ABSTRACT

RESONANCE RAMAN STUDIES OF OXYGENATED OF MYOGLOBIN AND CYP2B4 AND THEIR MUTANTS

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Important oxidative heme enzymes use hydrogen peroxide or activate molecular oxygen to generate highly reactive peroxo-, hydroperoxo- and more highly oxidized forms resulting from heterolytic O-O bond cleavage. Members of the cytochrome P450 superfamily catalyze difficult chemical transformations, including hydroxylations and C-C bond cleavage reactions. In mammals, these enzymes function to reliably produce important steroids with the required high degree of structural precision. On the other hand, certain other mammalian P450s serve a different role, efficiently metabolizing xenobiotics, including pharmaceuticals and environmental pollutants.

Though so important, the precise mechanisms involved in such transformations are incompletely understood, because of difficulties in structurally characterizing the fleeting intermediates. This dissertation exploits a unique combination of techniques to address this issue, cryoradiolytically reducing the relatively stable dioxgen adducts to generate and trap the reactive species at low temperatures, followed by resonance Raman (rR) spectroscopic interrogation to effectively characterize key molecular fragments within these crucial intermediates.

One essential goal of this work is to evaluate the rR spectral response to structural variations of such species employing an accessible model that can be systematically manipulated. Myoglobin (Mb) serves this purpose because its readily accessible site-directed mutants are useful for investigating the effects of heme site environment on the structure and function of heme proteins. In the present work, horse heart Mb and 6 site-directed mutants are employed to study the effects of active site environment on the structure and behavior of the Fe-O-O and Fe=O fragments of the peroxo-, hydroperoxo- and ferryl forms that can arise.

In addition, these methods are used to structurally define the dioxgen adduct of the drug-metabolizing Cytochrome P450 2B4 (CYP2B4), which is an important mammalian enzyme. Much effort in this work has been devoted to developing strategies to effectively trap the especially unstable dioxgen adduct of CYP2B4. Corresponding studies of two key mutants, the dioxgen adducts of E301Q and F429H variants, were also conducted, where the former mutation alters distal pocket interactions, while the F429H variant alters the strength of the trans axial thiolate linkage that can modify the strength of the Fe-O and O-O linkages of the Fe-O-O fragments.