

Reversible Permeability Control of a Polypeptide Membrane by Electrochemical Redox Reactions in Side Chain Ferrocene Groups

Yoshio Okahata* and Kazuya Takenouchi

Department of Polymer Chemistry, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan

The permeability of a copoly(γ -ferrocenylmethyl-L-glutamate/ γ -methyl-L-glutamate) film cast on a platinum minigrad sheet could be reversibly controlled by the electrochemical redox reactions of ferrocenyl groups in side chains of the polypeptide.

In biological membranes, protein molecules in lipid bilayers may undergo reversible conformational changes to create a channel for specific substrate transport. Synthetic polypeptide membranes as structural models of proteins are known to be able to regulate their permeability by the conformational change of the helical structure¹ or the side chain mobility.² For example, the permeability of polypeptide membranes having carboxylate or azobenzene groups in side chains can be affected by pH changes^{1a} or photoirradiations,³ respectively.

In this work, we prepared a poly(glutamate ester) having varying amounts of ferrocenylmethyl groups in side chains and controlled the permeability across the polypeptide film cast on a platinum minigrad sheet by electrochemical redox reactions of side chains. A schematic illustration of the apparatus is shown in Figure 1.

Copoly(γ -ferrocenylmethyl-L-glutamate/ γ -methyl-L-glutamate), PFeLG-*X*, was prepared by an ester exchange reaction of poly(γ -methyl-L-glutamate) (average $D_p = 2500$, Ajinomoto Co., Tokyo) and excess amounts of ferrocenylmethanol⁴ (m.p. 74–76 °C) in 1,2-dichloroethane at 70 °C for 2–10 h. The polymer was repeatedly reprecipitated from 1,2-dichloroethane with excess of methanol to remove unreacted ferrocenylmethanol. The contents (*X*%) of ferrocenylmethyl groups in the polymers were determined by elemental analysis (C, H, N, and Fe). The obtained PFeLG was cast on a Pt minigrad sheet (150 mesh, film thickness 100 μm) from a

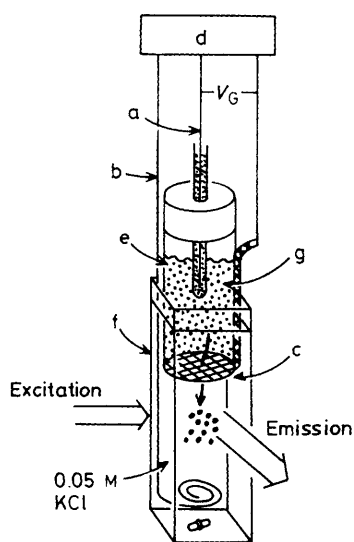
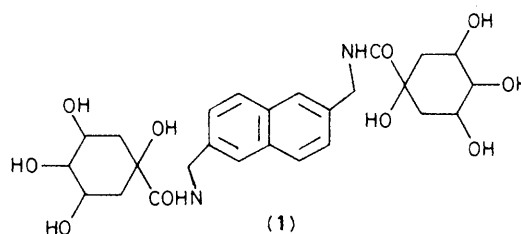
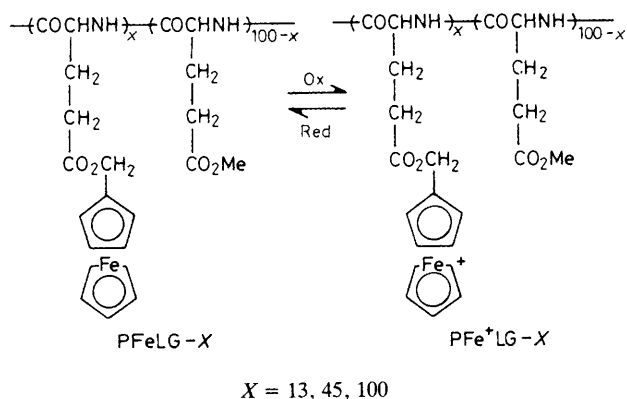


Figure 1. Experimental set-up for permeation of fluorescent probe (**1**) (5×10^{-4} M) through the PFeLG polypeptide film cast on a platinum minigrad sheet. (a) Reference electrode, Ag/AgCl in saturated KCl (-0.05 V vs. saturated calomel electrode); (b) Pt wire for counter electrode; (c) Pt minigrad sheet (150 mesh, 38 mm²) embedded in PFeLG films; (d) potentiostat; (e) polyethylene tube (diameter 7 mm); (f) 1 cm quartz cell; (g) permeant (**1**) in 0.05 M KCl.

chloroform solution. Cyclic voltammetry studies of PFeLG-13, -45, and -100 films cast on a Pt electrode in 0.05 M aqueous KCl showed two well known peaks at +0.42 V vs. saturated calomel electrode (s.c.e.) in the cathodic region (PFeLG \rightarrow PFe⁺LG) and at +0.32 V vs. s.c.e. in the anodic region (PFe⁺LG \rightarrow PFeLG). Cyclic voltammograms of PFeLG films in an oxidation–reduction cycle of ferrocenyl side chains were reversible.

Permeation of the freely water-soluble, nonionic fluorescent probe (**1**) (5×10^{-4} M) in aqueous KCl solution (0.05 M) through the PFeLG film/grid was followed fluorophotometrically at 340 nm (excited at 280 nm) according to the apparatus of the thermostated quartz cell in Figure 1. A nonionic permeant was chosen to avoid the effect of electrostatic interaction with the film/grid. Typical time courses of permeation of probe (**1**) under an intermittent redox potential applied to the Pt grid/film in a nitrogen atmosphere are shown in Figure 2. Apparent permeation rates ($P/\text{cm}^2 \text{ s}^{-1}$) were obtained from slopes of Figure 2 according to previous methods.⁶ The permeability of PFeLG films against probe (**1**) increased with increasing ferrocenyl content of the side chains.

Upon applying the potential +0.35–0.45 V vs. s.c.e. to the PFeLG-45 film on a Pt grid, the film turned from brown to dark green (λ_{max} , 630 nm) within 10 s. This indicates the oxidation of PFeLG to the cationic PFe⁺LG, upon which the permeability was increased by a factor of 8–10. Upon switching off the Pt grid/film potential (0 V), even after 10 min in the oxidised form, the dark green PFe⁺LG-45 film was reduced to the brown PFeLG film in 30 s and the permeability reverted to the original slow rate. The PFeLG-100 film, in



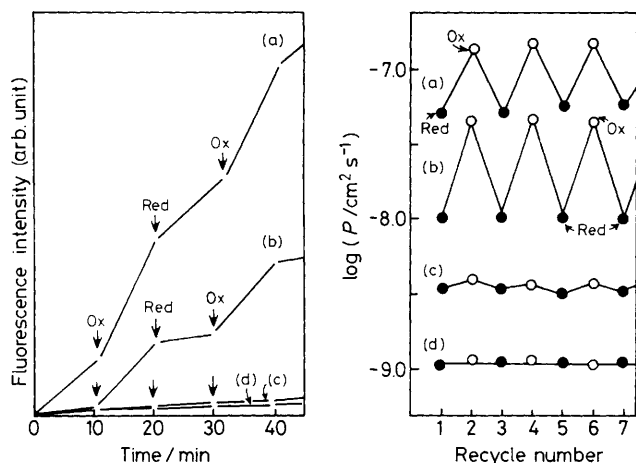


Figure 2. Reversible permeation changes of the probe (1) through the PFeLG film/Pt grid by electrochemical redox reactions. The potential of $+0.35$ — $+0.45$ V and 0 V vs. saturated calomel electrode was applied to Pt grid/film at Ox and Red, respectively. (a) PFeLG-100, (b) PFeLG-45, (c) PFLG-13, (d) poly(γ -methyl-L-glutamate).

which all side chain groups were substituted by ferrocenyl groups, showed similar permeability changes owing to redox reactions although the membrane was relatively permeable anyway. The permeability changes of these PFeLG/PFe⁺LG films on Pt grid could be reproduced repeatedly without damaging the film chemically or physically. In contrast, the permeability of PFeLG-13 film was hardly affected by redox reactions because of the low content of ferrocenyl side chains, neither was that of the poly(γ -methyl-L-glutamate) film.

The circular dichroism (c.d.) spectra of both PFeLG and oxidized PFe⁺LG films cast on a quartz plate showed the negative curve centred at 225 nm independent of the ferrocene content in side chains. This indicates that both PFeLG and PFe⁺LG adopt mainly right-handed α -helix coils, as does poly(γ -methyl-L-glutamate) in the solid state, independent of the redox form of the side chains. The α -helix content of the film decreased with an increase in the content of ferrocenyl

groups in side chains. In solid membranes of synthetic polypeptides, permeants are thought to diffuse not in the rigid helical skeleton but in the relatively fluid side chain region.¹⁻³ Upon applying the oxidizing potential to the PFeLG film on a Pt grid, the lipophilic ferrocenyl side chain is changed to the cationic, hydrophilic ferrocenyl group, and water-soluble permeants can easily diffuse and permeate through the hydrophilic, swelled side chain region of the peptide film. We suppose that the micromosaic and heterogeneous structure (rigid helical skeletons and fluid side chain regions) of polypeptide membranes is important in redox-sensitive permeation control, because a cast film of poly(ferrocenylmethyl acrylate) showed little permeation change upon redox reactions on a Pt grid.

Thus, the permeability of the PFeLG film is controlled by the change in the hydrophilic nature of fluid side chain regions due to the electrochemical redox reaction of ferrocenyl groups. This is the first example of reversible, highly responsive permeability control of polypeptide membranes by redox reactions. We have also studied the permeability control of a redox site-containing, bilayer-immobilized film by electrochemical redox reactions, in which the fluidity of the lipid bilayer membrane is regulated by redox reactions on a Pt minigrid.⁶

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