MATERIALS CHEMISTRY COMMUNICATION

Enantioselective adsorption of ferrocene-modified glutamic acids on helical poly(L-glutamic acid) self-assemblies at gold electrodes

Masazo Niwa,* Manabu Matsui, Kaoru Koide and Nobuyuki Higashi*

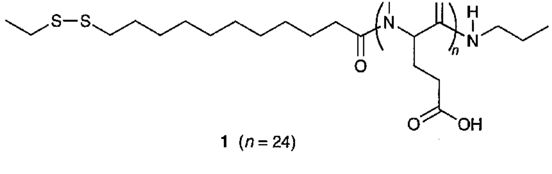
Department of Molecular Science & Technology, Faculty of Engineering, Doshisha University, Kyotanabe, Kyoto 610-03, Japan

A novel poly(L-glutamic acid) (PLGA) self-assembled monolayer was prepared on an Au electrode. The conformational transition of the PLGA segment between α-helix and coil could be electrochemically monitored by use of a redox couple of $Fe(CN)_6{}^{3-/4-}$. The helical PLGA monolayer was found to capture ferrocene-modified glutamic acids enantiomerically by means of an electron transfer reaction of the surface-confined ferrocenyl moiety.

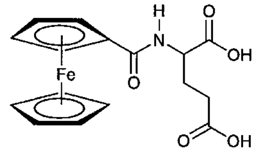
Synthetic polyelectrolytes have received much attention due to their importance as simplified models of natural polyelectrolytes such as protein and nucleic acid and also in many industrial applications. To fabricate the higher-ordered structures of such a biopolymer by using purely synthetic polyelectrolytes, we have devised a strategy in which polyelectrolytes such as poly(methacrylic acid) are aligned on two-dimensional media. Au-deposited plates have been employed as two-dimensional solid substrates since well organized monolayers can be prepared on Au by spontaneous adsorption of organic thiols and disulfides.[1–4] The polymeric amphiphiles, consisting of a poly(methacrylic acid) segment and long alkyl chains whose ends are modified with disulfide bonds to attach to the Au surface, have been found to successfully form spontaneously adsorbed monolayers on Au substrates. Lateral molecular orientations as well as molecular thicknesses of the resulting monolayer films have been then governed by the conformational size of the poly(methacrylic acid) segment during adsorption.[5] Furthermore, these molecularly controlled surfaces have provided an appropriate environment for molecular interactions.[6,7]

Poly(L-glutamic acid) (PLGA) is a typical synthetic polypeptide that should become another candidate for polyelectrolyte assembly and its structure is much more relevant to biopolymer models. Recently, we have described a secondary structural transition of a PLGA-functionalized monolayer on water.[8] This monolayer was also found to have the ability to capture α-amino acids enantioselectively.[9] Some reports[10,11] have appeared on the enantioselective permeation of α-amino acids through thick polymer films based on α-helical PLGA.

Here we describe the self-assembling properties of **1** and interactions with glutamic acid isomers, which are labelled with ferrocene (Fc-Glu) for electrochemical detection, on Au electrodes.



1 ($n=24$)



Fc-Glu (D,L)

A novel PLGA-based amphiphile **1** consists of a PLGA segment (number-average degree of polymerization, $n=24$) and an Au-adsorbable moiety (disulfide bond) which are connected with the long methylene chain as a spacer. First, polymerization of γ-benzyl-L-glutamate-*N*-carboxylic anhydride[12] initiated with *n*-propylamine was carried out and then the residual amino group at one chain-end of the polymer obtained was reacted with ω-(ethyldithio)undecanoic acid[6] in the presence of diethyl phosphorocyanide as a condensing agent, and finally the PLGA amphiphile **1** was obtained by removal of benzyl groups. Glutamic acids† chosen as guest amino acids were labelled with ferrocene (Fc-Glu) in order to detect electrochemically the amount of α-amino acids adsorbed at the **1** monolayer on the Au electrode.

The monolayer formation of **1** on the Au electrode was monitored by means of the previously established electrochemical method[13–16]‡ using the $Fe(CN)_6{}^{3-/4-}$ redox couple. The adsorption equilibrium was reached after 20 min immersion of the electrode into an aqueous solution containing **1** (1 mmol dm^{-3}) at pH 5.7, and the barrier capability towards the redox probe of the resulting monolayer was considerably high, implying that molecules of **1** are packed densely within the monolayer. Subsequently, the pH dependence of the electrochemical response (peak current, i_p) for this monolayer was examined since PLGA is well known to show a conformational transition between the α-helix and random coil structures upon changing the surrounding pH. Fig. 1 displays the i_p variation of the monolayer electrode in response to changes in the solution pH. The i_p values are evaluated from the cyclic voltammetry (CV) curves; the inset of Fig. 1 shows a typical CV curves obtained at pH 3.1 and 8.0, together with that for a bare electrode. With lowering pH from 8.0 to 3.1, the i_p value increased steeply at *ca.* pH 5.5. To reveal the secondary structure of the PLGA segment, FT-IR RAS spectra‡ were

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† The ferrocenyl-modified glutamic acids (Fc-Glu(D or L)) were synthesized as follows. L-Glutamic acid, benzyl alcohol and toluene-*p*-sulfonic acid were suspended in benzene, and the mixture was refluxed until the theoretical amount of water was removed by a Dean–Stark trap. The diester tosylate obtained was condensed with ferrocenoyl chloride in the presence of triethylamine, and then finally the removal of benzyl groups by reduction with $Pd(C)/H_2$ gave the objective Fc-Glu(L). Fc-Glu(D) was prepared in the same way.

‡ Cyclic voltammetry was performed using a CV-1B (BAS) coupled with an RW-12 X-Y recorder (Rikadenki). The Au electrodes were mounted in a conventional three-electrode cell. All potentials were measured and reported with respect to an Ag/AgCl (saturated KCl) reference electrode. FTIR RAS spectra were measured by a Nicolet System 800 spectrometer using a reflection attachment at an incident angle of 80°, together with a polarizer.

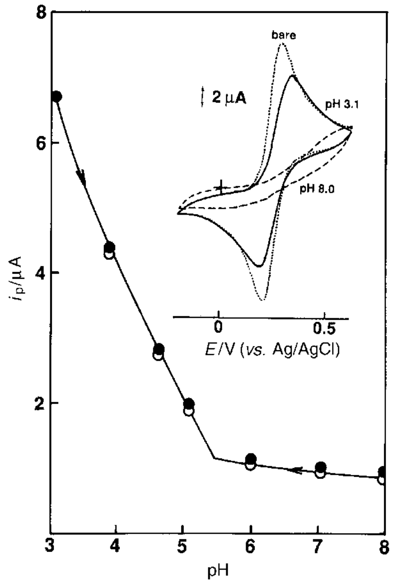

Fig. 1 pH Dependence of the peak current (i_p) of the redox couple, $Fe(CN)_6^{3-/4-}$ (3 mmol dm^{-3}), for the **1**-monolayer-covered electrode, pH 8.0→3.1 (○); pH 3.1→8.0 (●). Inset shows cyclic voltammograms for the **1**-monolayer-covered electrodes at pH 3.1 and 8.0, and for a bare electrode. All experiments were recorded in KCl (0.1 mol dm^{-3}); potential scan rate, 100 mV s^{-1}. Electrode area = 0.02 cm^2.

measured for the monolayers on Au plates treated with water at pH 8.0 and 4.0. The latter monolayer showed a peak assigned to the α-helix structure (1551 cm^{-1}, amide II) although the existence of small amounts of the random coil structure (1532 cm^{-1}, amide II) cannot be excluded, while the former monolayer was totally in the random coil form. Therefore, the relatively high i_p value at the lower pH suggests that contact of $Fe(CN)_6^{3-/4-}$ with the electrode surface became easier due to formation of a void as the result of the rod-like conformation of the helical PLGA segment. In contrast, at the higher pH, contact of $Fe(CN)_6^{3-/4-}$ with the electrode surface was suppressed, probably due to the electrostatic repulsion between the anionic redox probe and the ionized and expanded polymer segment, which resulted in the reduction of i_p. When the pH was elevated, the i_p value reverted to the original value without hysteresis, suggesting that the monolayer is immobilized stably at the Au surface.

Subsequently, the interaction of this monolayer with Fc-Glu(D) and (L) isomers was examined. The Fc-Glu adsorption was carried out by immersing the **1**-covered electrodes in aqueous solutions of Fc-Glu isomers (0.2 mmol dm^{-3}, pH 3.0) and allowing 3.5 h for equilibration. After equilibration, the electrodes were washed with water (pH 3.0) to remove physisorbed Fc-Glu isomers. Fig. 2(a) shows a typical CV obtained for Fc-Glu(D) adsorbed on the **1**-monolayer-covered electrode with 0.1 mol dm^{-3} KCl as a supporting electrolyte at pH 3.0, at which both carboxylic acid groups of Fc-Glu(D) and of the polymer (PLGA segment) are completely protonated and the PLGA segment adopts the α-helical conformation. A CV curve of Fc-Glu(D) in solution for the bare electrode was also recorded under the same conditions and is included in Fig. 2(a) for comparison. The redox potentials of Fc-Glu(D) in solution are almost the same as those for the absorbed Fc-Glu(D), which are due to the one-electron oxidation and reduction of the ferrocenyl group of absorbed Fc-Glu(D). Rinsing the electrode with water (pH 3.0) two additional times resulted in nearly identical CVs, which demonstrated that desorption is negligible. Fig. 2(b) displays a plot of peak current against scan rate. The peak current is found to vary linearly with scan rate. These results indicate that Fc-Glu(D) is surface-confined to the **1** monolayer and is not subject to diffusion to the electrode. When the **1**-monolayer-covered electrode was immersed into an aqueous solution of Fc-Glu(L) under the same conditions, there was no peak in the CV, suggesting that the helical PLGA assembly has the ability to capture Fc-Glu enantioselectively.

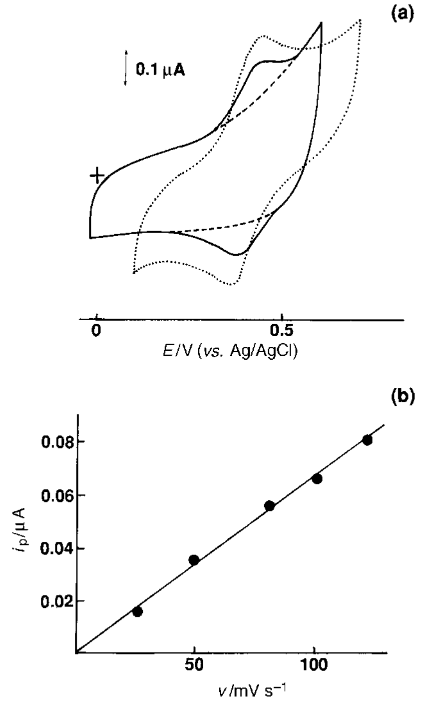

Fig. 2 (a) Cyclic voltammograms acquired at a scan rate of 100 mV s^{-1} of a **1**-monolayer-covered electrode (dashed) and the same electrode after adsorption of Fc-Glu(D) (solid line). The dotted curve indicates a bare electrode in Fc-Glu(D) solution (0.2 mmol dm^{-3}). In all cases, the supporting electrolyte is 0.1 mol dm^{-3} KCl at pH 3.0. (b) Plot of peak current (i_p) *vs.* scan rate.

We have demonstrated self-assembled monolayers based upon PLGA on Au and their specific interaction with α-amino acids, which has been successfully monitored electrochemically by covalently attaching a ferrocenyl group to the α-amino acid. Financial support from the aid of Dashisha University's Research Promotion Fund (1995) is gratefully acknowledged.

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