**Characterization of Cytochrome P450 from *Sulfolobus tokodaii* Strain 7 in Organic Solvents**

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**Introduction**

Cytochrome P450s are heme-thiolate enzymes involved in a wide variety of oxidative metabolic reactions. This versatility, as well as the broad range of substrates, makes P450 enzymes potentially useful catalysts for biosensors, biomedical devices, and bioreactors. However, there are mainly two problems for the application of cytochrome P450s to biodevices. (i)The P450 enzymes are relatively unstable. It is requiring that their thermal stability should be improved for their industrial application. (ii)The monooxygenation reaction requires specific redox partners (cytochrome P450 reductase and electron transfer protein) and electron donors such as NADH (Fig. 1a). We have recently reported overexpression and purification of cytochrome P450 from a thermo-acidophilic crenarchaeon *Sulfolobus tokodaii* strain 7 (cyt. P450st). The direct electron transfer between cyt. P450st and the electrode has also been accomplished by immobilization of enzyme on an electrode to construct a catalytic system without redox partners (Fig. 1b) [1]. The higher catalytic efficiency of cytochrome P450s would be achieved in organic media since most substrates for cytochrome P450s are hydrophobic compounds. In the present study, cyt. P450st has been modified with poly(ethylene oxide) (PEO) to dissolve in organic media. Effects of modification with PEO on the properties of PEO-modified cyt. P450st (PEO-P450st) has been investigated. We also report that the studies on effects of salts on thermostability and electrochemical properties of PEO-P450st in PEO.

**Experimental**

Cyt. P450st was expressed in *E. coli* BL21 (DE3) and purified by two-step chromatography [1]. To obtain PEO-P450st, cyt. P450st was stirred with activated PEO (average molecular weight of 750) in a 0.05 M borate buffer solution The average number of PEO molecules bound to one molecule of cyt. P450st was estimated to be 17. Raman spectra were obtained on a JASCO NRS-1000 spectrometer using a Kaiser Optical holographic notch-plus filter and a liquid $N_2$-cooled CCD detector at 413.1-nm excitation. Catalytic reaction of PEO-P450st was carried out at 37 °C for 30 minutes by "Shunt pathway". The products were analyzed by gas chromatography. The redox reaction of PEO-P450st was analyzed by cyclic voltammetry (ALS Electrochemical Analyzer 624A). All measurements were performed using a three-electrode cell containing a plastic formed carbon working electrode, an Ag wire reference electrode, and a Pt wire counter electrode.

**Results and discussion**

To investigate the effects of modification with PEO on the active site of PEO-P450st, resonance Raman spectroscopy was performed. Observed Raman spectrum of PEO-P450st was not significantly altered compared with that of wild type P450st. Catalytic activity of PEO-P450st was retained in the buffer solution. These results show that effects of modification with PEO on the active site structure and enzymatic activity of PEO-P450st are not serious. PEO-P450st could be dissolved in several organic media, such as PEO (average molecular weight of 200), DMF, and DMSO. The electrochemical properties of PEO-P450st in PEO containing 0.05M $KClO_4$ were investigated by cyclic voltammetry. The electrochemical response of PEO-P450st in PEO was obtained by using a plastic formed carbon electrode as a working electrode. The redox potential was -245 mV *vs.* Ag, assigned to the $Fe^{III}$ / $Fe^{II}$ redox couple. The separation between the anodic and cathodic peaks was ca. 50 mV. These results exhibit that cyt. P450st is expected for application in organic solvents by the use of the modifying enzyme with PEO. The effects of salts on thermostability of PEO-P450st in PEO were investigated by UV-vis spectroscopy. The Soret band of PEO-P450st near 400 nm was extremely decreased at 90 °C in PEO without KCl. On the other hand, the absorbance of the Soret band was not much change in PEO with 0.5 M KCl at 90 °C (Fig. 2). It is indicated that the thermostability of PEO-P450st in PEO was affected by the amount of salts. Further studies on the mechanism of stabilization are under way.

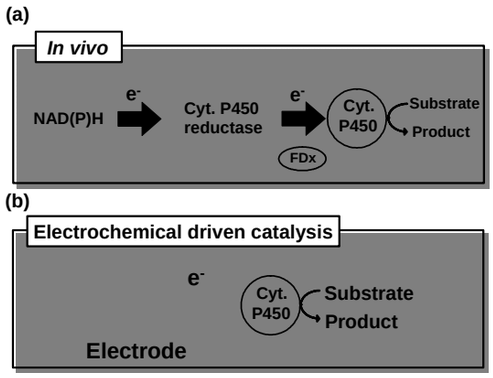


Fig. 1 Schematic illustration of electron transfer reaction for cyt. P450.

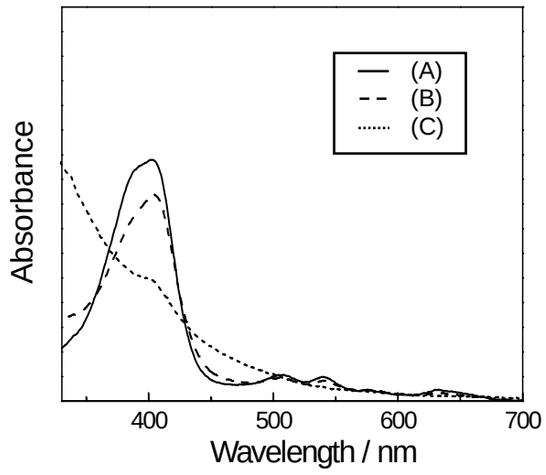


Fig. 2 UV-vis absorption spectra of PEO-P450st in PEO with 0.5 M KCl at room temperature (A) and at 90 °C (B) and without KCl at 90 °C (C).

**References**

1) Y. Oku, A. Ohtaki, S. Kamitori, N. Nakamura, M. Yohda, H. Ohno, and Y. Kawarabayasi, *J. Inorg. Biochem.*, in press.