Methane Hydroxylation by Methane Monooxygenase

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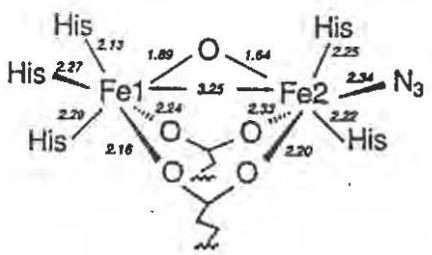
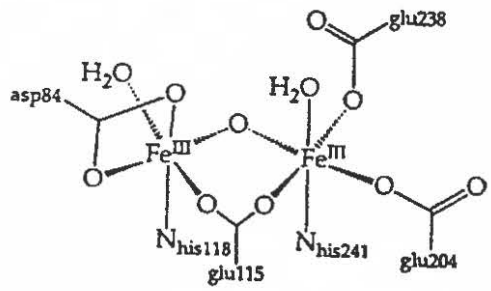
Methane monooxygenase (MMO) catalyzes the conversion of methane to methanol. This protein has been isolated from methanotrophic bacteria to a high degree of purity [1]. Additional equivalents of atmospheric oxygen and NADH are also needed for substrate turnover. Currently, the use of purified MMO is the only way to convert methane to methanol under common laboratory conditions. The broad interest in this area makes the mechanistic understanding of this process extremely important, which is the focus today.

Currently, an x-ray crystal structure of MMO is not available, leaving only the physical properties of MMO to determine the active site. Spectroscopic comparisons to hemerythrin (Hr) and ribonucleotide reductase (RR) indicate that MMO related to this class of diiron μ-oxo proteins [2]. The x-ray crystal structures of Hr [3] and RR [4] active sites are shown below. Mössbauer spectroscopy indicates either Fe(III) high spin or Fe(II) high spin in either of MMO's three oxidation states [5]. EPR spectra show a different situation for the met, Fe(III)·Fe(III), and semimet, Fe(II)·Fe(III), oxidation states where the S = 0 and S = 1/2 ground states exist due to antiferromagnetic coupling between the two iron nuclei [2]. The active site of MMO seems to be quite similar to those of Hr and RR.



Azidometmyohemerythrin (Hr)



Ribonucleotide Reductase (RR)

Small differences in physical properties of MMO from Hr and RR are thought to be due to a substituted bridging oxygen, derived from an amino acid [6]. Antiferromagnetic coupling in the met states indicates Fe-O bond length is considerably shorter in Hr and RR compared to MMO [6,7]. This longer Fe-O bond length in MMO is also supported by EXAFS [6]. Finally, the only model complex to have redox potentials close to MMO is $Fe_2(H_2Hbab)_2$(N-MeIm)$(DMF)_2$ [8]. Together these observations imply that MMO's bridging oxygen is derived from either a phenoxide (tyrosinato) residue or a carboxylate (aspartate or glutamate) residue.

Oxygen and hydrogen peroxide both turnover substrate from the reduced and met states, respectively [9]. Despite this, an oxygen bound adduct has never been observed for MMO, making analogies to model complexes the only current structural probe. Two complexes, $[Fe_2$(N-Et-HPTB)(OBz)$](BF_4)_2$ [10] and$[Fe_2$(HPTB)(OH)$(NO_3)_2](NO_3)_2$ [11], upon addition of O_2 and H_2O, respectively, will form an irreversibly bound 1:1 adduct. From Resonance Raman, Mössbauer, and ^{1}H-NMR spectroscopies this adduct is known to be antiferromagnetically coupled and have a peroxide ligation in a bidentate bridging fashion. A similar adduct in MMO seems logical in that MMO's next step is to activate and cleave the O-O bond.

Current results imply that hydroxylation by MMO occurs in a stepwise fashion rather than by a concerted process [12,13]. The proposed species is a high valent iron-oxo intermediate involved in an initial H atom abstraction step. Then after a time lapse, as indicated by allylic rearrangement [12], the substrate radical is hydroxylated. This intermediate has been shown to exist in $Fe_2O(TPA)_2(ClO_4)_4$ at -40 °C [14]. Currently the mechanism of MMO is proposed to go through O-O bond cleavage to form a high valent iron-oxo intermediate.

The question is now, if O_2 is cleaved from this peroxide adduct where does the non-iron-oxo oxygen atom go? Upon addition of O_2 to $[Fe_2(O_2CH)_4(BIPhMe)_2]$ the bridging oxygen of a formate has moved to a terminal position to make room for a μ-oxo ligand in the product [15]. There are two examples of a carboxylate ligation in metalloproteins, Concanavalin A [16] and RR [4]. Further, this lability has a substantial precedent in coordination chemistry and may be seen in other metalloproteins.

In summary, hydroxylation of methane by MMO is currently believed to come from an iron-oxo intermediate. This iron-oxo intermediate comes from a decomposition of an oxygen bound adduct of MMO. This adduct can be reached either by two-electron reduction and oxygen binding or by addition of hydrogen peroxide. Obviously, x-ray crystallography on MMO will add to our current state of knowledge.

References

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