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Electrochemical characterization of ferrocene-containing redox polymer and its application to biosensors

Satoshi Koide

*School of Materials Science,
Japan Advanced Institute of Science and Technology*

(Supervised by Associate Professor Kenji Yokoyama)

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Chapter 1 Introduction

Enzyme catalysis at modified electrodes provides selective molecular recognition and transduction of biochemical information into electrical signals. An enzyme electrode based on an electron transfer mediator had been reported by Cass and co-workers 15 years ago [1]. In recent years, biosensors based on reagentless mediated enzyme electrodes have been proposed [2-16]. They can be composed of a combination of a redox enzyme and a redox polymer. These devices rely on the electrical connection between an enzyme redox center and an electrode through the participation of redox centers in polymers.

An osmium complex containing redox polymers have been reported [2-8]. These redox polymers were prepared with polyvinylpyridine [2-4] or polyvinylimidazole [5-8] complexed with $[\text{Os}(\text{bpy})_2\text{Cl}]^+$ [2-5], $[\text{Os}(4,4'\text{-dimethyl-bpy})_2\text{Cl}]^+$ [6,7] and $[\text{Os}(4,4'\text{-dimethoxy-bpy})_2\text{Cl}]^+$ [8]. An electrochemical response of these redox polymers was stable. However, in practice it is difficult to form these redox polymers due to the formation of osmic acid in acidic conditions. Osmic acid has toxicity which is hence undesirable. Ferrocene containing redox polymers have been reported [9-16]. In this research, many redox polymers [10-12, 15] were immobilized onto electrodes by cross-linker (for example, glutaraldehyde: GA, epichlorohydrin and so on). However, the electrochemical response of these redox polymers were unstable due to leakage and/or over-swelling. Other redox polymers [13, 16] were mixed with carbon paste and enzyme. However, the use of carbon paste causes difficulty in evaluating electrochemical properties of redox polymers. In another case, a redox copolymer was prepared so that acrylamide and vinylferrocene was cross-linked with *N,N'*-methylenebisacrylamide; it was then entrapped in GOx containing hydrogel [14]. However, this redox hydrogel requires a dialysis membrane to be fixed on the electrode to stop leakage.

To improve above procedure, our group has previously proposed BSA-based immobilization that co-reticulates GOx and a redox polymer with GA on a glassy carbon electrode [15]. A cross-linked copolymer of acrylamide and acrylic acid with 11 bond side chains containing ferrocene at the ends was used. This immobilization method was highly effective to stabilize the electrochemical responses of the redox polymer. However, synthesis of this redox copolymer was complicated and yielded a low efficiency.

In this study, I investigated the electrochemical characterization of enzyme electrodes with a combination of GOx and a cross-linked redox polymer or a redox peptide. The redox polymer can be considered electrochemically stable, because the cross-linking of the redox polymer prevents it over-swelling on the electrode. Analysis of detailed catalytic electrochemistry of redox polymer-enzyme electrodes is necessary in order to fabricate biosensors based on redox polymers. The redox peptide can immobilize onto a gold electrode by SAM (Self-Assembled Monolayer method). The SAM maintains the flexibility of the redox peptides. Thus, a rapid electron transfer reaction can, in theory, occur among the redox sites of the redox peptide, between the redox site of the redox peptide and electrode, and the redox site of the redox peptide and the redox site of the enzyme.

Chapter 2 Electrochemical characterization of ferrocene-containing polyallylamine modified with hydroxyl groups

A ferrocene containing polyallylamine was condensed to mix with protein in buffer solution. This indicates that electrostatic repulsion of the redox polymer was weakened because of the increase of counter ions with increasing ion strength. Thus, the ferrocene containing redox polymer was undissolved in the buffer solution and reproducibility could not be obtained in the enzyme redox polymer electrodes. It is expected that a ferrocene containing polyallylamine dissolved owing to modification by hydroxyl groups. In this study, I prepared ferrocene containing polyallylamine modified with glycidol or L-lactate and polyallylamine modified with an oxidized form of ferrocene, and evaluated electrochemical characterizations of their redox polymers. The electrochemical response for ferrocene containing polyallylamine modified with hydroxyl groups, or for polyallylamine modified with an oxidized form of ferrocene, decreased with time. Therefore it is concluded that leakage and/or over-swelling of redox polymer occurred.

Chapter 3 Electrochemical characterization of ferrocene-containing polyallylamine cross-linked with GA

A redox polymer based on cross-linked polyallylamine with covalently attached ferrocene and GOx were co-immobilized on a glassy carbon electrode using GA and BSA. The use of the cross-linked redox polymer and the addition of BSA for enzyme immobilization provided the redox polymer-protein electrode with very stable electrochemical response. This indicates that the redox polymer in hydrogel was prevented from over-swelling. A linear correlation between the anodic peak current and the square root of the scan rate was observed for a redox polymer-protein electrode. This result indicates charge propagation in the polymer network by a diffusion-like process, such as electron hopping among neighboring redox sites and counter ion motion. The effect of pH has been investigated on the anodic peak current of this redox polymer-protein electrode. The anodic peak current of the redox polymer depended on the pH and was approximately constant in the pH range: 2.2 to 7, but increased with increasing pH for pH values over 8. The pH

dependence of the anodic peak current is associated with the charge status of the redox polymer. The pK_a value of the amino group of polyallylamine is expected to be around 9 [17]. The results were probably owing to the charge status in the redox polymer-enzyme hydrogel.

Chapter 4 Catalytic electrochemistry used ferrocene-containing polyallylamine cross-linked with GA

Catalytic electrochemistry of the enzyme electrode with the redox polymer was investigated. Catalytic current was observed by addition of glucose. The enzyme electrode with high redox polymer content showed prepeaks at around 260 mV at low glucose concentration. On the contrary, no clear prepeaks were observed with low redox polymer content. At high glucose concentration, a typical catalytic current with a sigmoidal shape was observed with low redox polymer content. However, peaks were still observed with high redox polymer content.

Appearance of these prepeaks can be clearly explained by cyclic voltammetric simulation. A cyclic voltammetric simulation for electrochemically mediated enzyme reaction has been reported by our group [18]. In this paper, a digitally simulated cyclic voltammogram (CV) demonstrates that a prepeak is observed at low substrate concentration, high mediator concentration and high enzyme activity. These electrochemical behaviors can be elucidated by the calculated concentration profiles of mediator and substrate. Appearance of the prepeak is due to the depletion of the substrate concentration in the vicinity of the electrode surface. Hence, the recycling rate of the redox site was reduced, and the catalytic current increase was suppressed. The concentration profiles can also elucidate whether the catalytic currents show a sigmoidal shape or a peak at high glucose concentration. The effect of enzyme content was investigated. No prepeak was detected at low enzyme content, whereas a prepeak was observed at high enzyme content. These results, together with the theory, led us to conclude that these prepeak phenomena occur at high enzyme activity.

Chapter 5 Electrochemical characterization of ferrocene-containing redox peptide

The immobilization of a redox polymer by cross-linking meant that rapid electron transfer could not be achieved. It is possible to immobilize a peptide onto a gold electrode by SAM. It is expected that the use of the SAM can maintain the high flexibility of redox polymer. Thus, I investigated for redox peptide modified with ferrocene. The electrochemical response of the electrode with the SAM immobilised ferrocene-containing redox peptide was observed. However, this electrochemical response was not clear. This is due to the condensation of the redox peptide onto the gold electrode. Then the n-decyl mercaptan was further modified on the electrode after being immobilized with redox peptide and hence the electrochemical response was clear. A thiol of long alkyl chain such

as n-decyl mercaptan was densely packed onto the gold electrode surface [19-22]. Thus, this indicates that the condensation of the immobilized redox peptide onto the electrode was improved by the modification of n-decyl mercaptan. The electrochemical response of the redox peptide modified gold electrode led to stability after soaking in buffer solution for 2 hours. A linear correlation between the anodic peak current and the square root of the scan rate was observed for the redox peptide modified electrode. This result also indicates charge propagation in the polymer network by a diffusion-like process, such as electron hopping among neighboring redox sites and counter ion motion. A peak-to-peak potential separation was 53 mV/s at scan rate of 10 mV/s. This suggests that charge propagation of the redox peptide was of a surface wave form. Catalytic electrochemistry of the enzyme electrode with the redox peptide was investigated. Catalytic current was observed by addition of glucose.

Chapter 6 Conclusion

In this work, I demonstrated electron transfer between the active site of GOx and both a ferrocene-containing redox polymer and a ferrocene-containing redox peptide. A redox polymer based on cross-linked polyallylamine with covalently attached ferrocene and GOx were co-immobilized on a glassy carbon electrode using GA and BSA. The use of the cross-linked redox polymer and the addition of BSA for enzyme immobilization provided the redox polymer-enzyme electrode with a very stable electrochemical response. This indicates that the redox polymer in hydrogel was prevented from over-swelling. The electrochemical response of SAM immobilized ferrocene-containing redox peptide was very stable.

Both the anodic peak current and the redox potential of the redox polymer-enzyme electrode depended on pH. These results were probably owing to the charge status in the redox polymer-enzyme hydrogel.

In catalytic electrochemistry, the enzyme electrode loaded with the high cross-linked redox polymer and high enzyme contents showed prepeaks at low glucose concentrations in CVs. This is due to the depletion of substrate concentration in the vicinity of the electrode surface. Hence the recycling rate of the redox sites were reduced and the catalytic current increase was suppressed. The enzyme electrode with the ferrocene containing redox peptide showed catalytic current.

In this paper, detailed electrochemical properties of an enzyme electrode based on ferrocene-containing redox polymer was exhibited. I demonstrated many interesting phenomena such as pH dependence of the redox property and prepeak appearance for catalytic electrochemistry. A prepeak appearance was first reported for the electrochemical behaviors of an enzyme electrode co-immobilized with a redox polymer. These results provide us with important information for future fabrication of biosensors based on redox polymers. I demonstrated new biosensors based on redox peptide.

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- [2] S. Koide, K. Yokoyama, “Development of the enzyme electrode based on novel redox peptide” (in preparation)

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