Electron Transfer Reaction of Poly(ethylene oxide)-Modified cyt.c in 1-Ethyl-3-Methylimidazolium bis(trifluoromethanesulfonyl)imide

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Introduction

Recently, ionic liquids (ILs) collect increasing attention as alternative to volatile organic solvents.[1] ILs have fascinating features such as non-volatility, thermal stability, electrochemical stability, and high ionic conductivity. Accordingly, these have been investigated as novel and safe ion conductive matrices as well as reaction solvents.[2] ILs are also expected as stable solvents in the biological field. However, most proteins are insoluble in general ILs. Structural improvement in either of proteins or ILs should be required to solubilize general proteins in ILs. We noticed that high solubility of poly(ethylene oxide) (PEO) into ILs, because of high affinity with ions. These PEO-modified heme-proteins are expected to be soluble in ILs without denaturation.

In this report, cytochrome c, modified with PEO chains (PEO-cyt.c), was dissolved in 1-ethyl-3-methyl-imidazolium bis(trifluoromethane-sulfonyl)imide, **1**, one of typical ionic liquids. The electron transfer reactions of thus dissolved PEO-cyt.c were analyzed. Furthermore, the effect of added salts on the redox response of PEO-cyt.c in ILs was also investigated.

Experimental

Horse heart cytochrome c (Type VI), was purchased from Sigma Chemicals Co. PEO monomethyl ethers with average molecular weight of 550, 1000, 2000, and 5000 were purchased from NOF Co. Ltd. Cyt.c was modified by our previous method[3] with 14.3 chains of PEO_{550} (PEO_{550}-cyt.c(14.3)), 15.4 chains of PEO_{1000} (PEO_{1000}-cyt.c(15.4)), 6.1 or 13.5 chains of PEO_{2000} (PEO_{2000}-cyt.c(6.1 or 13.5)), and 9.7 chains of PEO_{5000} (PEO_{5000}-cyt.c(9.7)), respectively. Lithium bis(trifluoro-methanesulfonyl)imide (LiTFSI), a gift from Sumitomo 3M, was used as received. The salt, **1** was synthesized according to our previous method.[2] The structure of the obtained **1** was confirmed with ^{1}H-NMR spectroscopy. A Karl Fischer moisture titrator (MKS-210; Kyoto Electronics Co.) was used to determine the water content of **1** as 0.37 wt% in average. The visible absorption spectrum and the electrochemical redox reaction of PEO-cyt.c dissolved in **1** (0.1mM) was studied with optical waveguide (OWG) spectrophotometer (SIS-50, System Instruments Inc.).[4] An optical glass waveguide LaSK n1 (20 x 65 x 0.4mm, refractive index; 1.75) was purchased from Sumita Optical Glass Co. The electrochemical cell system constructed on the optical glass waveguide with carbon working electrode (3 x 40 mm), together with Pt and Ag wires (0.5mm) as counter and reference electrode, respectively.

Results & Discussion

Native cyt.c was insoluble in **1**, but cyt.cs modified with PEO_{550}(14.3), PEO_{1000}(15.4) or PEO_{2000}(6.1) were partly soluble. On the other hand, cyt.cs modified with PEO_{2000}(13.5) or PEO_{5000}(9.7) were dissolved in **1**. The redox activity of dissolved PEO_{2000}-cyt.c(13.5) in **1** was analyzed by OWG spectroscopy. However, the spectral change based on the redox reaction of PEO-cyt.c was scarcely observed. This was explained as lack of small counter ions for heme in spite of extremely high density of ions in ILs. To carry out the redox reaction, small and suitable sized ion species were concluded to be essential for the electron transfer reaction in ILs. Since KCl is known to be a good electrolyte for the electrochemical reaction of heme-proteins in PEOs, we added KCl as supporting electrolyte in **1**. When negative potential was applied to the PEO-cyt.c in saturated **1** with KCl, the Soret band showed a red shift from 408nm to 414nm based on the reduction (Figure 1(A)).[5] After that, the λ_{max} returned to 408nm by applying positive potential. Figure 1(B) shows the effect of KCl on the absorbance change at 414nm with potential sweep.

Effects of species and amount of the added salts on the redox response of PEO-cyt.c in **1** were studied with several organic and inorganic salts. The redox response of PEO-cyt.c deeply depended on the anion radius of the added salt, and chloride anion was concluded to be the best anion to precede the redox response of PEO-cyt.c in **1**. As shown in Scheme 1, it was strongly suggested the chloride anions migrate between bulk and heme pocket of the cyt.c during the redox reaction.

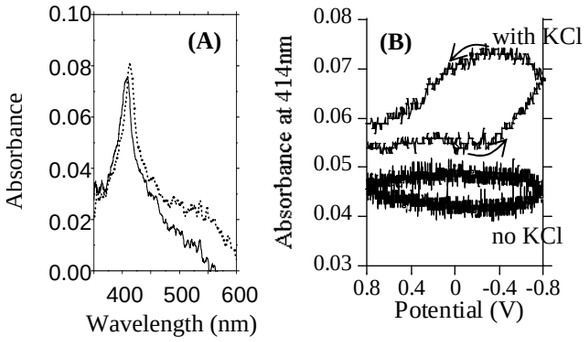
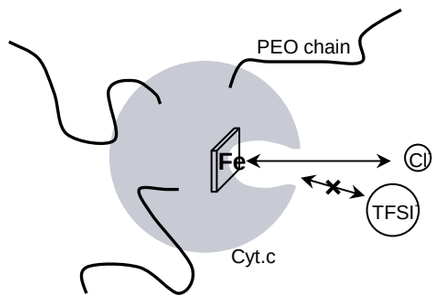


Figure 1 (A) OWG spectra of oxidized (solid) and reduced (dotted) PEO_{2000}-cyt.c (13.5) in **1** with KCl. (B) Effect of the given potential on the absorbance change at 414nm of PEO_{2000}-cyt.c dissolved in **1**.



Scheme 1 Size of ions is important for redox reaction of heme proteins in ILs.

References

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