Thermostability of Poly(ethylene oxide) Modified Heme Proteins in Ionic Liquids

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Introduction

Ionic liquids (ILs) were characterised by extremely high ion content, high ionic conductivity, low viscosity, non-volatility, flame resistance, and so on. Accordingly, these have been investigated as novel and stable ion conductive materials as well as reaction solvents. Some of these have relatively wide potential window to allow various electrochemical reactions. ILs are also expected as stable solvents in biological field. However, most proteins were denatured in the ILs. Since poly(ethylene oxide)s (PEOs) have strong affinity with ions, PEO-modification was expected to solubilize several molecules and macromolecules. We have already reported that PEO modified proteins were soluble in ILs.[1] PEO modified proteins are expected to keep their activity at wide temperature range[2) 3)]. In this study, we reported the thermostability of PEO modified cytochrome c and myoglobin in ILs.

Experimental

PEO modification of proteins was carried out according to our previous method.[2] Cytchrome c modified with PEO monomethyl ethers with average molecular weight of 2000 (PEO_{2000}-cyt.c) (modified with 13.5 chains) and myoglobin (PEO_{2000}-Mb) (modified with 12 chains) were dissolved in 1-ethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide ([EMIm][TFSI]) (Fig.1) containing 0.05M tetrabutylammonium chloride (TBACl). The spectra of the solution were carried out with optical waveguide (OWG) spectroscopy.[1] The electrochemical cell system constructed on the optical glass waveguide with carbon working electrode (2 × 50mm), together with Pt and Ag wires as counter and reference electrode, respectively. OWG spectra were analyzed with applying the potential (-800mV and +800mV vs Ag). The solutions were heated with silicon rubber heater.

Results & Discussion

At room temperature, the Soret band distinctive for heme proteins was observed at 408nm for oxidized PEO_{2000}-cyt.c in EMImTFSI. When a negative potential was applied to the working electrode, a red shift from 408nm to 414nm was found due to the reduction. To classify the redox response, the plot of Δ absorbance (I_{408nm}-I_{414nm}) which was given by subtracting absorbance at 414nm from that at 408nm was depicted against time. Although PEO_{2000}-cyt.c showed a small decrease in the Δ absorbance by continuous heating, it kept its redox activity up to 140°C (Fig.2). At 140°C, PEO_{2000}-cyt.c solution showed redox response repeatedly in EMImTFSI as shown in Fig.2 (right hand side).

Thermal stability of PEO_{2000}-Mb was also analyzed. At room temperature, PEO_{2000}-Mb showed similar spectral shift reflecting the redox reactions. However, the redox response received considerable damage by gradual heating. The spectral peak shift was diminished at around 120°C, and no spectral change was detected at and above 140°C as shown in Fig.3. By comparing PEO-cyt.c and PEO-Mb, former showed better thermal stability probably due to covalent bond of heme to globin chain.

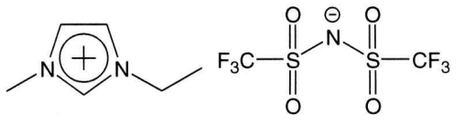

Fig.1 Structure of [EMIm][TFSI].

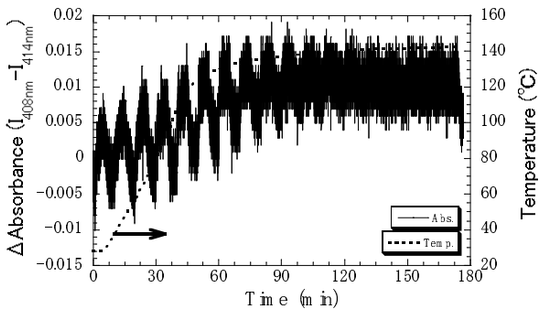

Fig.2 OWG spectral peak shift reflecting the redox reaction of PEO_{2000}-cyt.c dissolved in [EMIm][TFSI] containing 0.05M TBACl .

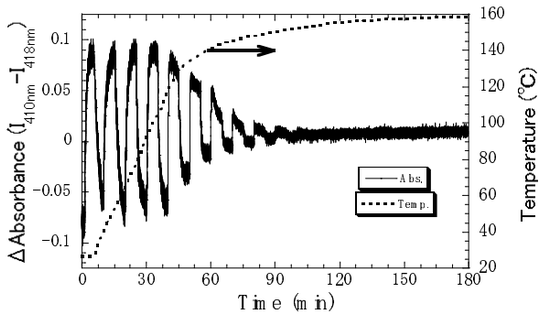

Fig.3 OWG spectral peak shift reflecting the redox reaction of PEO_{2000}-Mb dissolved in [EMIm][TFSI] containing 0.05M TBACl .

Reference

1) H. Ohno, C. Suzuki, M. Yoshizawa, and K. Fujita, *Chem. Lett.*, 32, 450 (2003)
2) N.Y. Kawahara, and H. Ohno, *Bioconjugate Chem.*, 8, 643-648 (1997)
3) N.Y. Kawahara and H. Ohno, *Solid State Ionics*, 113-115, 161-166 (1998)