Methane serves as the sole source of carbon and energy for methanotrophic bacteria. Methane monooxygenase (MMO) catalyzes the oxidation of methane to methanol at ambient temperature and pressure. Aside from methane, MMO can also catalyze the oxidation of a variety of other hydrocarbons including alkane, alkene, aromatic, alicyclic, heterocyclic and halogenated compounds. Studies of the structure and mechanism of MMO can provide insights for designing better industrial catalysts for methane hydroxylation, as well as its applications in bioremediation. The active site of soluble MMO contains a non-heme diiron center, which is located at the \( \alpha \) subunit of the hydroxylase component of MMO. The other two components are the reductase, which transfers electrons from NADH to the diiron center, and component B, which is a regulatory protein. Hydroxylase is capable of catalyzing hydroxylation reaction either upon chemical reduction and exposure to \( O_2 \), or upon addition of \( H_2O_2 \), suggesting that the complete active site required for oxygenase catalysis resides on the hydroxylase alone.

X-ray crystallography and a variety of spectroscopic techniques, including EXAFS, Mössbauer, EPR, ENDOR, MCD, optical, and Resonance Raman spectroscopy have been used to characterize the structure of the dinuclear iron active site. Mössbauer\(^7\) and EPR\(^8\) spectra demonstrated that the resting state of the native hydroxylase (H\(_{ox}\)) contains an antiferromagnetically coupled high-spin diferric center. This diferric unit can be fully reduced to a ferromagnetically coupled high-spin diferrous species (H\(_{red}\)), which is the active form of the enzyme reacting with dioxygen. The X-ray crystal structure of H\(_{ox}\) at 4°C (Figure 1A) reveals a carboxylate-rich ligand environment.\(^9\) The frozen-crystal structures of H\(_{ox}\) (Figure 1B) and H\(_{red}\) (Figure 1C) indicate that upon reduction, Glu243 undergoes carboxylate-shift.\(^10\) This kind of change in carboxylate coordination-mode has been observed in the R2 protein of ribonucleotide reductase and several synthetic model complexes.\(^11\) Carboxylate shifts can provide coordination site for the incoming dioxygen without causing large changes in the coordination sphere.

Figure 1

Fully reduced hydroxylase activates dioxygen and generates a highly active species that can oxidize the substrates. During this process, two diamagnetic intermediates, termed P and Q, have been detected with rapid freeze-quench Mössbauer and stopped-flow optical spectroscopy.\(^12\) P was assigned as a diiron(III)-peroxo species based on its Resonance Raman,\(^13\) optical, and Mössbauer parameters.\(^12\) Two synthetic non-heme diiron(III) peroxo adducts have been crystallized recently\(^14\) (Figure 2), providing certain insight into the nature of intermediate P. Intermediate Q was proposed to have a formal diiron(IV) oxidation state based on its diamagnetism and unusually small isomer shift (\(-0.18 \text{ mm/s}\)). This kind of non-heme diiron(IV) species has not been observed in either synthetic complexes or other proteins.
Several mechanisms of substrate hydroxylation have been proposed. One of them (Figure 3) is based on the similarity of MMO with cytochrome P-450 in substrate ranges and product-distributions.\textsuperscript{15} It involves the abstraction of hydrogen from the substrate to form a substrate radical, which then rebinds to the hydroxyl on one of the iron atoms, and yields the alcohol product. The observation of configuration inversion of a chiral substrate\textsuperscript{16} and the detection of EPR signals of several spin-trapping radical adducts\textsuperscript{17} support this radical-based mechanism. Evidence for other mechanisms, such as involvement of a cationic intermediate,\textsuperscript{18} has also been obtained. The exact mechanism is probably dependent on properties of the substrates, sources of the enzyme, regulation by Component B, and temperature of the reaction. Further studies are needed to obtain better understanding of the mechanism of methane monooxygenase.

![Figure 2](image)

![Figure 3](image)

References


