The Structure Biology Studies of Metallic Membrane Protein: Particulate Methane Monooxygenase from Methylococcus Capsulatus (Bath)

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The particulate methane monooxygenase (pMMO) is a membrane-bound protein found in all methanotrophic bacteria. It consists of a three subunits hydroxylase (43, 29 and 28 kDa). The enzyme exhibits unusual substrate specificity: only small normal alkanes are hydroxylated and similar alkenes epoxidated. The hydroxylase is a copper protein, with 15 copper ions arranged in copper clusters. A type 2 copper center, a dinuclear copper cluster, and a trinuclear copper cluster are involved in the hydroxylation chemistry of the enzyme. The remaining copper ions are associated with the 45 kDa subunit, and provide a buffer of reducing equivalents in the protein. Presumably, the electron transfer copper clusters (E-clusters) participate in mediating the electron input from the NADH reductase to the catalytic copper clusters (C-clusters). pMMO has proved difficult to isolate and purify for biochemical and biophysical characterization because of its instability in detergents used to solubilize the enzyme. We have devoted the bulk of our attention toward obtaining the purified pMMOdetergent complex with high activity, as well as the water-exposed domain of the PmoB subunit containing the E-clusters, and toward obtaining single crystals of them. Unfortunately, only a small number of diffraction spots have been observed, as the crystals are primarily "one-dimensional" or "two-dimensional". The pMMO-detergent complex thus isolated has a molecular mass of 220 kDa, consisting of an αβγ protein monomer encapsulated in a micelle of *ca.* 240 detergent molecules. It is a copper protein containing 13.6 mol of copper/mol of pMMO and essentially no iron. The activity of the enzyme has been measured by the standard propene epoxidation assay by injecting the reaction mixture into a Gas Chromatograph equipped with a Flame Ionization Detector (FID) for product analysis (Table 1). The specific activity of the purified C5 pMMO-detergent complex is as high as 16.87 nmoles propylene oxide/min·mg protein with NADH as the reductant. One observation of interest is that the activity of the pMMO-detergent complex is enhanced by ascorbate and copper-acetate, presumably because of more facile reduction of the E-cluster copper ions in pMMO-detergent complex compared to NADH, following turnover. High resolution X-ray crystallography requires well-ordered three dimensional crystals grown from protein solutions, but membrane protein structural biology is still a frontier area of modern biomedical research. Optimal conditions for nucleation and growth of protein crystals are difficult to predict as they depend on a large number of variables.

Table 1.

Sample	Specific Activity (nmol/mg/min)
C12 Purified pMMO-detergent complex	15.33
C5 Purified pMMO-detergent complex	10.51
C5 Purified pMMO-detergent complex+ Cu(II)-acetate	11.92
C5 Purified pMMO-detergent complex+ Cu(II)-acetate + ascorbate	12.23
C5 Purified pMMO-detergent complex+ Cu(II)-acetate + Cu(II)-acetate + ascorbate	16.87

Reassuringly, the membrane protein crystallization techniques are identical to those used for soluble proteins. We use the Hampton screen kits to screen for suitable crystallization conditions, and it allow us to quickly test wide ranges of pH, salt and precipitants. Crystallization screens have performed at 4oC and the vapor diffusion and micro-dialysis method. Small changes in pH, salt, precipitant concentration, additives, protein concentration, type of detergent has to be tested empirically. Recently, we have made some progress in the crystallization of pMMO, and some of important information has been derived. Three types of pMMO crystals have been obtained already under the suitable conditions (Fig. 1). However, only a small number of diffraction spots were observed when we tried to collect intensity data with these crystals. We suppose that the low resolution data are due to the crystals that are too small; and perhaps, they are one-dimensional or two-dimensional crystals only. It is necessary to improve the quality of the crystals before X-ray diffraction can be obtained at high resolution.

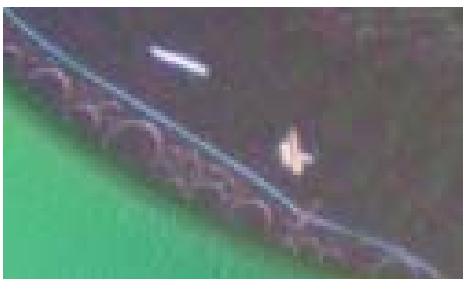

Figure 1

We are searching for better crystallization conditions to obtain three-dimension and larger crystals. The three dimensional structure of pMMO would clarify the configuration of the pMMO active site and allow more precise characterization of the chemistry at the copper sites during dioxygen activation and methane hydroxylation mediated by pMMO.