Communications to the Editor

Irreversible Adsorption and Electron Transfer Reaction of Cytochrome *c* on Poly(methacrylic acid) Segment-Carrying Triblock-Polyion-Complex Monolayer Electrodes

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In our recent reports,[1-3] we have described interfacial properties of the surface monolayers and the Langmuir–Blodgett (LB) multilayers from triblock-polyion complexes composed of triblock polyions (poly(styrenesulfonate)-*block*-poly(methacrylic acid)-*block*-poly(styrenesulfonate)) and cationic lipids. In those monolayer assemblies, the poly(methacrylic acid) (PMMA) segment has been used as a conformation-changeable moiety to model protein molecules embedded in biomembranes. In fact, the triblock-polyion complexes form well-behaved surface monolayers that are affected by pH change in the subphase due to conformational transition of PMAA segments, and also these PMAA segments in LB multilayers function as a permeating pathway for ions. In this paper we describe the preparation of a triblock-polyion-complex monolayer of an SH-carrying polyanion (1) and a cationic lipid (DOTU) on gold electrodes and cytochrome *c* (cyt *c*) adsorption at the monolayer surface. Cyt *c* is a typical redox protein that has been well investigated in terms of electron-transfer reactions between protein and gold electrodes modified with tin oxide[4,5] and bis(4-pyridyl) disulfide.[6] A highly desirable goal in this regard would be the development of electrode/adsorbed protein systems which contain the controllable and well-defined interface at the molecular level. Recently, Tarlov et al. showed the usefulness of carboxylic acid-terminated alkanethiol monolayer electrodes to the electron-transfer reaction of cyt *c*.[7] We report here preliminary results on the use of a triblock-polyion-complex (1/DOTU) monolayer on gold to study the electron-transfer of cyt *c*. In this approach, cyt *c* was irreversibly adsorbed on PMAA segments embedded in lipid monolayers, and the redox behavior of the cyt *c*-bound monolayer electrode was examined by using cyclic voltammetry.

The novel polyanion (1) was prepared according to the manner reported previously.[2] A telechelic PMAA having an isopropylxanthate group at both termini of the polymer chain was first prepared by polymerization of methacrylic acid in the presence of bis(isopropylxanthogen) disulfide with UV irradiation. The copolymerization of (mercaptomethyl)styrene and sodium styrenesulfonate was then carried out in water in the presence of the telechelic PMAA as a macrophotoinitiator upon UV irradiation. The number-average molecular weight and the copolymer composition were estimated by means of vapor-pressure osmometry and ^{1}H NMR spectroscopy, respectively. The resulting polyanion was confirmed to consist of a PMAA segment with an average chain length of n = 21 and copolymer segments composed of sodium styrenesulfonate and (mercaptomethyl)styrene (molar fraction, x = 0.08) with an average chain length of m = 51 as shown in Figure 1. Another polyanion (2) without the PMAA segment was also prepared by copolymerization of (mercaptomethyl)-styrene and sodium styrene sulfonate for comparison.

Polyion-complex monolayers of these polyanions with DOTU[8] were prepared on gold via two steps: the polyanion is first adsorbed and immobilized on gold, and then the cationic lipid, DOTU, is introduced into the polyanion-immobilized gold by an ion-exchange reaction. A typical procedure is as follows. The polyanion 1 was immobilized on gold by immersing the gold electrode in an aqueous solution of 1 ([SH] = 5×10^{-5} M) at pH 3.5. The steady-state adsorption was found to be reached after 40–50 h on the basis of measurements of cyclic voltammetric curves with 3 mM Fc-Gly [1-[(ferrocenylcarbonyl)oxy]-2,3-propanediol][9] as the electroactive species and 0.1 M KCl as the electrolyte. The 1-modified electrode thus obtained was immersed in an aqueous dispersion of DOTU (5×10^{-3} M) at pH 3.5, at which carboxylic acid groups in the PMAA segment are protonated and thus the cationic lipid is expected to complex preferentially with poly(sodium styrenesulfonate) segments of 1, and simultaneously traced with cyclic voltammetry. The peak current (i_p) immediately decreased and reached equilibrium after a few minutes, implying rapid adsorption of DOTU onto the 1-modified electrode through ion exchange. A further decrease in i_p took place by annealing this electrode in water at 60 °C for 30 min, and then finally no electrochemistry attributable to Fc-Gly was observed, indicating that the monolayer blocks the electrochemical communication of Fc-Gly with the electrode. Characterization of the resultant 1/DOTU monolayers was made by X-ray photoelectron spectroscopy and ellipsometry. As a result, the sulfur/nitrogen ratio of the 1-modified gold with DOTU, which includes two nitrogen atoms per molecule, was determined to be 1:2.1 (±0.3), in close agreement with that (1:2) expected from complete pairing of the sulfonate anion in 1 and the ammonium cation in TODU, and the ellipsometric thickness of the same monolayer was estimated to be 35 (± 1) Å.

To reveal the pH dependence of this 1/DOTU monolayer-modified electrode on electrochemical responses, cyclic voltammograms were measured at various pHs in an electrolyte solution with 3 mM Fc-Gly and 0.1 M KCl. Figure 2 shows plots of the maximum reduction current (i_{pc}) as a function of pH, in which data for a 2/DOTU monolayer-modified electrode are also included. For this electrode, as expected, the i_{pc} value was independent of pH and showed almost complete electrochemical blocking toward Fc-Gly. On the other hand, the 1-modified electrode exhibited a remarkable pH dependence: the i_{pc} value increased drastically in the region of pH 5–7 with increasing pH. Such a pH region is well consistent with that in which PMAA in an aqueous solution causes conformational transition from a compact hypercoiled to an expanded form.[10] When the pH was lowered, the i_{pc} value reverted to the original value with only slight hysteresis. The observed reversible change in i_{pc} should

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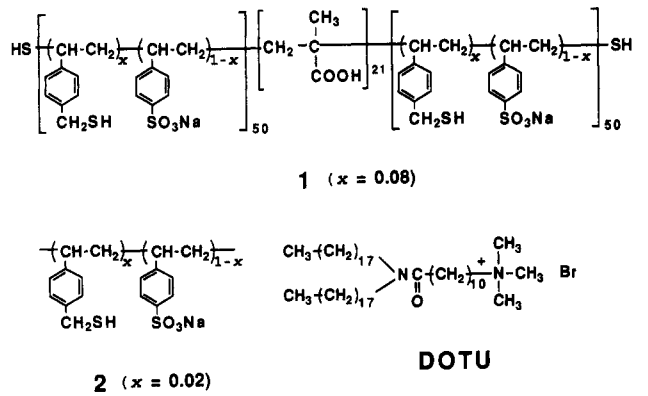

Figure 1. Structures of SH-carrying polyanions with or without the poly(methacrylic acid) segment and cationic lipid.

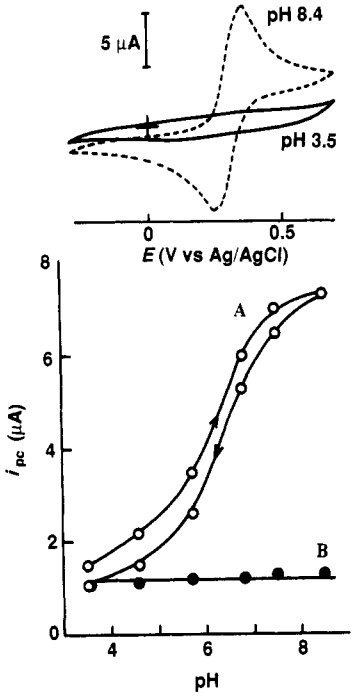

Figure 2. Changes in cyclic voltammetric curves for the 1/DOTU monolayer (A)- and the 2/DOTU monolayer (B)-modified electrodes by varying pH in electrolyte solutions (0.1 M KCl) with 3 mM Fc-Gly. The top shows typical cyclic voltammetric curves for the 1/DOTU monolayer-modified electrode at the prescribed pH. The bottom shows plots of i_{pc} as a function of pH.

be ascribed to the conformational change of PMAA segments in the 1/DOTU monolayer: i.e., at a low pH (e.g., pH 3.5), PMAA segments adopt a hypercoiled form in order to minimize hydrophobic interactions and thus exist in more hydrophobic sites such as the lipid monolayer phase, but at a higher pH, the PMAA segments prefer the water phase to the lipid monolayer phase due to their hydrophilicity.

On the basis of these results, a 1/DOTU monolayer surface at pH 7 was selected to provide favorable electrostatic binding with cyt *c*. At pH 7, the monolayer surface is expected to have negative charges of the deprotonated PMAA segment, which would lead to attractive interaction with positively charged lysins on the surface of cyt *c*, as well as hydrophobic domains of aligned DOTU alkyl chains. The cyt *c* adsorption process was performed by immersing the 1/DOTU monolayer-modified electrode in a solution with 30 μM ferricyt *c* (Sigma type VI horse heart cyt *c*) in 4 mM, pH 7 phosphate buffer and allowing 30 min for equilibration, after which the electrode was rinsed three times with the same buffer. Figure 3A shows a typical cyclic voltammogram obtained for cyt *c* adsorbed

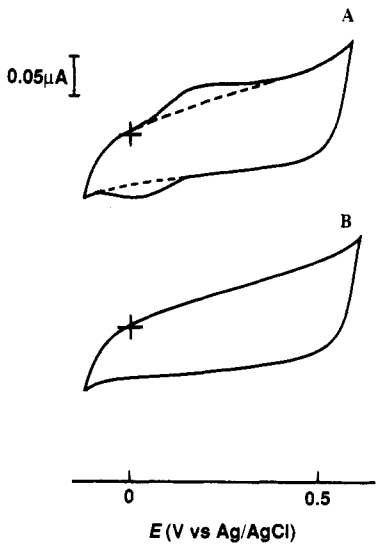

Figure 3. (A) Cyclic voltammograms obtained at a scan rate of 50 mV s^{-1} of a 1/DOTU monolayer-modified electrode (dashed) and the same electrode after adsorption of cyt *c* on the 1/DOTU monolayer (solid). The supporting electrolyte is 4 mM potassium phosphate buffer at pH 7. (B) The cyclic voltammogram of a 2/DOTU monolayer-modified electrode after adsorption of cyt *c*. Other experimental conditions are the same as in part A. Electrode area = 0.02 cm^2.

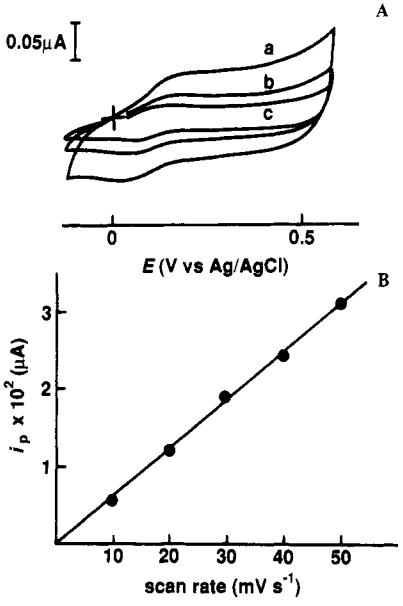

Figure 4. (A) Cyclic voltammograms of cyt *c* adsorbed on a 1/DOTU monolayer at scan rates of (a) 50, (b) 20, and (c) 10 mV s^{-1}. (B) Plot of peak current (i_p) vs scan rate. Solution conditions are the same as in Figure 3.

on a 1/DOTU electrode. The peaks are due to the one-electron redox reaction of adsorbed cyt *c*. Rinsing the electrode with buffer four additional times resulted in nearly identical current–potential curves, which implies that desorption is negligible. When the same experiment was undertaken for the 2/DOTU modified electrode, there was no peak due to cyt *c* adsorption in the voltammogram (Figure 3B). Thus, such cyt *c* adsorption on the 1/DOTU surface is considered mainly due to electrostatic binding with PMAA segments. The cyt *c*/1/DOTU surface formal potential was 55 mV (vs Ag/AgCl), which agrees well with that (60 mV) of native cyt *c*,[6] suggesting that the interaction of cyt *c* with 1/DOTU may be similar to that found in biological systems. Figure 4A shows cyclic voltammograms of the cyt *c*/1/DOTU electrode obtained at different potential scan rates, and the peak current based on those

voltammograms are plotted against scan rate in Figure 4B. The peak current is found to vary linearly with scan rate. This again indicates that cyt *c* is surface-bound to the 1/DOTU monolayer and is not subjected to diffusion to the electrode. From these voltammetric data, we attempted to estimate the electron-transfer distance (d). Peak separations in Figure 4A were used to calculate an apparent electron-transfer rate constant (k^0) of 0.4 s^{-1}.[11] Using the conventional expression,[7,12] we calculated an electron-transfer distance of d = 34 Å, which is well consistent with the thickness of the 1/DOTU monolayer (35 Å) estimated by ellipsometry.

In summary, we have demonstrated that triblock-polyion-complex monolayers containing PMAA segments provide a favorable environment for adsorption of cyt *c* and its effective electron transfer. These phenomena are obtained due to combined properties of the polyelectrolyte and the lipid monolayer, which may serve as useful analogues of biological membranes.

References and Notes

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(12) Gray, H. B.; Malmstrom, B. *Biochemistry* **1989**, *28*, 7499, in which the apparent electron-transfer rate constant, k^0, is expressed with the electron-transfer distance, d, and some thermodynamic parameters as in the following equation: $k^0 = \upsilon \exp[-\beta(d - d_0)] \exp(-\Delta G^*/RT)$, with $\upsilon = 10^{13}$ s^{-1}, $\beta = 1.0$ $Å^{-1}$, $d_0 = 3$ Å, and $\Delta G^* = 11$ kJ mol^{-1}.