ABSTRACT

RESONANCE RAMAN STUDIES OF OXYGEN ACTIVATION OF CYTOCHROME P450 2B4, MYOGLOBIN AND THEIR MUTANTS

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Marquette University, 2014

Heme proteins are one of the most versatile groups of proteins in living cells and vital to aerobic life. Heme proteins are one of the most versatile groups of proteins in living cells and vital to aerobic life.

Myoglobin (Mb) is a good model to study the heme macrocycle structure and iron-oxo ligand fragments of enzymatic intermediate in the monooxygenases cycle. Mb and its site directed mutants has long served as the fundamental model for investigating the effects of heme site environment on the structure and function of heme proteins. Many important oxidative heme enzymes effect chemical transformations by activating molecular oxygen, in the process generating highly reactive peroxo-, hydroperoxo- and more highly oxidized forms resulting from heterolytic O-O bond cleavage. In recent years it has been shown that these peroxo- and hydroperoxo- intermediates can be generated, trapped and spectroscopically characterized by employing the method of cryoradiolysis.

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 fragments, including the oxy-Mb precursors and the peroxo- and hydroperoxo- forms. A new vacuum system was used to make deoxy- and oxy-complex. The deoxy-, oxy- and transient oxygenated derivatives of myoglobin and its mutants are created and studied by resonance Raman spectroscopy.

Cytochrome P450 2B4 is an important mammalian membrane-bound enzyme which can catalyze the metabolism of pharmaceuticals and other xenobiotics. In order to make oxy-CYP2B4 at low temperature, the experimental methods were optimized, including the mixing time, mixing method and way how to add oxygen. The E301Q and F429H mutants were also used to try to make the oxy-complexes. Finally the oxy-complexes of wild-type CYP2B4 and E301Q mutant were made and measured by rR successfully. The F429H mutant became P420 before oxidization.