for 24 h at 4 °C in 0.1 M phosphate buffer (pH 8.5). The product (Figure 1) was separated by TLC (silica-gel alumina sheet, Merck) with 1-propanol:ammonia:water = 6:2:1 (v/v)%. Concentration of the product was determined by Ludowing-Benmaman method.[11] The structure was confirmed by ^{1}H NMR data. An Au electrode (Model No. 11-2012, Bioanalytical Systems (BAS)) was immersed for overnight at 4 °C in 0.1 M phosphate buffer (pH 7.0) with 1 mM modifier. The modified electrode was incubated for 60 min with stirring in 0.1 M phosphate buffer including lectin. CV-50 W analyzer (BAS) was used to all voltammetric measurements. A counter electrode was a platinum wire, and an Ag/AgCl electrode was used as a reference electrode.

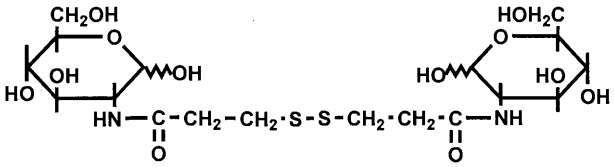

Figure 1. Modifier obtained by reaction between galactosamine and DTSSP.

Voltammograms of 0.5 mM potassium ferrocyanide in the presence or absence of added SBA, obtained by a bare Au electrode in 0.1 M phosphate buffer (pH 7.0) are shown in Figure 2. When the ferrocyanide ion was added in a solution, well-defined peaks were observed (curve b). However, the peaks disappeared in the presence of 30 μg/ml SBA (curve c). For 30 μg/ml Con A or 30 μg/ml WGA without sites for galactosamine, the wave of ferrocyanide ion was not also observed. This was due to inhibition of the electrode reaction produced from non-specific adsorption of the lectin on the electrode surface.

Figure 3 shows cyclic voltammograms of ferrocyanide ion and adding lectin at a galactosamine-modified electrode. Electrode response of ferrocyanide ion at the modified electrode decreased (curve b) compared to that at a bare electrode as shown in Figure 2(b). Reversibility of the electrode reaction also became lower. It is guessed that this reason is due to decrease of active area on an electrode surface or decrease of accessibility marker ion to the electrode surface. In various concentrations of the SBA, the measurements at the modified electrode were performed. The peaks of marker ions decreased with increasing concentrations of SBA (curves b-e). In contrast, the electrode response of marker ion did not change in the presence of 30 μg/ml Con A (curve b). Therefore, it was proposed that this phenomenon arose from the specific binding

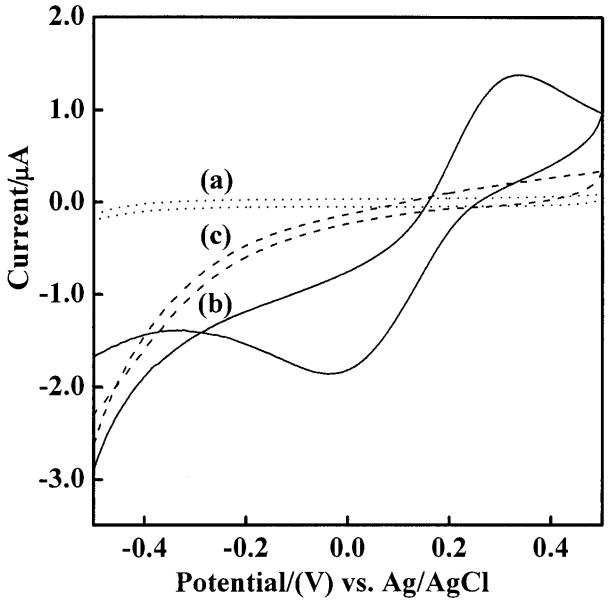

Figure 2. Cyclic voltammograms at a bare Au electrode.
(a) Blank, (b) 0.5 mM potassium ferrocyanide,
(c) 0.5 mM potassium ferrocyanide + 30 μg/ml SBA.
Measurements using scan rate 50 mV/s in 0.1 M phosphate buffer(pH 7.0).

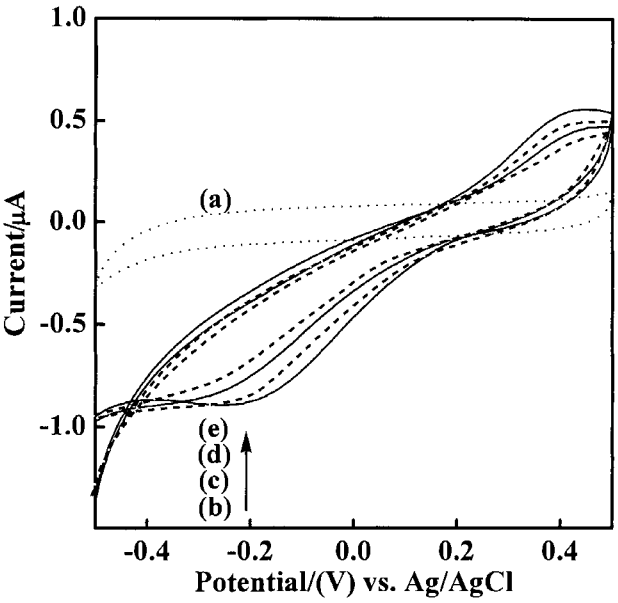

Figure 3. Cyclic voltammograms at galactosamine-modified electrode.
(a) Blank, (b) 0.5 mM potassium ferrocyanide or 0.5 mM potassium ferrocyanide + 30 μg/ml Con A, (c) 0.5 mM potassium ferrocyanide + 15 μg/ml SBA, (d) 0.5 mM potassium ferrocyanide + 30 μg/ml SBA, (e) 0.5 mM potassium ferrocyanide + 45 μg/ml SBA.
Measurements using scan rate 50 mV/s in 0.1 M phosphate buffer (pH 7.0).

between SBA and galactosamine but not from non-specific adsorption of the protein. Accordingly, this electrode exactly enables to evaluate the interaction between SBA and galactosamine.

In order to investigate an electrostatic interaction of SBA film with marker ion, the same experiments were attempted with hexaammineruthenium(III) ion having positive charge. As a result, voltammetric behavior of hexaammine-ruthenium(III) ion was similar to that of ferrocyanide ion. Furthermore, measurements were carried out in 0.1 M acetate buffer (pH 5.0) and 0.1 M phosphate buffer (pH 8.5) because isoelectric point of SBA is 6.0. In two buffer solutions, the decreases of peak currents were observed in the presence of SBA. That is, SBA combines with galactosamine on the electrode in pH from 5.0 to 8.5. The peak currents of ferrocyanide ion obtained by the modified electrode with SBA film were proportional to the square root of the scan rates in the range of 10 and 100 mV/s. Therefore, the electrode reaction of ferrocyanide ion depends on diffusion and not adsorption. It was apparent that the electrostatic interaction between ferrocyanide ion and SBA film was little. In avidin-biotin system, an electrostatic interaction between marker ion and avidin was very strong.[12] However, the interaction hardly influenced on the lectin-sugar system. Consequently, it is clear that the permeability of marker ion is controlled by the factor of steric hindrance of SBA.

In conclusion, a galactosamine-modified Au electrode by SAM was constructed. SBA selectively combined to the electrode surface on the basis of the interaction with galactosamine. Formation of the SBA film on the electrode surface controlled the electrode response of ferrocyanide ion. Moreover, it was found that galactosamine-SAM suppressed non-adsorption of protein to Au electrode. This procedure would be applied to evaluation of the interaction of many lectin-sugar systems.

References and Notes

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