# The Interaction of Cytochrome P450 2B4 with its Redox Partners

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For a number of years my laboratory has attempted to understand the mechanism by which cytochrome $b_5$ affects catalysis by cytochrome P450 2B4. We have approached this problem by comparing how cytochrome $b_5$ and cytochrome P450 reductase influence the various steps of the reaction cycle of the microsomal protein, cytochrome P450 2B4. It has previously been demonstrated in a purified reconstituted system under steady state conditions that cytochrome $b_5$ enhances the efficiency of cytochrome P450 2B4 catalysis by ≅ 15% irregardless of the substrate. Increased metabolite is formed at the expense of the side product superoxide (J.Biol.Chem. 270:27407). Further studies employing site-directed mutagenesis of cytochrome P450 2B4 established that cytochrome $b_5$ and cytochrome P450 reductase bind to the proximal surface of cytochrome P450 2B4 near the axial cysteine. The binding sites for cytochrome $b_5$ and reductase overlap but are nonetheless distinct (J.Biol.Chem. 273:17036). Consistent with the observation of distinct binding sites was the subsequent demonstration using stopped-flow spectrophotometry that cytochrome P450 2B4 catalysis in the presence of cytochrome b5 occurred without an observable intermediate. In striking contrast catalysis in the presence of cytochrome P450 reductase proceeded through an observable intermediate which is hypothesized to be the hydroperoxo intermediate ($Fe^{+3}OOH^{-}$) (Biochem. 42:11594). These experiments were possible because the cytochrome P450 reductase used in these experiments was only capable of transferring a single electron. The FAD molecule in the reductase had been replaced with deazaFAD which did not undergo redox changes under the conditions of the experiment

The experiments to be presented will compare the kinetics of the spectral changes observed by stopped-flow spectrophotometry with the kinetics of product formation by oxyferrous cytochrome P450 2B4 with the different electron donors, cytochrome $b_5$ and cytochrome P450 reductase. In order to measure the kinetics of product formation under single turnover conditions a rapid chemical quench apparatus has been used to interrupt cytochrome P450 catalysis at precisely known times. The pico-nanogram amount of product produced during the course of the reaction has been quantitated by gas chromatography-mass spectrometry. Specifically we have investigated whether product formation occurs more rapidly in the presence of cytochrome $b_5$ than in the presence of reductase.