

Intermediate Formation of WT and Y96F Variant of Ferric Cytochrome P450cam in the Reaction with Peracids

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Recently, we reported optimized conditions for obtaining spectroscopic evidence in reactions of P450cam with *m*CPBA for formation of classical Cpd I, consisting of Fe^{IV} plus a porphyrin π -cation radical, as well as a Cpd ES-like species, consisting of Fe^{IV}=O plus a Tyr radical (Spolitak, T., Dawson, J., Ballou, D. JBC, in press). Cpd ES arises from intramolecular electron transfer to Cpd I, and has been previously characterized by RFQ-Mössbauer and EPR studies using peracetic acid as oxidant (Schünemann et al., *J. Biol. Chem.*, 2004, **279**, 10919-10930). They suggested that the EPR signal came from the radical of Tyr96 for WT, and from the radical of Tyr75 for Y96F variant. According to our stopped-flow data, at pH > 7 a significant fraction of Cpd I is formed transiently, whereas at low pH only a species with a Soret at 406 nm, presumably Fe^{IV}=O + tyrosyl radical, is observed.

We probed the influence of Tyr96 in studies of the Tyr96Phe P450cam variant, which has a less oxidizable residue in that position. The kinetics of formation of intermediates and the distribution between heterolysis and homolysis pathways was examined with rapid scanning stopped-flow methods. Using a variety of conditions (temperature, pH), we followed the conversion of Cpd I to Cpd ES, which has a Soret maximum at 407 nm. This rate was about 80% that for WT. Whereas, Cpd I formed approximately twice as fast as for WT. With cumene hydroperoxide under appropriate conditions, we have obtained evidence for Cpd II.

Addition of ascorbate to either of the high-valent species reforms the Fe^{III} state with only a small loss of heme absorbance. These results indicate that typical peroxidase chemistry occurs with P450cam and offer an explanation for the contrasting results reported earlier. The delineation of improved conditions (pH, temperature, choice of peracid) for generating highly oxidized species with P450cam should be valuable for their further characterization.