Pyrene-Wired Heme Domain Cytochrome P450 BM3 Electrodes

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Cytochromes P450 (P450s) catalyze oxygenations of inert substrates under physiological conditions. Exploiting this activity *in vitro* would be greatly facilitated if reductants other than NADPH could be found. We are working on electrochemical methods for reduction of P450 from *Bacillus megaterium* (BM3), an attractive target for in vitro applications given its high turnover rates and broad substrate specificity.[1]

Prior work achieved rapid heme reduction photochemically (2.5 x 10^6 s^{-1} and 4.6 x 10^5 s^{-1} with and without substrate) by covalently tethering a ruthenium diimine to an engineered cysteine (N387C) on the heme domain of BM3 (hBM3).[2] It occurred to us that "wiring" the N387C hBM3 mutant to an electrode could also yield high electron tunneling rates. Previously, Katz utilized N-(1-pyrene)iodoacetamide (Py) (thiol specific) to anchor and electronically connect a photosynthetic reaction center to a basal plane graphite (BPG) electrode.[3] Thus, we made the N387C hBM3 single surface cysteine mutant, attached Py to the cysteine, and achieved rapid electron transfer (ET) with a BPG electrode. Cyclic voltammetry (CV) (Figure 1) revealed a couple centered at -340 mV, which we assigned to the heme $Fe^{III/II}$ redox couple.[4,5] A plot of the cathodic peak current versus the scan rate was linear, indicating a surface-confined species. The standard rate constant (k^o, $\Delta G^o = 0$) for the BPG-Py-hBM3 system was found to be 650 ± 50 s^{-1},[6] which is the fastest electrode kinetics reported for any P450 system.

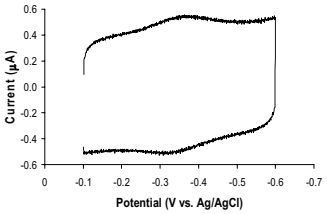

Figure 1. Cyclic voltammogram of the Py-hBM3 conjugate on BPG (0.07 cm^2) at 200 mV/s in 50 mM KP_i / 20 mM KCl / pH 7.

To characterize the surface, protein films were cast onto highly oriented pyrolytic graphite (HOPG) and imaged using AFM. Figure 2a shows a section of HOPG soaked in a Py-hBM3 solution, revealing a series of small islands 2-5 nm in height that likely represent protein clusters on the surface. Figure 2b shows the corresponding image of HOPG soaked in unlabeled hBM3. Clearly, no surface features are visible, implying that only the Py-hBM3 conjugate adsorbs to the surface. Regarding surface coverage, Figure 2a suggests that there is sub-monolayer coverage. CV experiments on HOPG with a Py-hBM3 film (for 0.25cm^2 HOPG, hBM3 monolayer = 1.4 x 10^{-12} mols) confirm this finding: integrating under the cathodic peak yielded 6.2 x 10^{-13} mols of electroactive protein, or ~ 44% surface coverage.

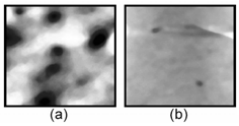

Figure 2. 800 nm x 800 nm AFM images of HOPG soaked in (a) Py-hBM3 and (b) hBM3.

CV's in the presence of dioxygen revealed large catalytic currents at the onset of the $Fe^{III/II}$ couple. To determine the number of electrons transferred to dioxygen, Py-hBM3 films were cast onto a BPG rotated-disk electrode (RDE). Using the Levich equation for a RDE, $i_L = 0.62nFAD_o^{2/3}\omega^{1/2}\nu^{-1/6}C$, theoretical lines for the one-, two-, and four-electron reduction of dioxygen were generated (Figure 3). RDE experiments were conducted by performing electrolysis at -0.5 V and determining the limiting current for each rotation rate. The results of these experiments (solid points, Figure 3) scatter around the theoretical line for n = 4, suggesting that the BPG-Py-hBM3 system converts dioxygen primarily to water. This finding is further supported by results from an Amplex Red fluorescence assay for hydrogen peroxide, which revealed that only a small fraction of the current (< 17%) was used to generate peroxide. This is in stark contrast to other P450 electrochemical systems, where peroxide is the primary product of dioxygen reduction.[7,8]

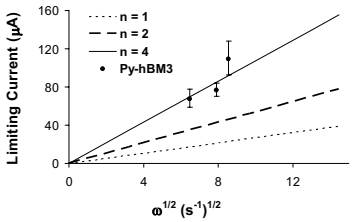

Figure 3. Solid lines: Levich plots derived for the 1, 2, and 4 electron reduction of dioxygen. The points represent the limiting current at 400, 600, and 700 rpm for Py-hBM3 films on BPG-RDE in the presence of dioxygen (250 μM).

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