Chemical nature of the hydroxylase enzyme of methane monooxygenase as revealed by the 2.2 Å crystal structure

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The reaction of methane with dioxygen to form methanol and water:

$$CH_4 + NADH + H^+ + O_2 \rightarrow CH_3OH + NAD^+ + H_2O$$

is the first step in the metabolic pathway of methanotrophic bacteria. Methanotrophs use methane as their sole source of carbon and energy, and are important in limiting atmospheric concentrations of this greenhouse gas. The oxidation of methane to methanol is a chemically difficult transformation which current industrial processes can only reproduce using high temperatures and pressures to achieve low yields [1]. Yet methanotrophic bacteria perform the reaction at ambient temperature and pressure. The enzyme system responsible for this chemistry is methane monooxygenase (MMO), which is composed of a hydroxylase, a reductase and a regulatory protein. Various approaches, including synthetic modeling chemistry, kinetics, and time-resolved spectroscopy, have been used in our laboratory and elsewhere in pursuit of a detailed understanding of the MMO catalytic mechanism.

Recently, we determined the 2.2 Å X-ray structure of the hydroxylase from *Methylococcus capsulatus* (Bath) (Fig. 1a) [2]. This protein is an $\alpha_2\beta_2\gamma_2$ dimer. There is a large canyon formed by the $\alpha\beta$ protomers, with an opening in the center of the molecule. Each of the two α subunits houses one catalytic diiron center. The coordination of the diiron center (Fig. 1b) is similar to what was predicted by physical studies of the hydroxylase.

The three-dimensional structure of the hydroxylase provides a new framework with which to interpret the results of biochemical, kinetic, and spectroscopic experiments. For example, the regulatory protein strongly affects the hydroxylation reaction by changing the redox potentials of the diiron center and by altering the rate and regioselectivity of substrate oxidation [3]. According to chemical cross-linking studies, this protein contacts the α subunit of the hydroxylase [4]. The structure suggests that the regulatory protein may bind in the canyon region near the dimer interface. Two of the iron-coordinating helices are exposed in the canyon, and several ligands, including Glu209 and Glu243, could be affected by conformational changes in this region. The canyon could also constitute the binding domain for the reductase, which, according to the cross-linking studies, interacts with the β subunit.

The structure also reveals several amino acid residues which may be important in catalysis (Fig. 1c). Cys151 is near the active site, in the same position as the functionally important tyrosyl radical in the structurally related ribonucleotide reductase R2 protein. A speculative mechanism has been proposed in which Cys151 undergoes redox changes during turnover [5]. In addition, a threonine residue, Thr213, is located near the active site at a distortion in one of the helices. This helical distortion resembles that in the longest helix in cytochrome P450CAM, which houses a conserved threonine residue that has been postulated to supply protons during catalysis. Thr213 may serve a similar function in the hydroxylase. Finally, the hydrophobic pocket in the active site cavity may be essential to position substrates near the diiron center during catalysis.

The hydroxylase is often compared to the analogous heme enzyme cytochrome P450, for which a mechanism involving a high valent iron oxo (ferryl) species and a substrate radical is widely accepted. Attempts to find evidence for substrate radical intermediates in MMO indicate that, if they do exist, they are extremely short-lived. Recent stopped flow optical spectroscopic measurements have detected intermediates in the reaction of reduced hydroxylase with dioxygen. Related investigations of model compounds have provided some insight into possible mechanisms and the chemical nature of these intermediates [5]. While the X-ray structure does not directly address such mechanistic questions, the detailed information now available about the active site and overall three-dimensional structure of the hydroxylase should contribute significantly to the design and interpretation of future mechanistic experiments.

References

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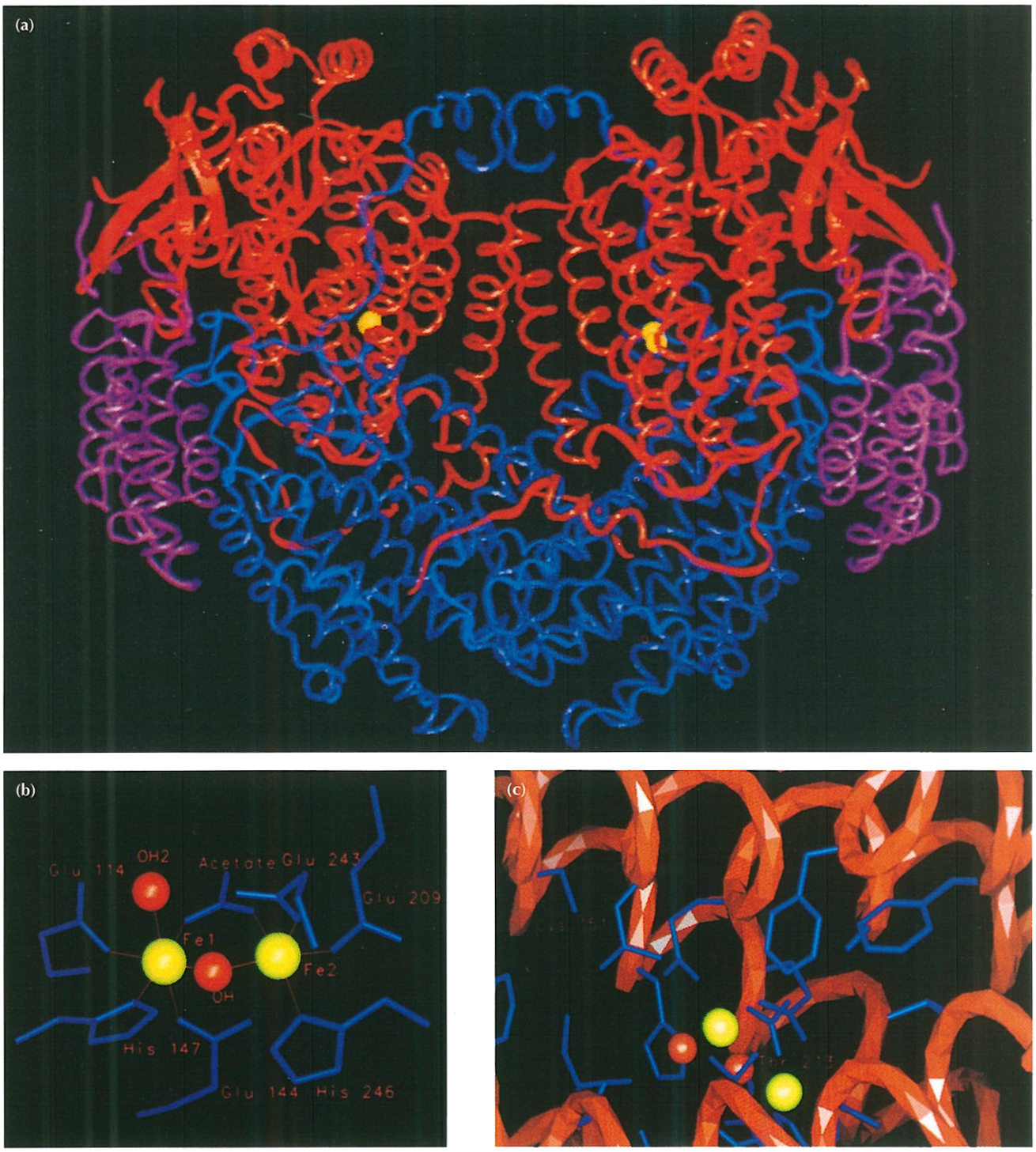

Fig. 1. The MMO hydroxylase. **(a)** The holo enzyme. The holo hydroxylase (MW 251 kDa) consists of two copies each of three different subunits in an $\alpha_2\beta_2\gamma_2$ polypeptide arrangement. The α subunits are shown in red, the β subunits in blue, and the γ subunits in violet. The iron atoms are represented as yellow spheres. The two αβγ protomers are related by a non-crystallographic two-fold symmetry axis, and together form a shape resembling a heart. In addition, an Fe-S/FAD-containing reductase (MW 38.6 kDa) and a regulatory protein (MW 15.5 kDa) are required for activity. **(b)** The coordination of the dinuclear iron center. The two iron atoms are triply bridged by one carboxylate ligand, Glu144, a monodentate hydroxide ion, and a bidentate acetate ion from the crystallization buffer. Each iron atom is coordinated to the δ-N atom of a histidine residue, Fe1 to His147 and Fe2 to His246. Fe1 is also ligated to one monodentate carboxylate, Glu114, and to a water molecule. Fe2 has two additional monodentate carboxylate ligands, Glu209 and Glu243. The diiron center is located in a cavity containing a hydrophobic pocket. **(c)** The active site cavity.