Synthesis of Self-Assembled Redox-Active-Polymer on Electrode Surface for Biosensor Application

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To develop a stable electrochemical biosensor device, a gold electrode surface was modified with a polymerization initiator by the self-assembled monolayers (SAMs) method for surface polymerization. Poly(vinylferrocen-co-acrylamide) as a redox active polymer was living-radical-polymerized at the electrode surface, avoiding a homogeneous polymerization in a solution and physical adsorption of free polymer on the electrode. The living-radical-polymerized redox active polymer showed stable electrochemical response and catalytic enzyme response. The SAM-based synthesis and immobilization of the redox active polymer was carried out. The redox-active-polymer-modified electrode showed a stable electrochemical response after several potential sweeps. The current value increased with increasing in polymerization time. The surface polymerization can be used to control the polymer chain growth on the electrode surface.

1.   Introduction

Synthetic and intravital polymers are usable material in the analytical[1–3] and medical fields[4,5] owing to the multiple molecular interactions compared with smaller compounds. Particularly, reduction/oxidation (redox) active polymers have been studied as electron transfer mediators for medical biosensors.[6–10] These polymers can be used to construct the reusable sensor. The immobilization of an electron transfer mediator and enzyme is very important to avoid desorption from the electrode surface. Immobilization based on the physical adsorption or adhesion of a redox active polymer and an enzyme on an electrode has been carried out.[8,9] This method suffers from desorption or elimination of the polymer and enzyme from the electrode during measurement.

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Self-assembled monolayers (SAMs) have been studied for the chemical immobilization of various materials on the surface. SAMs of organic molecules on Au(111) are becoming increasingly important not only in the areas of fundamental surface science and interfacial electrochemistry[11,12] but also in other areas. For example, in many biomedical applications, SAMs separate a metal surface from a solution.[13–16] Alkanethiols adsorbed on gold are closely packed via thiolate-gold covalent bonds and van der Waals forces between the alkyl chains. This can prevent the desorption of materials from the substrate surface.[17,18] Our previous work showed that a self-assembled polymer is easily immobilized on a gold substrate and can be applied to a biosensor.[19–21] SAM-based and ion-complex enzyme electrodes have showed that a catalytic current value increases upon glucose addition. Also, the electrode showed a stable response after several scans. Therefore, the desorption of the materials from the electrode was prevented when the SAM and ion complex were used for immobilization. The SAM-based and ion-complex immobilization of redox active polymer and enzyme could lead to the construction of reusable enzyme sensors.[22] However, we were constrained to handle several steps before the immobilization of the polymer on the surface. Synthesis of polymer films from only the substrate surface via SAM-based surface living radical polymerization can simplify the experimental procedure and can avoid unworthy polymerization in solution. Scheme 1 shows that SAM-based surface living radical polymerization produces only a polymer chain covalently immobilized to the electrode surface, whereas, conventional solution polymerization yields both immobilized and free polymers. The surface confined redox-active-polymer can smoothly achieved electron transfer. In this study, we report that a gold electrode was modified with a redox active polymer using the SAMs technique and surface living radical polymerization. 4-(Chloromethyl)benzoic acid as a polymerization initiator was attached to cystamine which was immobilized on the gold electrode surface using the SAMs technique. Benzyl chloride groups of 4-(chloromethyl)benzoic acid bonded to the surface form a controlled number of radicals by atom transfer to a Cu-2,2'-bipyridyl as catalyst.[23] Equilibrium is maintained between the active and inactive chain ends, R• and RCl with the equilibrium favoring the inactive species, minimizing termination and radical transfer reactions, therefore the length of the polymer chain can be controlled by adjusting the polymerization time. Hence, polymerization only occurs on the electrode surface. The chemical immobilized redox-active-polymer by SAM has flexibility unlike the physical immobilization of the polymer via the crosslinking reagent.[9] The obtained electrode shows a stable electrochemical response.

2. Experimental

2.1 *Materials*

Cysteamine, 4-(chloromethyl)benzoic acid, and vinylferrocene (Vfc) were purchased from Aldrich (USA). Copper(I), $_D$(+)-glucose 2,2'-bipyridyl chloride and dimethylformamide, acrylamide (AAm) monomer, sodium dihydrogenphosphate dihydrate, *N*-methylmorpholine (NMM), dimethylformamide (DMF), and disodium hydrogenphoshate 12-water were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The glucose solution was equilibrated overnight prior to measurements.

(a)

(b)
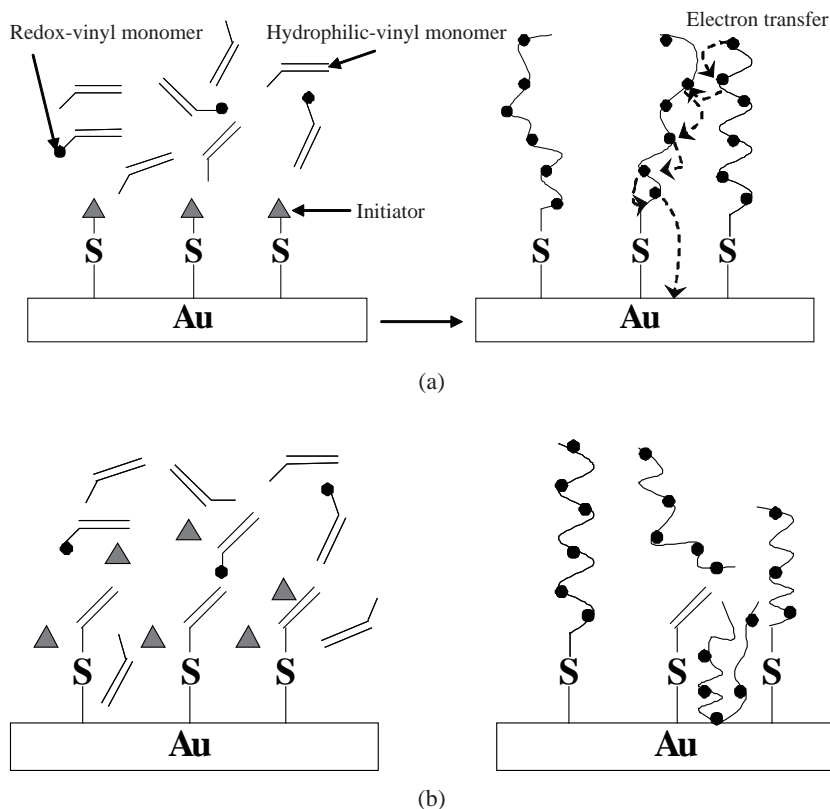
Scheme 1.   Schematic illustration of (a) SAM-based surface-confined living radical polymerization and (b) solution polymerization.

Glucose oxidase (GO*x*) from *Aspergillius niger* type XS 25,000 units/g was purchased from Sigma (USA).   GO*x* content was determined to be 6.71×10^{-9} [mol/mg-solid] by measuring the FAD content.   The FAD concentration was determined from the absorbance at 450 nm using $\varepsilon 450 = 14.1$ mM^{-1} cm^{-1}.[24]   The ratio of GO*x* bound to total FAD molecules was measured using gel filtration chromatography (TOSOH HPLC, TSK-gel G3000SWXL, Japan) and was found to be 86%.   Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) and *N*-hydroxybenzotriazole (HOBt) were purchased from Merck (Tokyo, Japan).   Ultrapure water (18.3 MΩ-cm) from Millipore was used in all the experiments.

2.2   *Homogeneous polymerization of redox active polymer*

4-(Chloromethyl)benzoic acid (3.0 mM) as initiator, Vfc (17 mM), AAm (0.86 M), copper(I) chloride (2.9×10^{-1} mM) and 2,2'-bipyridyl (7.9×10^{-2} mM) as catalysts

were dissolved in DMF. Radical polymerization was carried out at 130°C for 3 or 6 h. The molecular weight of the obtained redox active polymer was determined by Gel Permeation Chromatography (GPC). GPC was carried out using Tosoh TSKgel G4000PWXL column and a UV detector (254 nm) at 40°C. Poly(ethylene oxide) (PEO) (MW: 920,000, 540,000, 250,000, 140,000, 100,000, 50,000, 240,000, 2,000 and 200) was used to prepare calibration curve.

2.3 *Surface-initiated radical copolymerization of Vfc and AAm*

A gold disk electrode of 6 mm diameter was used. The gold electrode was rinsed with methanol, followed by soaking in cysteamine solution (1 µM) for 2 h. The amino group of cysteamine on the gold electrode surface was modified with 4-(chloromethyl)benzoic acid (4.6 mM) using PyBOP (9.2 mM), HOBt (9.2 mM), and NMM (9.2 mM) (Fig. 1(a)). Then, Vfc (18 mM), AAm (0.9 M), copper(I) chloride (0.3 mM), and 2,2' -bipyridyl (7.9×10^{-2} mM) were sonicated for 1 min to accelerate the dissolution in DMF. 4-(Chloromethyl)benzoic acid-immobilized electrode was immersed in this solution. Surface radical polymerization was allowed to proceed at 130°C for 3, 6 and 12 h. After the reaction, the electrode was washed several times with distilled water to remove the physically adsorbed monomer reagent. A schematic description of the polymerization of the redox active polymer on the gold electrode surface is given in Fig. 1(b).

2.4 *Electrochemical measurement*

A three-electrode configuration was used. A SAM-based redox-active-polymer-modified electrode, a platinum wire, and Ag/AgCl/KCl were used as the working, auxiliary, and reference electrodes, respectively. All the potentials herein are quoted with respect to these electrodes. Unless otherwise stated, cyclic voltammetry (CV) was carried out in a deoxygenated phosphate buffer (0.1 M pH 7.0) with a computer-controlled potentiostat (CV-50W BAS) at room temperature.

3.   Results and Discussion

3.1 *Characterization of homogeneous polymerized redox polymer*

We investigated the effect of polymerization time on the molecular weight (Mw) of the redox active polymers. GPC elution curves showed that the Mw of the redox active polymers did not increase with the increasing in the polymerization time. The Mw of both redox active polymers was estimated as Mw 450,000 (Fig. 2). The result indicated that the reaction rate was very fast and the termination reaction occurred in a homogeneous system.

Homogeneous polymerized redox active polymers were easily dissolved in buffer solution on showed a typical electrochemical response: polymerization time of 3 on 6 h, respectively. We investigated the effect of polymerization time on the peak-to-peak potential separation (ΔEp) of redox active polymer. Both ΔEp values showed almost same (~80 mV) which did not correlate with polymerization time.

The catalytic electrochemistry of GO*x* with the redox-active-polymer-based enzyme electrode with glucose was investigated. A catalytic current was observed upon glucose
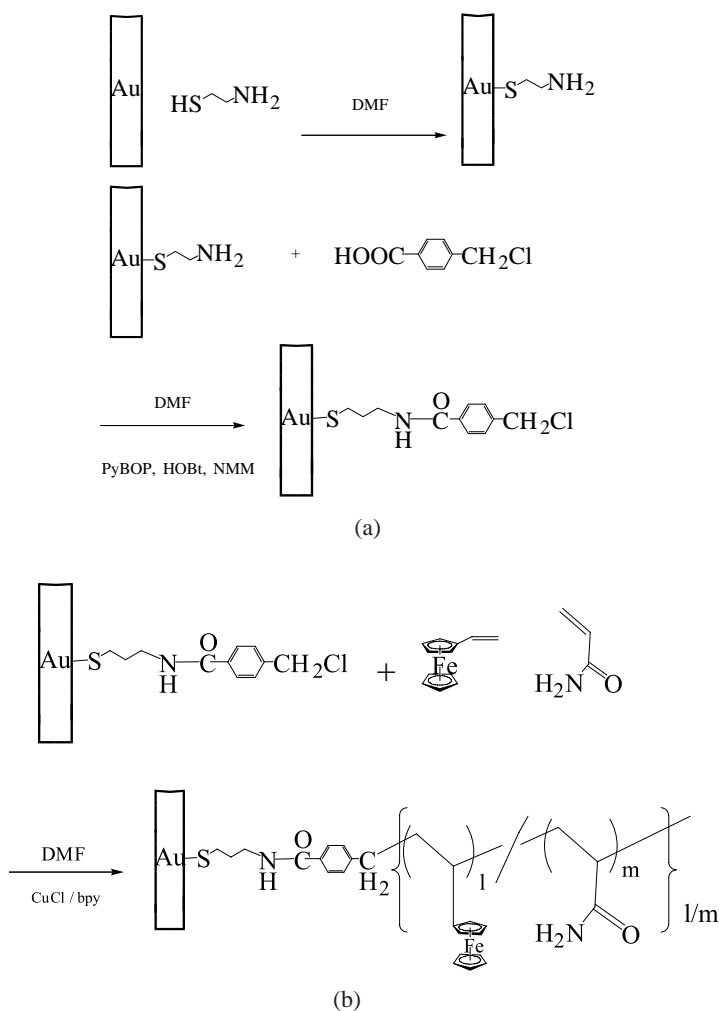
Fig. 1.   (a) SAM-based immobilization of cysteamine and 4-chloromethyl benzoic acid on gold surface, (b) SAM-based co-polymerization on gold surface.

addition (Fig. 3). The peak current value of the catalytic response showed a positive shift compared with that without the glucose substrate. The phenomenon occurred owing to the increase in concentration of the reduced mediator at the electrode of as high as the substrate concentration (0.1 M). Catalytic current values at +280 mV increased to 2 (Fig. 3(a)) and 1.4 (Fig. 3(b)) times, respectively. The copolymer of Vfc and AAm has redox and mediator abilities.
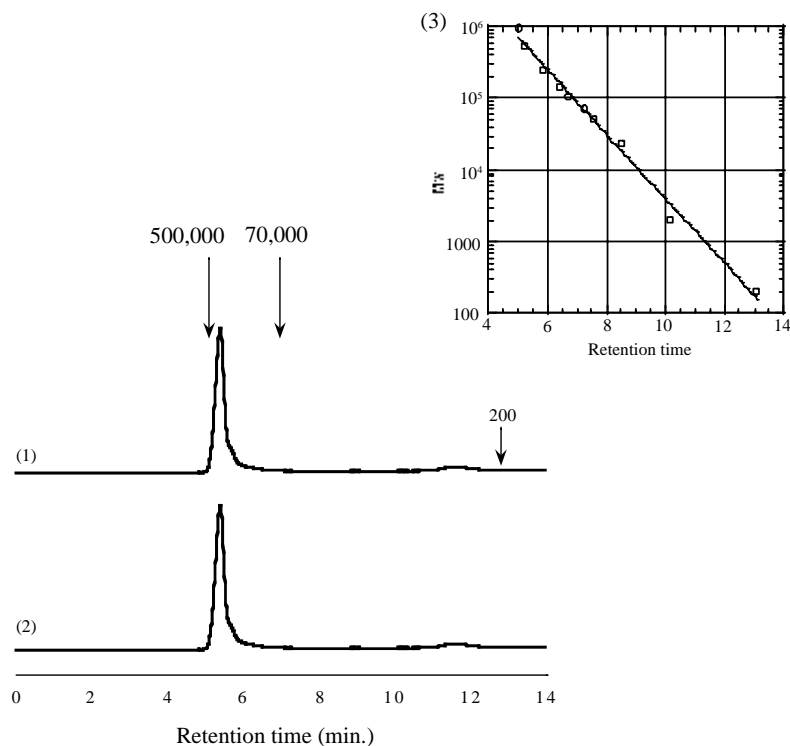
Fig. 2.   GPC elution curves of poly(VFc-co-AAm); polymerization time of (1) 3 on (2) 6 h. (3) GPC calibration curve of standard PEO.

3.2   *Stability of electrochemical reaction for SAM-based redox-active-polymer-immobilized electrode*

The redox-active-polymer-modified electrode was characterized by CV. Figure 4 shows typical cyclic voltammograms of the redox active polymer modified electrode that was surface-polymerized for 3 (Fig. 4(a)) on 6 h (Fig. 4(b)). To remove the physically adsorbed Vfc monomer, the electrode potential was held at 500 mV for 8 min, followed by changing the buffer solution; viz., Vfc monomer become hydrophilic by maintaining the positive potential on the electrode. Thus the only chemically adsorbed redox active polymer using SAM was retained on the electrode. Both electrodes showed a stable response after the 5th measurement because of the no difference in the response between the 5th and the 6th measurements (Figs. 4(a) and 4(b) (lines (3) and (4)), although the response decreased compared with the 1st response. We deduce that the first large abatement occurred owing to the absolute elimination of the excess physical adsorption of redox monomers on the surface. For Fig. 4(b), the rate of change of the electrochemical response was smaller than that shown in Fig. 4(a). We compared the
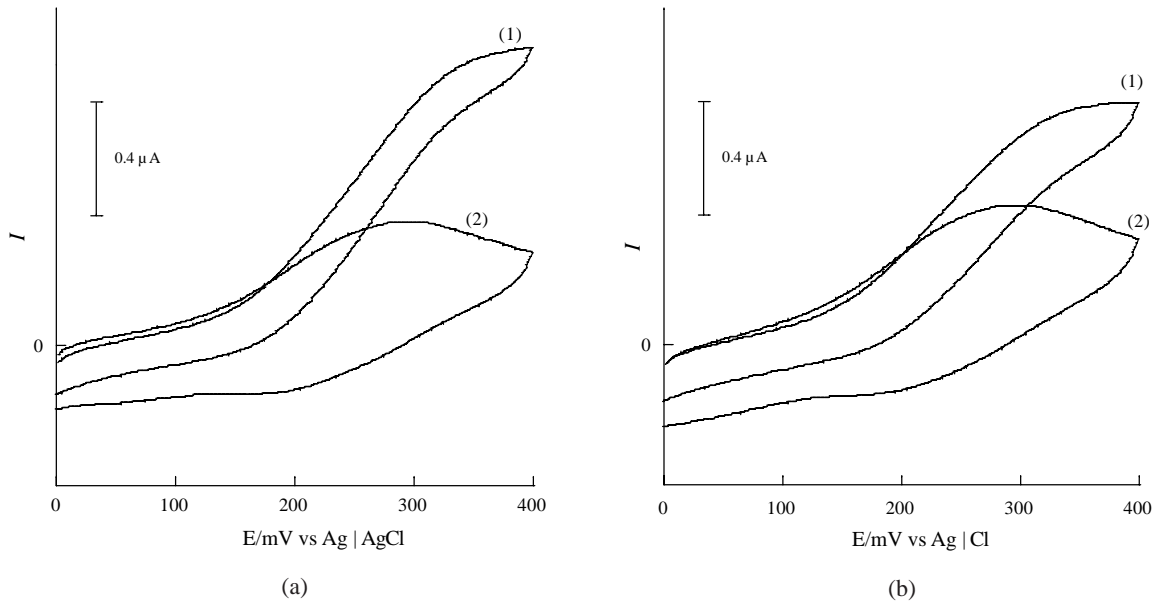
Fig. 3. Catalytic electrochemistry of enzyme with 2 mg of redox active polymer: polymerization time of (a) 3 on (b) 6 h. Cyclic voltammetries were carried out at scan rate of 5 mV/s in 0.1 M phosphate buffer (pH 7.0) and 0.1 M glucose (1) with and (2) without 33.5 µM GO*x*.
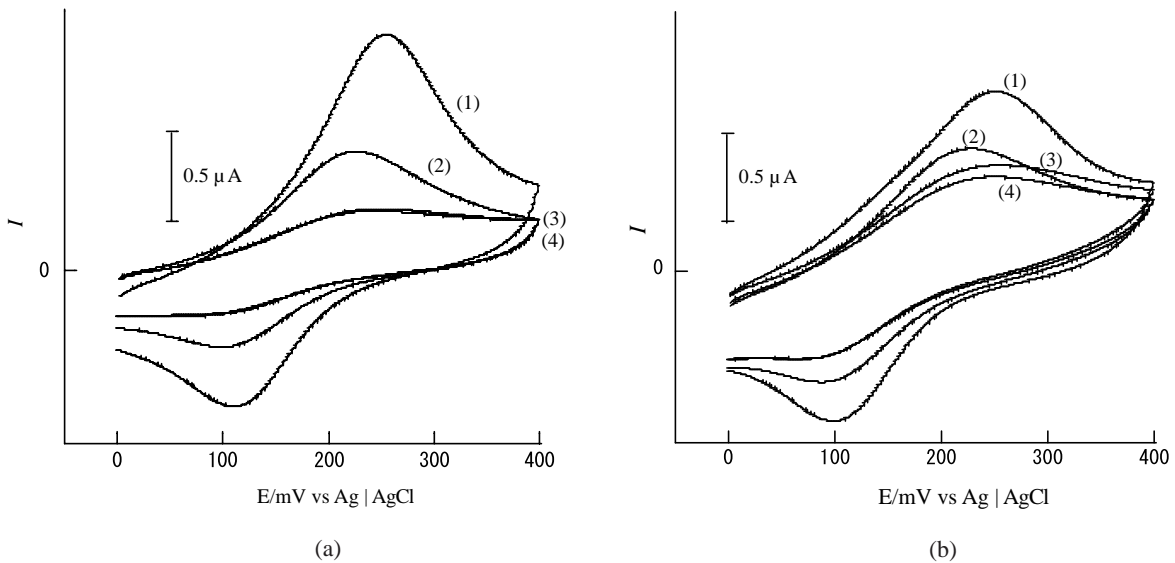

Fig. 4. CV curves of SAM of poly(VFc-co-AAm)-immobilized Au electrode: polymerization time of (a) 3 on (b) 6 h. Cyclic voltammetries were carried out at a scan rate of 5 mV/s in phosphate buffer (pH 7.0). The electrode was held at 500 mV for 480 s after the 1st measurement. The buffer solution was changed after the 1st, 3rd, and 5th measurements. CV measurements of (1) 1st, (2) 2nd, (3) 5th, and (4) 6th.

1st oxidation peak value and that of the 6th measurement. The abatement ratios were 74% (Fig. 4(a)) and 47% (Fig. 4(b)), respectively. This presumably indicated that the longer polymerization can avoid the excess physical adsorption of the monomer reagent namely the polymer density increases with increasing polymerization time. Nonetheless, this decrease in response owing to settling redox ability of Vfc does not affect the use of this electrode as sensor device. The oxidation and reduction peaks of Figs. 4(a) and 4(b) at 6th measurement were observed at (+243, +102 mV) and (+246, +100 mV) (vs Ag/AgCl), respectively. For $\Delta E p$ of the redox-active-polymer-immobilized electrode, these values (141 and 146 mV) increased in comparison with that of the homogeneous system (~80 mV) at the given scan rate. Electron transfer on the redox-active-polymer-immobilized electrode was slower than that on the homogeneous one owing to the limitation of polymer flexibility by the SAM-based immobilization.

3.3   *Effect of polymerization time*

The effect of surface polymerization time was investigated by CV. In the homogeneous polymerization system, the obtained polymers showed almost the same Mw and CV responses. These results are suggested that the termination reaction occurred in the early stage of polymerization. We carried out surface living polymerization for 3 and 6 h to confirm the effect of polymerization time on the electrochemical response. We compared these stable electrochemical responses after the 6th measurement (Fig. 5). These CV curves showed the current value dependence on the polymerization time. Polymer chain growth on an electrode depended on the polymerization time. For this reason, surface-initiated polymerization occurred more slowly than the homogeneous system. In addition, a very long polymerization time (12 h) did not increase the electrochemical response compared with the polymerization time of 6 h,
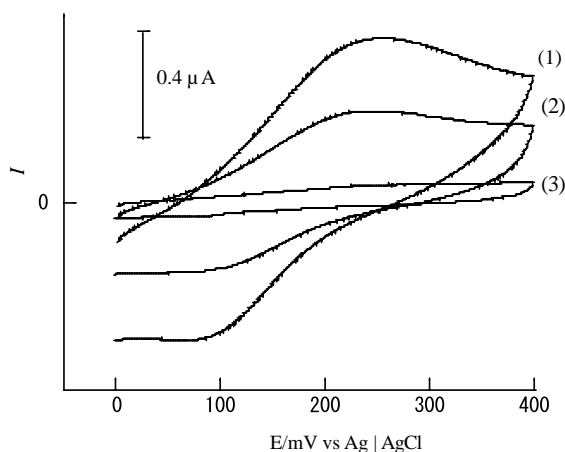

Fig. 5.   CV curves of SAM of poly(VFc-co-AAm) immobilized Au electrode: surface polymerization time of (1) 6, (2) 3 on (3) 0 h. Cyclic voltammetries were carried out at a scan rate of 5 mV/s in 0.1 M phosphate buffer (pH 7.0).

because the termination reaction also occurred for excess long synthesis time (data not shown). During this time, the electrochemical current value increased with increasing polymerization time within 6 h. It is noteworthy that the amount of redox active polymer on an electrode surface can be controlled by adjusting the polymerization time owing to the adoption of the surface living polymerization method.

4. Conclusions

In this work, our electrode can retain redox-active-polymer after washing owing to the covalently attachment between the initiator and electrode surface by SAM. These polymer-immobilized electrodes showed a stable response after six measurements. We could control the electrochemical response by adjusting the surface polymerization time. Particularly, the electrode that is polymerized for 6 h showed a stable electrochemical response (Fig. 5, line (1)). The limit for the mediator active has these SAM-based redox-active-polymer-immobilized electrodes. In the homogeneous system, we demonstrated the redox-active-polymer-mediated enzyme reaction; we expect that future optimization will enable us to analyze the enzyme reaction. Our current effort to solve this problem is to elucidate the factors involved in the effect of polymer surface density and the ratio of Vfc to AAm in one polymer chain. In this time, we synthesized poly(VFc-co-AAm) (i.e., the molar ratio of Vfc to AAm was 1:50). Thus, the amount of redox active sites in the polymer chain was presumably small. Nevertheless, we foresee a large potential of living surface polymerization for the very practical preparation of reusable redox active electrode, because this electrode shows stable electrochemical response after several measurements. Hence, it is expected that this electrode can detect the reaction between the enzyme and substrate by detecting the electron transfer between the enzyme and redox polymer on the electrode surface.[9,22]

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