Studies of the Soluble Methane Monooxygenase:

Heterologous Expression and Reactions with Nitric Oxide

by

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ABSTRACT

In the first chapter, the determination of the correct sequence of the hydroxylase genes from the *M. capsulatus* soluble methane monooxygenase (sMMO) system is reported. This data was used in a comparison of all the sequenced members of the sMMO system family of proteins. Sequence alignments of the α subunits reveal absolutely conserved residues in the active site region that act as iron ligands, participate in a hydrogen bonding network and provide a source of protons in the largely hydrophobic substrate binding region. Further examination of the rest of the conserved residues in the α and β subunits reveal a possible binding site for protein B, two possible binding sites for the reductase and several regions of intersubunit contact. Possible models for the effects of interaction between all the sMMO proteins are suggested based on the alignment data.

The second chapter describes methodologies used in attempting to express the sMMO hydroxylase. This protein forms insoluble, intractable inclusion bodies upon expression in *E. coli* . The insoluble material was collected and subjected to refolding procedures while varying temperature, reduction potential, thiol concentration, protein concentration, and the identity of the denaturant. Production of the inclusion bodies could not be averted via changing the nature of the expression system by changing media conditions, *E. coli* strains, and expression vector. Conclusions about the folding pathway of the sMMO hydroxylase are drawn from the data collected. Possible future courses of action to express the sMMO hydroxylase are suggested.

The third and final chapter describes the reaction of nitric oxide (NO) with the reduced iron center in the sMMO hydroxylase. The majority of the iron centers react with NO to form a dinitrosyl adduct, termed $H_{dinitrosyl}$, that models the H_{peroxo} intermediate in the reaction of O_2 with the reduced sMMO hydroxylase. This reaction is characterized by Mössbauer, EPR and optical spectroscopies using both continuous and discontinuous kinetic techniques. The $H_{dinitrosyl}$ intermediate

forms whether protein B is present or not, in contrast to the H_{peroxo} intermediate which forms in detectable amounts only in the presence of protein B. This is interpreted in terms of changes in the open coordination sites in the reduced sMMO diiron site upon protein B binding. The $H_{dinitrosyl}$ species decays to form a complex mixture of products, including further reaction with NO to form $Fe(NO)_2$ units and reductive coupling of bound NO molecules to form N_2O and oxidixed sMMO hydroxylase. The effects of protein B, methane and fluoride ion on these reactions are investigated. In addition to formation of $H_{dinitrosyl}$, NO reacts with a small percentage of sMMO hydroxylase sites that are depleted in one iron to form an S=3/2 mononuclear iron-nitrosyl species.

Thesis Supervisor: Stephen J. Lippard
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Finally, my dad Gerry, mother Shirley and sister Kim are the greatest family one could have hoped for. I love them all very much.

Dedicated to my family,
and to the memory of my grandfather
Earl Graham
and my grandmother
Rachel Coufal.

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"Qvid me anxivs svm?"