Geometrical Variability in the Diferrous Active Site of the Soluble Methane Monooxygenase Hydroxylase from *Methylococcus capsulatus* (Bath)

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Introduction

The biological oxidation of methane to methanol is performed at transition metal centers located within methane monooxygenase enzymes. The soluble methane monooxygenase from *Methylococcus capsulatus* (Bath) is a three-component enzyme; the 251-kDa hydroxylase component (MMOH) contains a carboxylate-bridged dinuclear iron active site in each α subunit of an $(\alpha\beta\gamma)_2$ structure. During the course of catalysis, the dinuclear iron center is reduced from the diiron(III) oxidation state to the diiron(II) state by transfer of electrons from the reductase component to MMOH, as mediated by protein B. Reduced MMOH reacts with dioxygen in the first step of the catalytic cycle. The resulting superoxo and peroxo diiron(III) structures then convert to the high-valent diiron(IV) intermediate Q, which reacts with methane, forming methanol and releasing the second dioxygen atom as water.[1,2]

The structure of MMOH from *M. capsulatus* (Bath) has been reported from crystals in both the diiron(II) and diiron(III) states.[3] Despite high-resolution structures of MMOH and substantial biochemical data, however, a detailed understanding of catalysis by the enzyme is still lacking. Previous x-ray crystallographic studies of MMOH demonstrated the ability of several side chains lining the active site cavity to adopt alternate rotamer conformations.[3,4] Density functional theory calculations and the results of pH studies with MMOH also suggested an important role for hydrogen bonding and proton transfer during catalysis by the enzyme.[5,6] In an effort to understand better the hydrogen bonding networks at the active site and the role of conformational flexibility during catalysis, we undertook a systematic study of the structures of a second crystal form of MMOH from *M. capsulatus* (Bath) that has also been used for substrate analog and product binding studies.[7,8] This report presents the high-resolution structure of the second crystal form in the reduced diiron(II) oxidation state and highlights the insights gained from this structure.

Materials and Methods

Crystals of MMOH from *M. capsulatus* (Bath) in space group $P2_12_12_1$ having unit cell dimensions a = 71 Å, b = 172 Å, and c = 222 Å were grown as described previously.[9] To generate crystals of the diiron(II) state, oxidized crystals were brought into an anaerobic chamber and soaked for 1 h in a stabilizing solution (10% PEG 8000, 25% glycerol, 0.2 M $CaCl_2$, 50 mM MOPS, pH 7.0) containing 200 μM methyl viologen and ~4 mM sodium dithionite. The crystals were then removed from the anaerobic environment and frozen as described previously,[9] and transported to Argonne for data collection.

Data were collected at beamline 19-ID using a 3 × 3 CCD detector and x-radiation with λ = 1.0331 Å. Diffraction intensities were indexed in DENZO and scaled with SCALEPACK.[10] Initial phases for data refinement were taken from the oxidized MMOH crystal form II model.[4] Data refinement was performed with CNS and manual rebuilding was done in O.[11,12]

Results

In comparison to the oxidized active site of MMOH, the reduced active site contains a previously observed carboxylate shift of Glu243 from a monodentate binding mode to Fe2 to a position bidentate chelating to Fe2 and monodentate bridging between the two iron ions (Fig. 1).[3] This shift displaces the μ-OH^- bridge found in the oxidized active site. Glu144 continues to bridge the two iron ions, albeit in a more symmetric fashion than is found the in the oxidized active site, and a water molecule occupies the bridging position distal to the two histidine ligands. The remaining ligands to iron are unchanged relative to their positions in the oxidized structure. The dimeric MMOH molecule contains two dinuclear iron active sites; the oxidation state-dependent changes are reproduced in each.

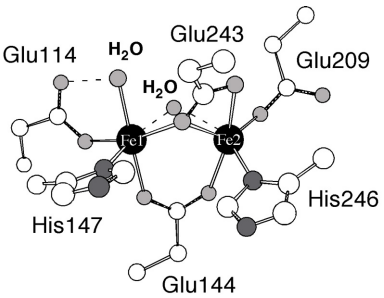

FIG. 1. Reduced MMOH active site.

The active site cavity in each protomer contains one or two well-ordered solvent molecules, respectively, in addition to the solvent located within the coordination spheres of the iron ions. These solvent molecules enforce one particular orientation of Leu110, a residue with a potential "gating" role in controlling substrate access to the active site,[4,7] but leave the active site cavity largely empty and provide no direct hydrogen bond pathway to the exterior surface of the enzyme. The carboxylate shift of Glu243 does, however, cause a disruption of the regular hydrogen bonding arrangement in helix F of MMOH, altering the position of Leu244 on the solvent-exposed surface of the enzyme. Asn214 also adopts a different rotamer, altering the MMOH surface.

Discussion

Reduction of MMOH from the diiron(III) to the diiron(II) oxidation state results in changes in both the coordination sphere of the diiron center and at the surface of the enzyme. The combination of the largely vacant active site cavity and the weakly coordinated solvent in the bridging position distal to the His residues is consistent with the active site being ready for reaction with

dioxygen. Alterations of the surface of MMOH in the vicinity of the active site may have implications for the binding affinity of the reductase and protein B components of the enzyme[13,14] and may help account for the differential accessibility of small molecules to the active site of MMOH relative to the oxidation state of the iron atoms.[15] A more comprehensive discussion of this work can be found in Ref. 9.

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