Conformation and Redox Reaction of Poly(ethylene oxide)-modified Horseradish Peroxidase in Poly(ethylene oxide) Oligomers

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Horseradish peroxidase (HRP) was modified with poly(ethylene oxide) (PEO) with Mw of 750, 2000, or 5000. In PEO oligomer, the redox reaction of PEO-modified HRP at indium tin oxide electrode was clearly detected with both visible spectrometry and cyclic voltammetry.

Since poly(ethylene oxide) (PEO) has low glass transition temperature and polar repeating unit similar to water molecule, salts are soluble in PEO and the dissociated ions are mobile. Thus, the salt-containing PEO is expected to be useful as an excellent solvent for electrochemistry.[1] Several metalloproteins such as hemoglobin,[2] myoglobin,[3,4] and cytochrome *c* [5] were studied electrochemically in PEO oligomers. To solubilize these proteins in PEO, chemical modification of PEO chains on the proteins was essentially required.[3] This PEO modification has been also used to solubilize cytochrome *c* and superoxide dismutase in a non-aqueous medium, and electrochemistry of these proteins has also been investigated.[6,7] However the effect of PEO modification on the electrochemical activity of the protein was not the same for individual protein, because of different higher-ordered structure and the environment of active center. It was therefore important to analyze this effect individually when one desired to use the corresponding proteins in PEO or equivalent solid state polymers. Horseradish peroxidase (HRP) is one of heme proteins being studied extensively. PEO-modified HRP (PEO-HRP) was developed to solubilize them in organic solvents,[8] and structure around heme in PEO-HRP was reported by Mabrouk.[9] However, there was no study on the electrochemistry of PEO-HRP in PEO. PEO oligomer are recently noticed to be quite interesting solvent for proteins. For example, thermostability of PEO-modified hemoglobin was extremely improved by the use of PEO as a solvent, keeping their redox activity at even 120 ℃.[10] In this study, we discuss the conformation and redox reaction of PEO-HRP in PEO oligomers.

HRP (121units mg^{-1}) was the gift from Toyobo Co. Ltd. PEO monomethylethers with av. Mw of 750, 2000, and 5000 were purchased from Aldrich Co. Ltd. PEO-HRP was synthesized according to the conventional PEO-modification method.[3] The degree of PEO modification on one HRP molecule was determined from the titration of amino groups of HRP by 2,4,6-trinitrobenzene sulfonic acid.[11] The average number of the modified PEO molecule with Mw of 750, 2000, and 5000 attached onto one HRP molecule was determined to be 1.2±0.5, 3.8±0.5, and 3.2±0.5, respectively.

The α-helix structure of native HRP and PEO-HRP in 0.1 M phosphate buffer, pH7.0 containing 0.2 M KCl, was studied with circular dichroism (CD) spectropolarimetry (JASCO, J-720) at 25 ℃ as shown in Figure 1(a). The α-helix content of HRP modified with PEO (Mw:750) (PEO750-HRP) was almost the same as that of native HRP in buffer solution. However, considerable change in the CD spectra was found for HRP modified with other PEOs (PEO2000-HRP and PEO5000-HRP). This suggests that a higher-ordered structure of HRP was changed by modifying PEOs with Mw of 2000 and 5000. The effect of degree of PEO modification on this structural change should not be zero. The detailed analysis on this change is now under progress. Figure 1(b) shows CD spectra of PEO-HRP in PEO200 (Mw:200) containing 0.5 M KCl (η=65cP at 30°C). PEO200 containing 0.5 M KCl was used after drying at 60 ℃ *in vacuo*. Overall water content was detected to be less than 0.3 wt% with Karl Fischer titration method. The α-helix content of HRP decreased with increasing Mw of modified PEO in PEO200. PEO750-HRP was highly soluble in PEO200 but almost insoluble in PEO400, the electrochemical redox

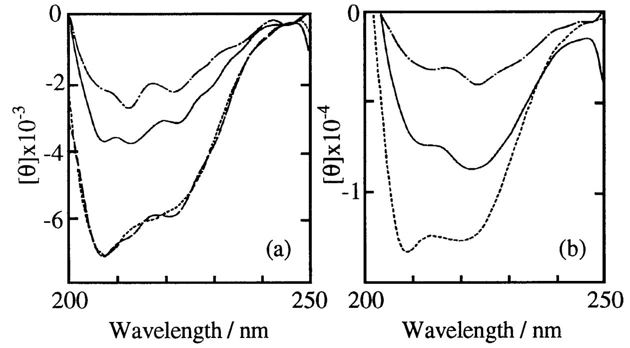

Figure 1. CD spectra for HRP (— —), PEO750-HRP (- - -), PEO2000-HRP (——), and PEO5000-HRP (—-—). (a) in 0.1 M phosphate buffer, pH7.0 containing 0.2 M KCl. (b) in PEO200 containing 0.5 M KCl.

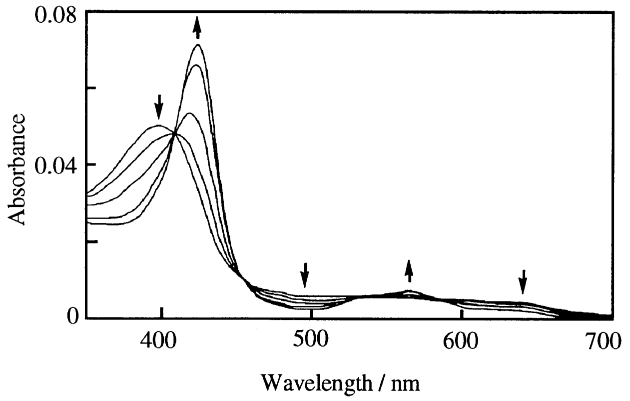

Figure 2. Visible spectral change of PEO750-HRP in PEO200 containing 0.5 M KCl under applying potential of -1.0 V (vs. Ag) for 0, 6, 19, 44, and 89 min at 25 ℃.

reaction of PEO-HRP was considered to be studied in PEO200.

Figure 2 shows UV-Vis spectra of PEO750-HRP (1.0x10^{-4} M) in PEO200 by applying potential of -1.0 V (vs. Ag) with thin layer cell (light path length=100 μm, W.E. : indium tin oxide glass (ITO, layer thickness:130 nm, surface resistance: 30.9 Ω/square), C.E. : Pt). The λmax for oxidized PEO750-HRP (401 nm) in PEO200 was 2 nm shorter than that of native HRP in buffer solution. This small shift of the Soret band means a little change in a chemical environment around heme of HRP in PEO oligomers. The absorbance of oxidized PEO750-HRP decreased and that of reduced PEO750-HRP (λmax=420 nm) increased in PEO200 by applying -1.0 V (vs. Ag). This reduced PEO750-HRP was re-oxidized by applying positive potential of +0.5 V (vs. Ag). The apparent reduction rate constant of PEO750-HRP in PEO200, estimated from the absorbance change at 420 nm, was 1.1 x 10^{-3} s^{-1}. Visible spectrum of PEO5000-HRP was not observed in PEO200 because of poor solubility. Also no spectra of PEO-HRP were confirmed in PEO400 regardless the Mw of the modified PEO by the same reason.

Figure 3 shows cyclic voltammogram of PEO750-HRP (2.0x10^{-4} M) in PEO200 containing 0.5 M KCl. The ITO glass (working area: 1.2 cm^2), Ag wire (ϕ=0.5 mm), and Pt wire (ϕ=0.5 mm) were used as working, reference, and counter electrode, respectively. Typical redox peaks of PEO750-HRP were obtained in PEO200 with cyclic voltammetry (Fuso Seisakusho: HECS 972, 321B) at 25°C under nitrogen atmosphere. The formal redox potential for PEO750-HRP, estimated from the anodic (-105 mV vs. Ag) and the cathodic (-110 mV vs. Ag) peak potentials, was -107.5 mV (vs. Ag). The electrochemical response for PEO750-HRP at ITO electrode in PEO200 was consistently detected even after continuous potential cycling for 24h at 25°C. The ratio of the anodic peak current to the cathodic one is 0.77. The half-widths of the anodic and the cathodic peaks were 200 and 150 mV, respectively. The peak potential separation (ΔE) for PEO750-HRP in PEO200 was only 5 mV. Since ΔE for PEO750-HRP was much smaller than 59 mV, PEO750-HRP was strongly suggested to be adsorbed on the ITO electrode in PEO200. The quantity of electricity of PEO750-HRP was constant in the sweep rate range of 10-100 mV s^{-1}. The quantity of PEO750-HRP adsorbed on the ITO electrode, estimated from the quantity of electricity, was 3.8 x 10^{-11} mol cm^{-2}. The peak current increased linearly with increasing the sweep rate. All these results suggest that the PEO750-HRP, adsorbed on the ITO electrode, shows fast redox response even in PEO oligomers.

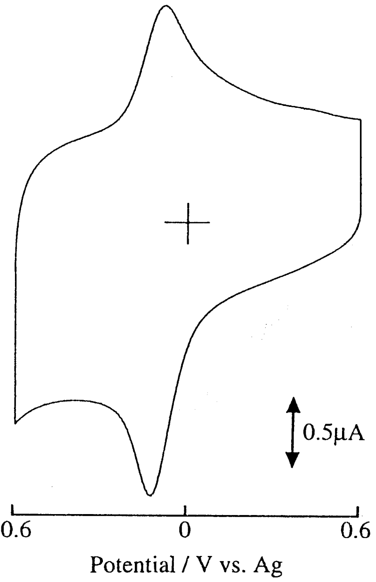

Figure 3. Cyclic voltammogram of PEO750-HRP in PEO200 containing 0.5 M KCl with a sweep rate of 33 mV s^{-1} at 25 °C.

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