THERMAL STABILITY AND ELECTROCHEMICAL CHARACTERIZATION OF POLY(ETHYLENE OXIDE)-MODIFIED MYOGLOBIN IN ORGANIC SOLVENTS

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Introduction

When placed in nonaqueous solvents instead of water, protein is expected to exhibit new properties such as the improvement of activity, enhanced thermostability, and change enzyme specificity. However, most native-proteins themselves are insoluble in the relatively hydrophobic solvents. Recently, it has been shown that homogeneous solutions of heme proteins in organic solvents could be prepared by the modification of amino groups with poly(ethylene oxide) (PEO). We have also reported that PEO-modified heme proteins [1, 2] are dissolved in a wide variety of organic solvents and showed the electrochemical reaction. In this study, thermal stability and electron transfer of PEO-modified myoglobin (PEO-Mb) have been investigated in various organic solvents.

Experimental

PEO-Mb in organic solvents Myoglobin (Mb) from horse skeletal muscle was modified with about ten PEO (molecular weight of 2000) chains and dissolved in organic solvents such as PEO oligomers with molecular weight of 200, methanol, *N*,*N*-dimethylformamide, chloroform, and dimethyl sulfoxide.

UV-vis and Raman spectroscopy The visible spectrum of PEO-Mb was studied from room temperature to 80 °C. UV-vis spectra were taken after holding the cell at desired temperature for 20 minutes. Raman spectra of PEO-Mb were obtained on a Jasco NRS-1000 spectrophotometer using a Kaiser Optical Holographic Notch- Plus^{TM} filter and a liquid N_2-cooled CCD detector with 4 cm^{-1} spectral resolution. The excitation source was a Coherent Innova 90C Kr^+ laser. Spectra were collected using a back-scattering geometry at an excitation wavelength λ_{ex} = 413.1 nm at various temperatures. Peak frequencies were calibrated relative to an indene standard and were accurate to ± 1 cm^{-1}.

Electrochemistry The electron transfer reaction of PEO-Mb on a plastic formed carbon electrode was investigated by cyclic voltammetry. Ag and Pt wires were used as reference and counter electrodes, respectively. Tetra-butylammonium perchlorate (TBAP, 0.1 M) was used as an electrolyte.

Results & Discussion

UV-vis spectra showed shifts of Soret bands (390-415 nm) for oxidized form of PEO-Mb (Fe^{3+}) in different solvents. It was indicated that the structure around heme in each solvent is different from that in water. Even after PEO modification, the solvent affects the electronic structure of heme molecules in PEO-Mb. It was known that the intense peak (ν_4) at around 1360-1370 cm^{-1} is sensitive to the oxidation state of iron and porphyrin skeletal modes (ν_2 and ν_3) are reliable indicators of spin and coordination states. The heme core marker bands from Raman spectrum in PEO oligomer represented a pentacoordinate high spin heme, although the native met-Mb has a hexacoordinate high spin heme at 25°C (Fig. 1). The dominant spectrum (ν_4) at 1365 cm^{-1} of PEO-Mb in PEO oligomer at 50°C (Fig.1) indicated the reduction of the ferric to ferrous form of PEO-Mb in PEO oligomer at higher temperature. These structural features facilitate the electron transfer of PEO-Mb in PEO oligomer onto the carbon electrode as shown in Fig. 2. The loss of the sixth ligand water could cause changes of the properties of PEO- Mb.

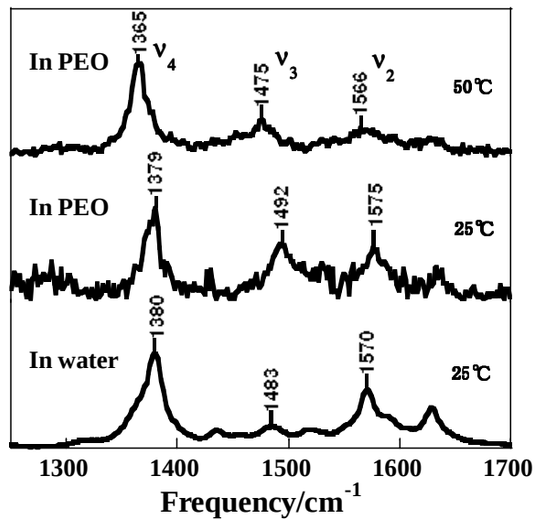

Fig. 1 Raman spectra of PEO-modified Mb in water and in PEO_{200} at 25°C and at 50°C. Excitation at 413.1 nm.

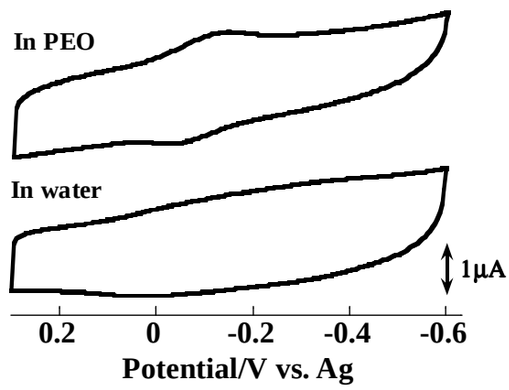

Fig. 2 Cyclic voltammograms of PEO-modified Mb in water and in $PEO_{200.}$

References

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2) H. Ohno, *Electrochim. Acta*. **43**, 1581-1587 (1998).