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# Redox properties of ferricyanide ion on layer-by-layer deposited poly(glutamic acid) film-coated electrodes and its use for electrocatalytic sensing of ascorbic acid

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## Abstract

Layer-by-layer (LbL) thin films composed of poly(allylamine hydrochloride) (PAH) and poly(glutamic acid) (PGA) were prepared on the surface of a gold (Au) electrode to study the redox properties of $[Fe(CN)_6]^{3-}$ ion on the modified electrodes. The LbL film-coated electrodes exhibited redox response to $[Fe(CN)_6]^{3-}$ ion when the outermost surface of the LbL film was covered with PAH while the response was suppressed on the LbL film-coated electrodes whose outermost surface was covered with PGA due to an electrostatic repulsion between $[Fe(CN)_6]^{3-}$ ions and the negatively charged PGA layer. The redox properties of $[Fe(CN)_6]^{3-}$ ion on the LbL film-coated electrodes significantly depended on the thickness of the LbL film and on pH of the $[Fe(CN)_6]^{3-}$ solution. The thicker film-coated electrodes gave higher response than the thinner film-coated electrodes. The higher response was observed in pH 9.0 solution than in pH 7.4 and 4.0 media. The PAH-terminated LbL film-coated electrodes which had been pretreated in the $[Fe(CN)_6]^{3-}$ solution at pH 9.0 or 7.4 for 15 min exhibited redox response even in a $[Fe(CN)_6]^{3-}$ ion-free buffer solution, suggesting that $[Fe(CN)_6]^{3-}$ ions are confined in the films. The $[Fe(CN)_6]^{3-}$ ion-confined electrodes can be used for the electrocatalytic determination of a mM range of ascorbic acid in solution.

*Keywords:* LbL films; Poly(glutamic acid); Ferricyanide ion; Ascorbic acid

## 1. Introduction

It has been established that a layer-by-layer (LbL) deposition of anionic and cationic polyelectrolytes on a solid surface gives nanometer-sized multilayer thin films [1–3]. Many kinds of polyelectrolytes including synthetic polymers, polysaccharides, proteins, nanoparticles and DNA have been used for this purpose by taking advantages of an electrostatic force of attractions of polyelectrolytes [4–8]. A hydrogen bonding and biological affinity have also been utilized as driving forces for constructing LbL thin films [9,10].

The LbL thin films have found applications to chemical sensors and biosensors for detecting specific gases, metal ions, humidity, biological components, etc. [11–14]. In this context, we have recently studied redox properties of ferricyanide ion ($[Fe(CN)_6]^{3-}$) on the LbL film-coated gold (Au) electrodes and found that the electrodes coated with LbL thin films composed of polysaccharide and poly(allylamine hydrochloride) (PAH) exhibit a redox response in the $[Fe(CN)_6]^{3-}$ solution, depending on the thickness of the LbL films [15]. It was also found that $[Fe(CN)_6]^{3-}$ ions can be confined in the film and the confined $[Fe(CN)_6]^{3-}$ ions are electrocatalytic active for oxidizing ascorbic acid in solution [15]. The characteristic feature of the LbL films composed of PAH and polysaccharide is that $[Fe(CN)_6]^{3-}$ ions freely diffuse into the film even if the surface of the film is covered with a negatively charged polysaccharide. This is a clear contrast to a limited permeability of the LbL films prepared using a conventional type of vinyl polymers that are densely packed in the LbL film [16]. These results suggest that the redox properties of $[Fe(CN)_6]^{3-}$ ion strongly depend on the type of polymer materials from which LbL film is constructed. In this situation, it may be interesting to study the redox properties of the electrodes coated with LbL films prepared by polypeptides because polypeptide-based LbL films are known to show peculiar properties due to the limited conformations including α-helix, β-sheet and random coil [17]. Polypeptide-based LbL films may be interesting from the view point of developing biocompatible nanomaterials for biosensors and drug delivery systems. For this

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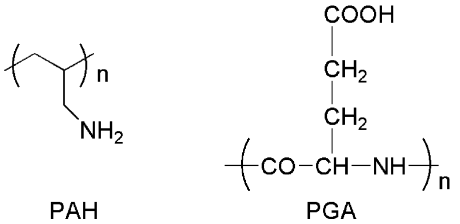

Fig. 1. The chemical structures of PAH and PGA.

reason, in the present study, we have prepared LbL thin films on the surface of Au electrode using poly(glutamic acid) (PGA) and PAH and the redox properties of $[Fe(CN)_6]^{3-}$ ion on the modified electrodes were studied. The present paper reports that the redox properties of $[Fe(CN)_6]^{3-}$ ion on the LbL film-coated electrodes depend on the sign of the electric charges on the outermost surface of the LbL film. It was also found that $[Fe(CN)_6]^{3-}$ ions can be confined in the LbL films whose outermost surface is covered with PAH. In addition, an electrocatalytic determination of ascorbic acid was successfully carried out using the $[Fe(CN)_6]^{3-}$ ion-confined LbL film-coated electrodes.

## 2. Experimental

### 2.1. Materials

Poly(allylamine hydrochloride) [PAH] was purchased from Nittobo Co. (Tokyo, Japan). Poly(glutamic acid) (PGA; molecular weight, 15,000–50,000) was obtained from Aldrich Chemical Co. (Milwaukee, WI). The chemical structures of the polymeric materials are shown in Fig. 1. Sodium 3-mercapto-1-propanesulfonate (MPS) was purchased from Tokyo Kasei Co. All other reagents were of the highest grade available and were used without further purification.

### 2.2. Apparatus

A quartz-crystal microbalance (QCM) (QCA 917 and QCA 922 systems, Seiko EG & G, Tokyo, Japan) was employed for the gravimetric analysis of the LbL films. A 9 MHz AT-cut quartz resonator coated with a thin Au layer (surface area, 0.2 $cm^2$) was used as a probe, in which the adsorption of 1 ng of substance induces a −0.91 Hz change in the resonance frequency. Circular dichroism (CD) spectra of the LbL films were recorded on a CD spectrometer J720 (JASCO, Japan). For the CD measurements, the LbL films were prepared on the surface of a quartz slide (1 mm × 10 mm × 40 mm). All electrochemical measurements were carried out using an electrochemical analyzer (ALS, model 660B).

### 2.3. Preparation of LbL film-coated electrodes

The LbL films were prepared on the surface of a gold (Au) disk electrode (3 mm diameter). The surface of the Au electrode was polished thoroughly using alumina powder and rinsed in distilled water before use. The polished Au electrode was further treated electrochemically in a 0.5 M $H_2SO_4$ solution by scanning the potential from −0.2 to 1.5 V at a scan rate of 0.1 V $s^{-1}$ for ca. 30 min. The Au electrode thus cleaned was dipped in a freshly prepared aqueous MPS solution (5 mM) overnight to form a negatively charged self-assembled monolayer of MPS on the Au surface. The surface of the MPS-modified electrode was further coated with an LbL film by dipping it alternately in 0.5 mg $mL^{-1}$ PAH and 0.5 mg $mL^{-1}$ PGA solutions for 15 min with an intermediate 5 min rinse in the buffer. The pH 9.0 and 7.4 solutions of PAH and PGA were prepared using 10 mM Tris–HCl buffer containing 150 mM NaCl, while 10 mM acetate buffer was used for preparing pH 4.0 solution. The multilayer films were prepared by repeating above procedure.

### 2.4. Gravimetric analysis of the deposition of LbL films

The Au-coated quartz resonator was thoroughly rinsed in water before use. The surface of the quartz resonator was cleaned electrochemically in 0.5 M $H_2SO_4$ in a similar manner to in the case of cleaning of Au disk electrode. The surface of the Au-coated quartz resonator was first modified with a MPS monolayer and the PAH layer was deposited by immersing the quartz resonator in a 0.5 mg $mL^{-1}$ PAH solution for 15 min and rinsed in the buffer solution for 5 min and in pure water for 1 min. The next PGA layer was deposited by immersing the PAH-deposited probe in a 0.5 mg $mL^{-1}$ PGA solution for 15 min. This procedure was repeated for preparing multilayer films. The LbL films were deposited on the both surfaces of the cleaned resonator (i.e., the total area of Au-coated surface was 0.4 $cm^2$) and the film-deposited probe was dried in air after each deposition until the frequency showed a steady-state value to estimate the weight of the film.

### 2.5. Electrochemical measurements

Electrochemical response was measured in a glass cell using an LbL film-modified electrode as a working electrode, a platinum wire as a counter electrode and a Ag/AgCl electrode as a reference electrode. All measurements were performed at room temperature (∼20 °C).

## 3. Results and discussion

### 3.1. Deposition behavior of PAH–PGA LbL films

The PAH–PGA LbL films were deposited on the MPS-modified Au-coated quartz resonator to monitor the loading of the films.

Fig. 2 plots the decrease in the resonance frequency ($-\Delta F$) observed upon depositing PAH–PGA LbL films as a function of the number of depositions. In all the solutions of different pHs tested, $F$ decreased with increasing the number of depositions, suggesting the LbL films were successfully formed on the quartz resonator. The film formation relies on the electrostatic force of attraction between the positive charges on the PAH chain and the negatively charged carboxylate ions in PGA because the films were prepared in pH 4.0–9.0 media, in which PAH