Cytochrome *c* Cross-linked with Glutaraldehyde (1) ; Electrochemical Response in Poly(ethylene oxide) Oligomers

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For rapid electron transfer of cytochrome c(cyt.c) in poly(ethylene oxide)(PEO) oligomers, higher-ordered structure of cyt.c was cross-linked intramolecularly with glutaraldehyde, and the redox reaction of this cyt.c was analyzed. The cross-linked cyt.c was soluble in PEO oligomers and showed better electrochemical redox response than PEO-modified cyt.c. Little conformational change of the cross-linked cyt.c was suggested even after adsorption on the electrode.

We reported that poly(ethylene oxide)(PEO)-modified heme proteins such as hemoglobin or myoglobin were dissolved in PEO oligomers and showed quasi-reversible redox reaction.[1-5] Furthermore, PEO oligomers enable the electrochemical redox reaction of heme proteins at high temperature(up to 120 °C).[6] Such improved condition of proteins in PEO oligomers should be a great help for new applications such as solid state sensory system. However, the redox reaction of heme proteins in PEO oligomers was slow than that in an aqueous phase. To improve the electron transfer process, we analyzed the electrochemical response of cytochrome c(cyt.c). Cyt.c is known as a typical electron transport protein *in vivo*. The electrochemical behavior of cyt.c is being studied vigorously with several electrodes in an aqueous medium, and fast electron transfer is well analyzed.[7] For non-aqueous study, the redox reaction of native cyt.c in polymer electrolytes was reported by Murray in 1988.[8] However, the conformation of cyt.c in polymer electrolytes has not been analyzed at all. We have been studying the electrochemical redox reaction and conformation of PEO-modified cyt.c(PEO-cyt.c) in PEO oligomers.[9] However, the electrode reduction rate constant of PEO-cyt.c was small compared with that of PEO-modified hemoglobin or myoglobin. Certain conformational change of cyt.c in PEO oligomers was suggested. In the present study, higher-ordered structure of cyt.c was cross-linked with glutaraldehyde, expecting little conformational change of cyt.c in the PEO oligomers.

The cyt.c cross-linked with glutaraldehyde(G-cyt.c) was synthesized according to a previous method.[10] The concentration of cyt.c solution was 0.10g · l^{-1} to prevent intermolecular cross-linking of cyt.cs. The mole ratio of glutaraldehyde in feed to lysine residues on cyt.c was 0.5. The Schiff base formed between ε-amino groups and glutaraldehyde was reduced with sodium tetrahydroborate. The molecular weight of G-cyt.c was determined with TOF mass spectroscopy(Shimadzu/Kratos, Kompact MALDI-Ⅲ). The spectrum of G-cyt.c gave a peak at 12561 dalton as shown in Figure 1(b). The increase of the molecular mass (150 dalton) was attributed to the glutaraldehyde cross-linking. The titration of unreacted amino groups of cyt.c with 2,4,6,-trinitrobenzene sulfonic acid[11] suggested that 4.7 amino groups of cyt.c were consumed in average. Moreover, G-cyt.c gave no peak based on dimer or further aggregates of cyt.c till 30000 dalton in the mass spectrum. From above results, about 2 glutaraldehyde molecules were reacted per cyt.c through the intramolecular cross-linking.

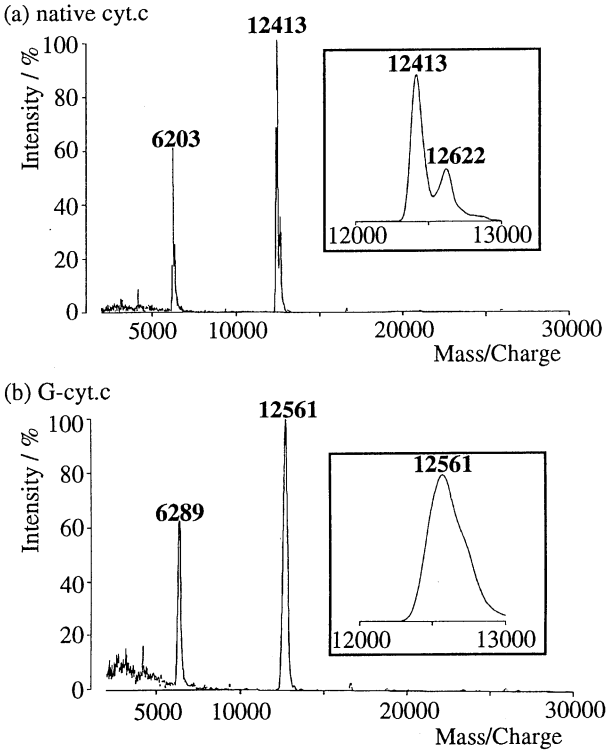


Figure 1. Mass spectra of native cyt.c(a) and cyt.c cross-linked with glutaraldehyde(b).

G-cyt.c was soluble in PEO oligomers without PEO-modification. The redox reaction of G-cyt.c was analyzed with cyclic voltammetry in PEO_{200}(average molecular weight of 200) containing 0.5mol · l^{-1} KCl as a supporting electrolyte. PEO-cyt.c, having 7.4 PEO chains with average molecular weight of 750, showed no peaks at first, but the peak current increased gradually with scanning time. The cyclic voltammogram(CV) after 24 h scanning was shown in Figure 2(a). The peak separation in the CV for PEO-cyt.c was 51 mV. The slope of relation between logarithm of peak current versus that of sweep rate was about 0.7. The PEO-cyt.c was considered to be denatured by the adsorption on the ITO electrode. From these results, PEO-cyt.c enabled the electron transfer only when it was adsorbed on the electrode surface, and the electron transfer between PEO-cyt.c molecules could not be carried out in PEO_{200}. Against this, G-cyt.c showed clear redox peaks even at the first scan in PEO_{200}. The quantity of electricity(anodic) of G-cyt.c(2.18×10^{-5}C · cm^{-2}) was about 3 times larger than that of PEO-cyt.c(7.82×10^{-6}C · cm^{-2}). The peak separation in the CV for G-cyt.c was 99 mV(Figure 2(b)). The slope of the relation between logarithm of peak current versus that of sweep rate was 1.0. This clearly shows that the redox reaction of G-cyt.c in PEO_{200} was controlled by the electron transfer between the cyt.c

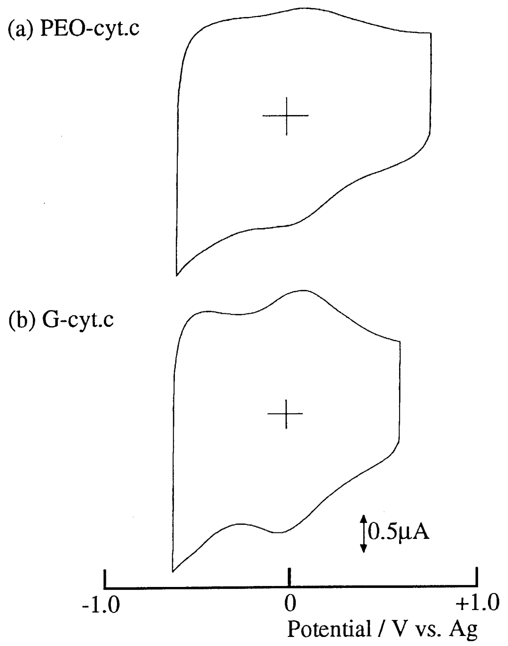
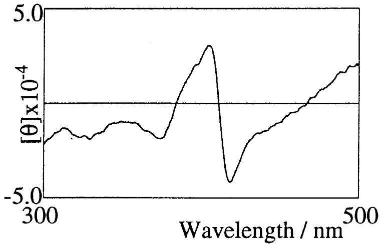


Figure 2. Cyclic voltammograms of PEO-cyt.c(a) and G-cyt.c(b) in PEO$_{200}$ after 24 h scanning. Sweep rate: 33 mV/s. W.E. and C.E. were ITO glass electrode (2.0 cm^2). R.E. was Ag wire.

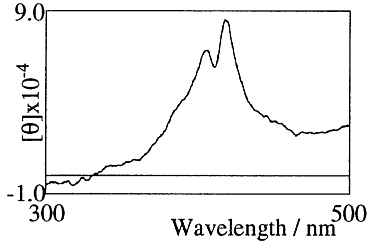
and the ITO electrode. The quantity of electricity of G-cyt.c was independent of the sweep rate suggesting adsorption. The working electrode was rinsed with pure PEO$_{200}$ after measurement, and CV was measured in PEO$_{200}$ containing only supporting electrolyte. Since the peaks based on the redox of G-cyt.c were detected, the adsorption of G-cyt.c on the electrode surface was strongly suggested.

The conformation of G-cyt.c was analyzed with circular dichroism(CD) spectroscopy in phosphate buffer and in PEO$_{200}$. In phosphate buffer, although α-helix content of cyt.c was decreased only 6.0% after cross-linking, the spectrum around 400 nm reflecting the chemical environment of the heme was different from that of native cyt.c. On the other hand, the spectrum of PEO-cyt.c around 400 nm was almost identical to that of native cyt.c, and the α-helix content was decreased less than 5.0% by PEO-modification in phosphate buffer. These CD results were different from those for PEO-cyt.c reported by Mabrouk.[12] This might be attributed to the different molecular weight of the modified-PEO as well as method of PEO-modification. In phosphate buffer, the redox potential of native cyt.c was 56.5 mV(vs. Ag), that of PEO-cyt.c and G-cyt.c was 52.5 mV and 159.5 mV, respectively. The chemical environment of the heme of cyt.c was suggested to be changed by cross-linking. In PEO$_{200}$, although the CD absorption based on α-helix structure was observed for G-cyt.c, the spectrum around 400 nm was different from those of native cyt.c in phosphate buffer and PEO-cyt.c in PEO$_{200}$ as shown in Figure 3.

(a) native cyt.c in phosphate buffer



(b) PEO-cyt.c in PEO$_{200}$



(c) G-cyt.c in PEO$_{200}$

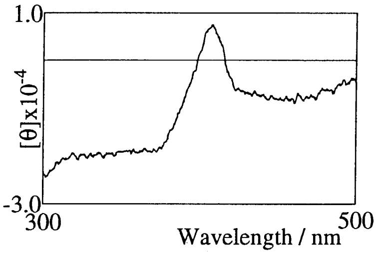


Figure 3. CD spectra of native cyt.c in phosphate buffer(a), PEO-cyt.c in PEO$_{200}$(b), and G-cyt.c in PEO$_{200}$(c).

However some conformational change of cyt.c occurred due to cross-linking, further conformational change after adsorption on the electrode might not occur in PEO oligomers. The cross-linking with glutaraldehyde might suppress further conformational change after adsorption on the electrode surface. Design of G-cyt.c with higher-ordered structure similar to that in an aqueous medium is now in progress.

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