Film Voltammetry of Wild-Type and Mutant Cytochrome P450 BM3

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The cytochromes P450 (P450's) catalyze chemically challenging oxidation reactions under physiological conditions. Of the P450's, the variant isolated from *Bacillus megaterium* (BM3) is particularly interesting owing to its high turnover rates and similarity to the little-understood mammalian forms. We are investigating the redox chemistry of the heme domains of wild-type P450 BM3 (WT) and its mutant form, 1-12G.

The 15 mutations that distinguish 1-12G from WT result in enhanced catalytic activity, including the ability to hydroxylate linear hydrocarbons regio- and stereoselectively.[1] Notably, the heme of 1-12G is mixed spin (absorbances at 390 and 418 nm) while that of WT is low spin (λ_{max} = 418 nm). To assess the effect of these mutations on the electrochemical properties of 1-12G, films of both WT and 1-12G proteins were cast onto basal-plane-graphite electrodes using the surfactant didodecyldimethylammonium polystyrenesulfonate (DDAPSS) to facilitate electron transfer at the surface.

Both proteins yield well resolved voltammograms assigned to the Fe(III/II) couples at nearly identical potentials (~ -250 mV *versus* AgCl/Ag). Standard ET rates (k^o, $\Delta G = 0$) were calculated from the voltammetry data, revealing an order-of-magnitude drop in k^o moving from WT to 1-12G (250 s^{-1} *versus* 30 s^{-1}). Like wild-type P450 BM3 in DDAB,[2] both proteins reduce dioxygen at the onset of the Fe(III/II) response. Analysis by rotating-disk voltammetry reveals a catalytic rate constant for WT that is 10-times larger than that for the 1-12G mutant, 5 x 10^5 $M^{-1}s^{-1}$ *versus* 2 x 10^{-5} $M^{-1}s^{-1}$. Strikingly, this analysis also reveals that WT P450 mediates primarily the two-electron reduction while 1-12G catalyzes the four-electron reduction of dioxygen to water. These results are correlated with the biochemistry of the two proteins, and suggest that a longer-lived peroxy species in the 1-12G protein may explain its high regio- and stereoselectivity.

References:

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(2) Fleming, B.D.; Tian, Y.; Bell, S.G.; Wong, L.; Urlacher, V.; Hill, H.A.O. *Eur. J. Biochem.* **2003**, *270*, 4082.