Electrocatalysis by Thermophilic Cytochrome P450 CYP119 in Surface-Modified Electrodes

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Cytochrome P450 CYP119 is a thermostable enzyme from *Sulfolobus solfataricus*, an extremophilic, archaebacteria found in sulfurous volcanic hot springs.[1] Although the factors contributing to its stability have not yet been identified, the crystal structure of CYP119 reveals that the enzyme is smaller and more compact than typical P450 enzymes, Figure 1.[2] The thermal and pH stability of CYP119, as well as its smaller size, makes CYP119 a good candidate for protein film voltammetry.[3]

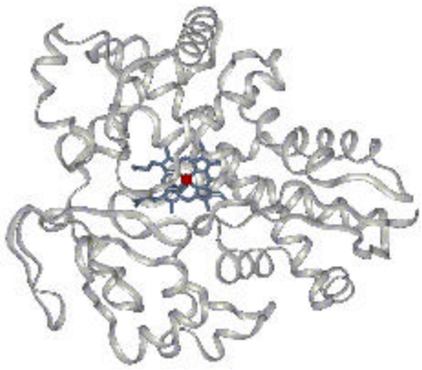

Figure 1. Protein structure of cytochrome P450 CYP119 from *Sulfolobus solfataricus*.[2]

It has been shown that CYP119 has an unusually high denaturation point (~90°C), and remains active in solution for extended periods at high temperatures.[4] We find that CYP119 in sol-gels and certain thermostable surfactant films retains much of its electrochemical activity up to 80°C, Figure 2. The Fe(III/II) couple decreases with temperature in a manner consistent with increased solvent access to the heme pocket.

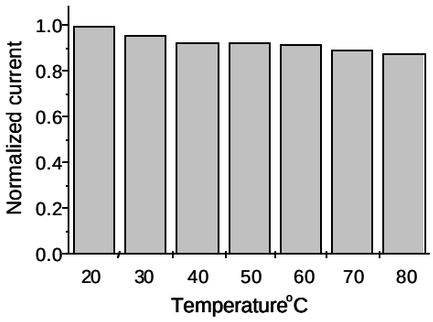

Figure 2. Temperature dependence for anodic currents of the Fe(III/II) couple of thermophilic P450 CYP119 in a thermostable film on PG electrode.

P450 enzymes are known to decompose halogenated substrates, and other groups have studied their use as electrocatalysts for dehalogenation of chlorinated hydrocarbons.[5] We posited that the thermal and pH stability of CYP119 may have added advantages in regard to substrate solubility and reactivity. Thus we have examined the use of CYP119 films to electrocatalytically decompose a variety of chlorocarbons.

At room temperature, CYP119 films demonstrate high turnover in the reduction of pentachloroethane, Figure 3. Preliminary mass-spectral analysis shows multiple degradations of substrates at room temperature down to mono-chlorinated products. The dehalogenation activity is enhanced at elevated temperatures, and full dechlorination of methylene chloride is observed at 55°C. Interestingly, catalytic currents change from diffusion limited at low temperatures to substrate limited at elevated temperatures despite the increase in substrate solubility.

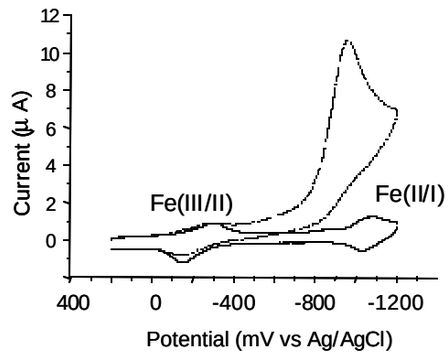

Figure 3. Cyclic voltammograms of CYP119 surfactant film electrode in a pH 7 buffer (solid line) and after saturation with pentachloroethane (dashed).

We have previously used myoglobin-modified electrodes to electrochemically model heme-based NOx reductases.[6,7,8] This method allowed identification of several distinct steps in NOx pathways, including the characterization of a reduced nitrosyl adduct similar to that proposed in the P450 nitric oxide reductase catalytic cycle.[7] The histidine-coordinated heme in myoglobin is a poor model for that in cytochrome P450nor, which is ligated by a cysteine thiolate. By comparison of the electrocatalytic rediuctions of nitrite, nitric oxide and nitrous oxide by CYP119 and myoglobin, we hope to delineate the effect of the axial ligand on these reactivities.

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