EXAFS Studies of the Tricopper Cluster in the Particulate Methane Monooxygenase (pMMO)

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The particulate methane monoxygenase (pMMO) is an enzyme that mediates the facile conversion of methane to methanol in methanotrophic bacteria under ambient conditions of temperature and pressure. It is a complex membrane protein consisting of three subunits (PmoA, PmoB, and PmoC) and many copper cofactors [1]. The protein has been notorious for its tendency to lose essential metal cofactors during isolation and purification. For this reason, it has been difficult to isolate and purify the pMMO to homogeneity for biochemical and biophysical characterization.

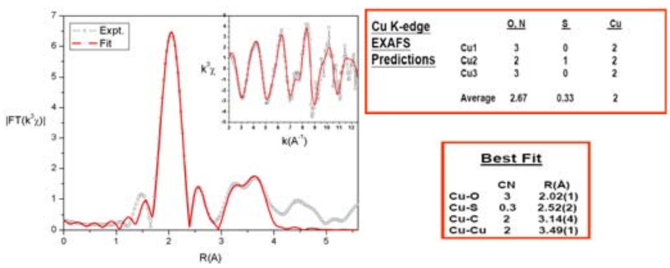
Our laboratory has previously presented experimental evidence for a tricopper cluster in active preparations of pMMO that contain the full complement of ~15 copper ions [2]. Although a tricopper cluster has not been observed in the recent crystal structure of the protein isolated from membranes of *Methylococcus capsulatus* (Bath)[3], we have identified an empty hydrophilic cavity in the structure containing a number of potential metal binding ligands (residues His38, Met42, Asp47, Asp49 and Glu100 from PmoA, and Glu154 from PmoC) to accommodate this cofactor [2]. In fact, it is possible to model a tricopper cluster into this hydrophilic pocket within the PmoA transmembrane domain (D site).

Given that all but two of the potential ligating residues that form the putative tricopper cluster at the D site would originate from a single fragment from the PmoA subunit, PmoA(38-49), it is possible to verify that the D site is a copper-binding domain by synthesizing the this fragment, HIHAMLTMGDWD, and examining its affinity for copper ions. With respect to the model tricopper cluster that we have rebuilt into the D site, this peptide lacks only the carboxylate side chains provided by Glu100 from PmoA and Glu154 from PmoC as ligands. However, these carboxylates could be provided by exogenous anionic ligands in the peptide-copper solution.

Following this strategy, we have shown that PmoA(38-49) is indeed a copper-binding domain, and it readily forms a tricopper cluster with a ligand structure identical to the structure that we have modeled into the D site earlier. Aside from characterizing the thermo-dynamics of formation of the tricopper-peptide complex by isothermal titrating calorimetry, identifying the copper species formed by FT ICR mass spectrometry, and confirming by EPR that the tricopper complex is a triad of ferromagnetically coupled Cu(II) ions in its ground electronic state, we have also deduced its ligand structure using mutant peptides as well as by X-ray spectroscopy *via* extended Cu K-edge X-ray absorption fine structure (EXAFS) (Fig. 1) on the wiggler beamline BL-17C1 at NSRRC in Hsinchu, Taiwan.

The best EXAFS data were obtained on a TEL-SAM-PmoA(38-49) fusion peptide consisting of a TEL-SAM domain fused to a peptide sequence comprised of PmoA(38-49), and an additional proline and aspartate (**H**IHA**M**LTMG**D**W**D**-PD). Best fitting results are displayed in Fig. 1 and the corresponding parameters are also summarized. These data are totally consistent with the ligand structure predicted from the tricopper cluster that we have rebuilt into the D site. By taking Cu-O and Cu-Cu distances of 2.02 Å and 3.49 Å, respectively, we estimate a Cu-O-Cu bond angle of 119.5°, consistent with a capping "μ_3-oxo" group located at the center of the triad.

Fig. 1: Fourier transformed amplitudes FT[$k^3\chi(k)$] (open circles) obtained for the peptide-copper complex together with the best fit (solid curve) based on the ligand structure proposed for the tricopper cluster. Insets show the respectively fitted $k^3\chi(k)$ data.



In this study, we have provided direct experimental evidence that the D site in pMMO is a tricopper-cluster binding domain. The biophysical and structural evidence presented here should resolve the long-standing debate over the existence and the location of the tricopper cluster in this enzyme.

References

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