Preparation of Oligopeptide SAM Modified Electrode

Sayaka Oka, Katsuhiko Nishiyama, Isao Taniguchi
Department of Applied Chemistry and Biochemistry,
Kumamoto University, 2-39-1,
Kurokami, Kumamoto 860-8555, Japan

1. In these years, protein engineering has been developed to prepare novel functional proteins. By immobilizing functional protein on the electrode surface, new type of chemical sensing device using the secondary structural change would be developed (Fig. 1). In this study, we synthesize oligopeptide having a α-helix structure and evaluate the secondary structure of the oligopeptide SAM on a gold electrode.

2. The oligopeptide of L-lysine (K) and L-cysteine (C) (CK_n; n (polymerization degree) = 4, 10, 30) having a thiol group at the terminal was prepared using a peptide synthesizer (P. Biosystems, Pioneer) by the Fmoc solid phase method. For example, the ck_{10} sequence is CKKKKKKKKKK. Molecular weight of CK_4, CK_{10}, CK_{30} were determined 642 (calcd. 642), 1445 (calcd. 1403), 4055 (calcd. 3966) by MALDI-TOF/ MS.

3. Effects of polymerization degree and solvent on the secondary structure of the oligopeptide were evaluated by circular dichroism (CD) spectra. CK_{30} showed typical negative peaks at 208 nm and 220 nm for α-helix in 2,2,2-trifluoroethanol (TFE) and methanol (Fig. 2 (a), (b)). On the other hand, CK_{30} showed random coil in H_2O and 1,1,1,3,3,3-hexafluoro-2-propanool (HFIP) (Fig. 2 (c), (d)). In contrast, both CK_4 and CK_{10} showed random coil. α-Helix content of CK_{30} at $[\theta]_{222}$ in TFE and methanol was almost 100%.

The structure of adsorbed CK_n SAM on Au (111) surface was evaluated by FT-IRRAS. The CK_n SAM modified electrodes were prepared by immersing an electrode into a CK_n-SH solution (1 mM (M = mol dm^{-3})). CK_{30} modified Au (111) prepared from TFE and methanol solutions by dipping for 24 hr showed IR signals at 1670 cm^{-1} (C – O: amide I) and 1540 cm^{-1} (N – H: amide II) corresponding to an α-helix structure (Fig. 3). From absorbance ratio of amide I to amide II, the angle of peptide-axis was determined to be 42° (prepared from TFE) and 51° (prepared from methanol), respectively, from the normal line of Au (111) surface (Fig. 3, (a), (b)). On the other hand, CK_{30} modified Au (111) prepared from H_2O and HFIP solutions by dipping for 24 hr showed a β-sheet structure. These results exhibit the secondary structure of CK_{30} at the electrode surface can be controlled by selecting solvent for modification.

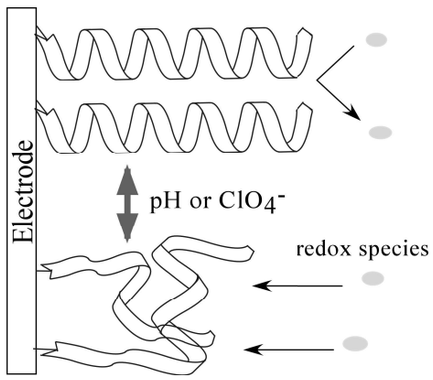

Fig. 1 Schematic representation of structural change for polypeptide modified electrode triggered by pH or ClO_4^-.

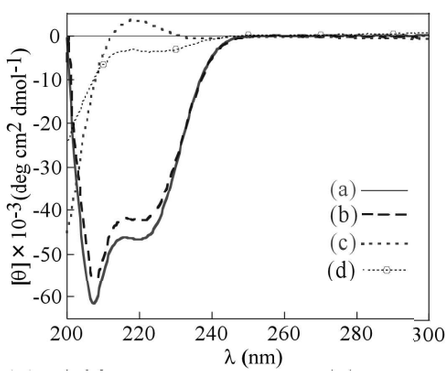

Fig. 2 Circular dichroism (CD) spectra of 1 mM CK_{30} in (a) TFE, (b) methanol, (c) H_2O and (d) HFIP.

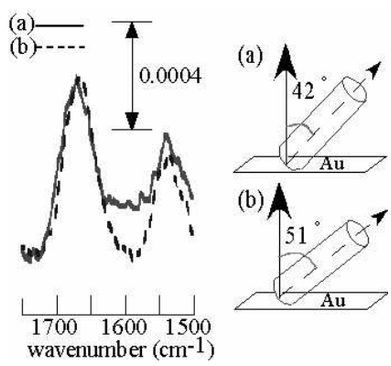

Fig. 3 FT-IRRAS spectra of CK_{30} modified Au (111) prepared from (a) TFE and (b) methanol.