Electrochemistry

— Communication —

Effect of Secondary Structure of Polypeptide SAM on Redox Reaction of Fe(CN)$_6^{3-}$

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Received March 1, 2008 ; Accepted July 16, 2008

We synthesized an α-helix forming polypeptide (CKS30) and a random-coiled peptide (CdIK30) in order to clear the effect of secondary structure of the modifier on the electrochemical reaction in solution. By changing the surface structure of the CKS30 from random-coil to α-helix, the electrochemical reaction of Fe(CN)$_6^{3-}$ was suppressed. In contrast, on the CdIK30 modified electrode, the oxidation and reduction peak currents of the cyclic voltammogram increased.

Key Words : Secondary Structure, Switching Device, Modified Electrode, Polylysine

1 Introduction

In order to construct surface structures to control the electrochemical or chemical properties, the self-assembled monolayer (SAM) with the thiol group has been widely used. In such SAM, it has been shown that the intermolecular interaction and the molecules-substrate interactions effect cooperatively and/or interactively to form a highly ordered monolayer structure. Polycl-lysine is one of the most popular polypeptides which shows structural change between the random coil and α-helix by pH or concentration of ClO$_4^−$. Recently, we synthesized three different length of poly-lysine containing a -SH group at the terminal. The synthesized polypeptides were adsorbed on an Au(111) surface in order to characterize their structure and the formation process of SAMs on the Au(111) surface through the use of AFM and FT-IR RAS. We discussed the correlation between the degree of polymerization and the assembled surface structure, and the molecular orientation within the self-assembled molecular layer. On the other hand, in the case of amino acid-modified electrodes having ionic moieties, the electrochemical reactions depend on the pH and/or ionic strength of the solution. The electron transfer rate is very sensitive to the properties of the electrode surface and several types of switching or sensing function have been reported. However, it has not been cleared whether the secondary structure of the modifiers on the electrode plays a significant role to the redox reaction in the solution or not.

In this study, we synthesized two types of polypeptides shown in Fig. 1. One is CKS30, which shows structural change between the random coil and α-helix by controlling pH or concentration of ClO$_4^−$. The other is CdIK30, which shows no structural change, since l-lysine and d-lysine moieties are connected alternatively in series. Using these two types of polypeptide-modified electrode, we will show the effect of secondary structure of the polypeptide on the redox reactions in solution.

2 Experimental

CKS30 and CdIK30 were prepared by a peptide synthesizer (PE Biosystems, Pioneer) according to the Fmoc solid phase method reported previously. The circular dichroism spectra of the polypeptides were measured at 25°C on a JASCO J-720 spectrophotometer using an optical cell of 1.0 cm path length. An Initiun AFFINIX Q quartz crystal microbalance (base frequency: 27 MHz) was used to estimate the amounts of the adsorbed polypeptides on the electrode surface.

Electrochemical measurements were carried out by a BAS-50 W electrochemical analyzer (BAS Co.). A Pt plate and Ag/AgCl (saturated KCl) were used as a counter and reference electrodes, respectively. An evaporated gold film (ca. 150 nm) electrode on a mica substrate, annealed above a hydrogen flame for 30 s, was used as a working electrode. Modified electrodes were prepared by dipping the gold film electrode in an 1 mM the peptide of methanolic solution for 1 hour. After the modification, the peptide-modified electrode was further modified with 20 μM hexanethiol (HT) of methanolic solution by dipping it for 20 min.

NH$_2$-(l-Lys)$_{30}$-Cys-CONH$_2$ : CK30

NH$_2$-[(d-Lys)-(l-Lys)]$_{15}$-Cys-CONH$_2$ : CdIK30

Fig. 1 Chemical Structure and abbrevation of polypeptides with a cystein at the terminal as an anchor to the electrode surface. All the lysines in CK30 are l-form (a) and d- and l-lysines are bonded alternatively in CdIK30 (b).
3 Results and Discussion

Figure 2 shows CD spectra of CK30 in (a) 1.0 M NaClO₄, (b) methanol, and (c) 0.1 M NaClO₄ + 0.9 M NaCl, and CdlK30 in (d) 1.0 M NaClO₄, (e) methanol, and (f) 0.1 M NaClO₄ + 0.9 M NaCl. From the CD spectrum of CK30, it can be seen that CK30 mainly forms the random-coil structure in aqueous solution containing 0.1 M ClO₄⁻ shown in Fig. 2(c). In addition, the spectra of CK30 in 1.0 M NaClO₄ and methanol show the two negative peaks at 208 and 222 nm corresponds to the α-helical structure (Fig. 2(a), (b)). In contrast, the CD spectra of CdlK30 in all the solvent or solution used in this experiment showed no signal from 180 nm to 700 nm as we expected (Fig. 2(d)-(f)). These results show that CK30 changes its secondary structure by changing the concentration of the ClO₄⁻. Since l-lysine and d-lysine are connected alternatively and the number of the l-lysine equals to that of d-lysine in CdlK30, the CD signals due to l-lysine were cancelled by that of d-lysine. Therefore, CdlK30 formed the random-coiled structure under all the conditions used here. So far, we have not studied the structural change in CK30 adsorbed on the electrode surface, however, the structural change in CK30 in solution was shown clearly.

Figure 3 shows the time course of frequency change in the QCM, indicating that the adsorption of (a) CK30 and (b) CdlK30 in methanol. When CK30 was injected to the QCM cell, the frequency decreased abruptly. In order to adsorb CK30 in α-helical structure, methanol was used for the QCM measurements. Generally, the frequency stability of the QCM in organic solvent was not so good as that in aqueous solutions. Therefore, maximum frequency change within 10 s after the injection was used to evaluate the amount of the peptides adsorbed. In the case of CK30, the change in the frequency was obtained to be −74 Hz, which corresponds to 46.8 ng of mass change. The cross sectional area of CK30 in the form of the α-helical structure, which was obtained by the space filling model, was calculated to be 2.5 × 10⁻¹⁴ cm². On the other hand, for the CdlK30, the frequency change was −106 Hz, which corresponds to 62.4 ng. Since CdlK30 forms random-coil structure in all the solvents as described before, we did not define the cross sectional area of the CdlK30. Anyway, since the mass change due to the adsorption of CK30 was close to that of CdlK30, the surface concentration of CK30 should be close to that of CdlK30. When the both of cross sectional areas of CK30 and CdlK30 were assumed to be 2.5 × 10⁻¹⁴ cm², the surface excess of CdlK30 and CdlK30 were calculated to be 1.2 × 10⁻¹¹ and 1.6 × 10⁻¹¹ mol cm⁻², respectively.

Figure 4 shows cyclic voltammograms (CVs) of 1 mM of Fe(CN)₆⁴⁻ at CK30/HT and CdlK30/HT modified Au electrode in aqueous solution containing ClO₄⁻ to induce the α-helical structure. As shown in Figs. 4(a) and (c), in the case of low concentration of ClO₄⁻, redox waves of Fe(CN)₆⁴⁻ were clearly observed at CK30/HT and CdlK30/HT. Under these solution conditions, both of the peptides form the random coiled structure. Therefore, the influence of CK30/HT and CdlK30/HT as the modifier on CVs is essentially identical to each other. In contrast, in high concentration of ClO₄⁻, the CV at CK30/HT modified electrode was different from that at CdlK30/HT modified electrode. As shown in Fig. 4(b), at CdlK30/HT modified electrode in 1.0 M NaClO₄, both the anodic and cathodic peak currents were increased, compared with those in 0.1 M NaClO₄ + 0.9 M NaCl. Since CdlK30 forms random coiled structure independent of solution, the change in the CV at the CdlK30/HT modified electrode will be attributed to the change induced by the complexation of the NH₃⁺ group and ClO₄⁻ not accompanied with the structural change in the peptide. On the other hand, as shown in Fig. 4(d), at CK30/HT modified electrode in 1 M NaClO₄, both the oxidation and reduction currents were decreased, compared with those in 0.1 M NaClO₄ + 0.9 M NaCl, and also the peak potential separation value was increased. These results indicate that at CK30/HT modified Au electrode, the apparent diffusion constant and electron transfer rate of Fe(CN)₆⁴⁻ were effected by the structural change from random coil to α-helix. At the HT-modified Au(111) electrode, no redox waves of
Fe(CN)$_6^{3+}$ were observed. Therefore, at the peptide/HT modified electrode, the reaction of Fe(CN)$_6^{3+}$ occurs through the peptide layer. By increasing the concentration of ClO$_4^-$, the peak separation of the CVs increased for both CK30/HT and CdIK30/HT system. The peak separation of Fig. 4(b) was almost equal to that of Fig. 4(d), indicating that the heterogeneous electron transfer rate constant at the random coiled peptide-modified electrode was almost equal to that at the α-helical peptide-modified electrode. So, the differences in CVs were mainly attributed to the mass transfer process through the peptide layer. In this case, the α-helical structure suppresses the diffusion of Fe(CN)$_6^{3+}$ than the random coil does. The observed CV was stable for repeated scans, and when the electrolyte solution was exchanged with 0.1 M NaClO$_4$ + 0.9 M NaCl, the electrochemical response was recovered and the CV similar to Fig. 4(c) was obtained. The change in CV at CK30/HT modified electrode induced by the change in the concentration of ClO$_4^-$ was reproducible for at least 5 times. When the Au electrode was modified with the peptides only (CK30 and CdIK30), similar results with a relatively small current change were obtained.

4 Conclusion

By comparing an α-helix forming polypeptide (CK30) and a random-coiled peptide (CdIK30) as the polypeptide modified electrode, we have found, for the first time, the secondary structure of the peptide plays an important role to control the electrochemical reaction of Fe(CN)$_6^{3+}$.

References