Short communication

Double-layered polyion complex for application to biosensing electrodes

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A B S T R A C T

Polyion complex (PIC) layers are formed via self-assemble ion pairing by alternately placing polycation/polyanion solutions on platinum electrodes. Their molecular sieving behavior for electrochemically reactive species through the layers is examined by electrochemical methods. Different molecular-sieve sizes are achieved by the selection of polycations with different intramolecular distances of neighboring cationic groups. Amperometric glucose sensing electrodes are fabricated by immobilizing glucose oxidase in a PIC double-layer. The proper double-layer configuration is efficient to improve the glucose response, blocking interferents due to the molecular sieving effect.

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1. Introduction

Demands for simple, reliable, and inexpensive assay for glucose have been increasing for clinical and nourishment applications. Glucose biosensors utilizing electrochemical transduction with enzymatic and non-enzymatic electrodes enable to monitor glucose concentration in analytes readily and precisely according to a literature by Toghill and Compton [1]. For enzyme-immobilized electrodes, it is known that polyion complex (PIC) with ionically-bonded polymer network structures is one of the most suitable matrixes for enzyme immobilization without any additional covalent modification of enzyme, which is beneficial to avoid denaturation of enzyme [2]. Enzyme immobilized PIC electrodes for lactate-biosensing were first developed in 1995 [3]. The polycomplex exhibited unique characters due to its specific physical and chemical properties [2,4,5], and recently, the enzyme-immobilized PIC electrode with high-performance has been realized in the study of biofuel cells [6,7].

Mizutani and coworkers reported that the PIC membrane which consisted of poly-L-lysine and poly(4-styrenesulfonate) showed permselectivity with a cut-off molecular weight of ca. 100 [3]. Thus the PIC layer was effective for eliminating electrochemical interferents in analyte, such as ascorbic acid (M.W. 176.1) and uric acid (M.W. 168.1), whereas smaller molecules like hydrogen peroxide produced by enzyme-catalyzed reaction easily permeated through the PIC [8,9]. PIC is known to have high hydrophilicity and forms nanosized-pores [2–4] that allow certain smaller molecules (M.W. <100) to penetrate and diffuse inside the pores while blocking relatively larger molecules. Furthermore, the pore size can be controlled by selecting polyanions/-cations combination based on the ladder polymer structure

for PIC [10–12], and one of the successful examples of nanopore regulation was reported on a potentiometric biosensor for creatinine which is not permeable in typical PIC due to its larger molecular size [11].

Because of strictly limited permeation of glucose (M.W. 180) in typical PIC, glucose oxidase (GOx)-containing PIC electrode could not show acceptable anodic response to glucose. This problem was successfully solved by the use of covalent immobilization of GOx on the surface of NH_3^+-terminated PIC with glutaraldehyde [13], however, the covalent immobilization possibly results in enzyme denaturalization. In this study, we evaluate the combination of polycation and polyanion for the PIC membrane and examine each molecular-sieving character. Since the molecular sieve is controlled by side-chain length of polycations used, we successfully demonstrate high selectivity/stability biosensing for double-layered PIC electrodes in which GOx is physically entrapped without any covalent bonds. The multi-layer PIC electrode is expected to have high potential for the broad application to high selectivity, sensitivity, and stability molecular functional electrodes including biosensors.

2. Experimental

Poly(L-lysine)-hydrobromide (PLL, Sigma-Aldrich, Mw > 300,000), poly(allylamine)-hydrochloride (PAm, Nittobo Co., Mw ≈ 150,000), poly(acrylic acid) (PAA, Sigma-Aldrich Inc., Mw ≈ 750,000), Nafion® (as 5% m/m alcohol–H$_2$O solution, Sigma-Aldrich), glutaraldehyde (GA, as 50% v/v of aqueous solution, Kanto Chemical Co.) and GOx from *Aspergillus niger* (EC 1.1.3.4, Toyobo Co. Ltd.) were purchased and used without further purification.

For preparation of PIC electrodes, Pt disk electrodes (1 mm in diameter) were polished with waterproof abrasive paper no. 2000, carefully rinsed and then sonicated in distilled water. A PIC layer was formed on the electrode as follows. Concentration of PAm, PAA and PLL in aqueous was adjusted to 30 mmol dm^{-3} (mM) based on a monomer unit, and

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GOx solution was prepared in a concentration of 80 mg cm^{-3}. 5 mm^3 of polycation and 5 mm^3 of polyanion solutions were alternately dropped on the electrode surface 5 times, and then dried in vacuum to form a PIC layer attached on the electrode. For double-layered PIC electrodes in which GOx is immobilized, two different PIC layers were prepared and stacked as follows: (i) 5 mm^3 of PAm and 5 mm^3 of PAA solutions were successively dropped on the Pt electrode surface 3 times, and then dried in vacuum to form the inner PIC layer, (ii) 15 mm^3 of GOx solution containing 40 mM phosphate buffer was dropped and dried, and (iii) 15 mm^3 of PLL and 15 mm^3 of PAA solutions were successively dropped on the surface 3 times and dried to deposit the outer PIC layer. The GOx electrodes were also prepared by using GA and Nafion® instead of PIC for comparison; 15 mm^3 of GOx solution was dropped on the Pt electrode. After drying in vacuum, 15 mm^3 of 1.0 vol.% GA aqueous solution or the Nafion® solution was dropped on the electrode and then dried. The GOx-immobilized electrodes were stored in 40 mM phosphate buffer solution (PBS, pH 7.0) when not in use.

Electrochemical evaluation was conducted with a three-electrode beaker-type cell which consists of the PIC working electrode, platinum wire counter electrode, and Ag/AgCl electrode as reference electrode with 5 mM K$_4$[Fe(CN)$_6$] and/or other interferents such as uric acid and ascorbic acid dissolved in the PBS. The amperometric response to glucose was measured in 40 mM PBS (pH 7.0) at 37 °C under atmospheric condition.

3. Results and discussion

Fig. 1a shows molecular structures of PICs used in this study. To obtain the nano-pore formation in ionically bridging structure of PIC [11,12], two polycations with $-$NH$_3^+$ groups, PAm or PLL, are ionically paired with PAA, hereinafter denoted as following abbreviation; PAm–PAA and PLL–PAA. PLL has a longer side chain and interval to the $-$NH$_3^+$ groups than PAm. PLL–PAA is, therefore, expected to have ladder polymer network structures with larger mesh (pore) size [12].

Fig. 1b shows cyclic voltammograms of the PLL–PAA and PAm–PAA modified and bare Pt electrodes in the ferrocyanide solution. Clear redox peaks of [Fe(CN)$_6$]$^{4-/3-}$ couple centered at 0.2 V are observed for the bare Pt electrode, and the steady redox current without significant peak-separation is also seen for the PIC-modified Pt electrode,

indicating that the insoluble PIC layer effectively modifies the Pt surface. Compared to bare Pt, the current is lower for the PIC electrode, especially for PAm–PAA. Since the loading amount of PIC layers is fixed to be almost the same, it is presumed that the mesh size of PAm–PAA is smaller than that of PLL–PAA and hinders diffusion of redox species through the PIC. The result is reasonably consistent with the difference in side-chain and interval length between every $-$NH$_3^+$ groups of the polycations, and thus the permeability of electrochemically reactive species in the PIC is suppressed.

To elucidate diffusivity of ferrocyanide, potential-step chronoamperometry was conducted by stepping the potential from rest to 0.55 V and the apparent diffusion coefficients were estimated by using a Cottrell equation;

$$i = \frac{nFAc(D)^{1/2}}{\pi^{1/2}} \times t^{-1/2} \qquad (1)$$

where F, A, c, and D are the Faraday's constant, geometrical electrode area (cm^2), bulk molar concentration of ferrocyanide, and diffusion coefficient (cm^2 s^{-1}), respectively. Diffusion coefficients were calculated by substituting the slope values of linear Cottrell plots, current (i) vs. inverse square root time ($t^{-1/2}$) into Eq. (1). As a result, the apparent diffusion coefficients of ferrocyanide are 5.3×10^{-6}, 1.9×10^{-6}, and 0.3×10^{-6} cm^2 s^{-1} for bare Pt, PLL–PAA, and PAm–PAA electrodes, respectively. The calculated data indicates the suppression of ferrocyanide diffusion (permeation) in the PIC, resulting in the lower redox current for PAm–PAA observed, which also agrees with the difference in the molecular structure between PAm–PAA and PAA–PAA as described above.

To demonstrate the molecular sieving ability of the PIC directly, molecular weight dependency for the PIC electrodes was examined by measuring oxidation current of electrochemically reactive species in the neutral PBS, as summarized in Fig. 1c. Anodic limiting current in adding various reactive species into electrolyte solutions was monitored and the ratio of current for the PIC electrodes (j_{PIC}) to that for the bare Pt electrode (j_{Pt}) was plotted as an oxidation current ratio, j_{PIC}/j_{Pt}. The constant potential of 1.0 V was applied in the case of electrolyte solutions containing 5 mM reactive species, except for oxalic acid (1.5 V and
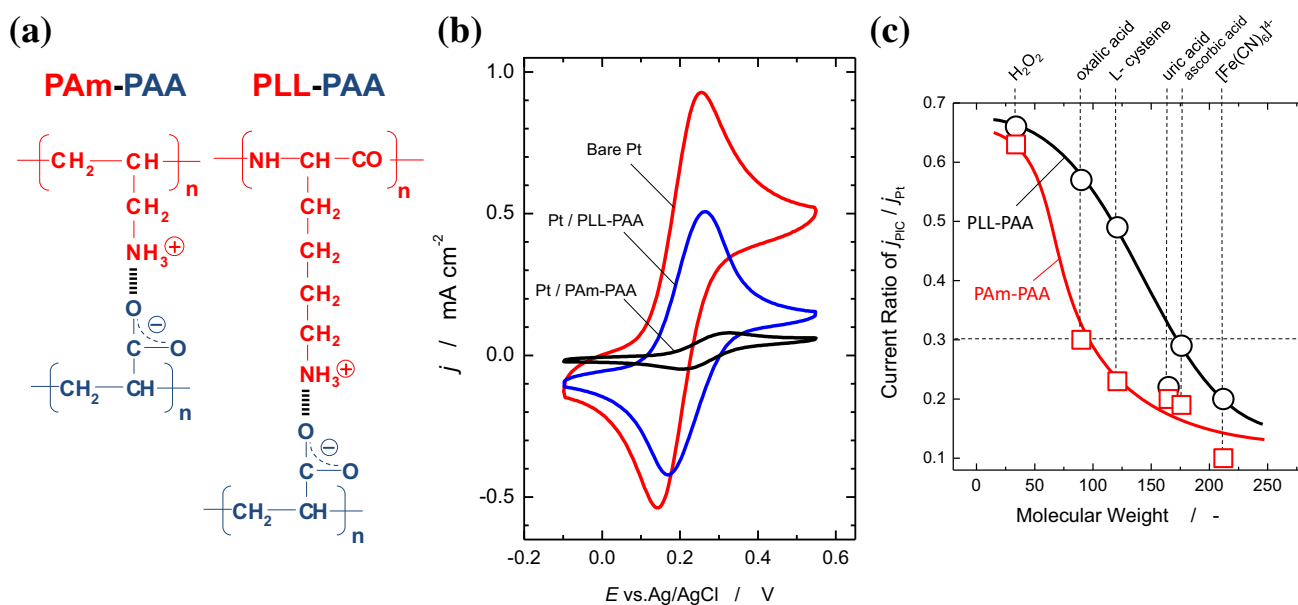

Fig. 1. (a) Molecular structures of polycation–polyanion complex, (b) cyclic voltammograms for bare and PIC-modified Pt electrodes in 5 mM K$_4$[Fe(CN)$_6$] solution at 50 mV s^{-1}, and (c) ratios of anodic current of the PIC-modified Pt electrodes.