# The XAS Studies of the Copper Ions in the Aqueous-exposed Domains of the 45 KDa Subunit of Particulate Methane Monooxygenase

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The crystal structure of the particulate methane monooxygenase (pMMO) from *Methylococcus capsulatus* (Bath) has recently been reported [Lieberman, R. L., and Rosenzweig, A. C. (2005) Nature 434(7030), 177-182]. These results support the 15 putative transmembrane α-helices predicted earlier by amino-acid sequence analysis of the three pMMO subunits (28, 45 and 29 kDa) using the program TMHMM (TransMembrane topology with a Hidden Markov Model), and the three-dimensional Fold Recognition Server based on the 3D Position Specific scoring Matrix Method [Vinchurkar, M. et. al Biochemistry 43(42) 13283-13292]. A prediction of the theoretical modeling was that the C-teminal water-exposed sequence (N255-M414) of the 45 kDa subunit matched the β-sheet structure of the multi-domain cupredoxins, suggesting that this might be the putative binding domain for the ca. 10 Cu(I) often referred to as the E-clusters. However, only three copper ions (1 mononuclear copper center and 1 dinuclear copper cluster were observed in the crystal structure. The type and number of copper ions revealed by the x-ray structure were also not in accord with earlier findings of ca. 15 copper ions in pMMO-enriched membranes and the purified enzymes. Towards resolving this conflict, we have cloned and over-expressed in *E. coli* the two aqueous-exposed domains (residues 1-186 and 254-414) of the 45 kDa subunit, the only regions of the three dimensional fold where presumably the water-soluble NADH can access the E-cluster to transfer reducing equivalents after these copper ions have become oxidized by turnover. The corresponding spectroscopic data have shown the recombinant *C*-terminus water exposed domain (residues 257-349, V322E E343D) behaving like a Cu(I) sponge , taking up to ca. 10 Cu(I) ions cooperatively with Hill constant αH = 4.3. From the circular dichroism studies, we showed that the folding of the *C*-terminus was highly Cu(I)-dependent, and the reconstituted Cu(I)-enriched peptides exhibited β-sheet structure, as predicted from the earlier modeling studies. To have a better understanding in terms of the incorporated copper ions in the corresponding genetic recombinant proteins, we carried out the XAS studies at the proposals 2005-2-017-1 and 2005-2-017--2. The X-ray spectroscopic data were collected using synchrotron radiations at NSRRC 17C1 W20 beam lines.

We first added 50 eqivalent of $CuSO_4$ to the *C*-terminal water exposed domain (residue 257-349, V322E E343D) without the addition of DTT. The copper enriched proteins were dialyzing with a 10,000 Da MW cut-off dialysis membrane against solution I containing 1.0 mM dithiothreitol, 20 mM Tris-HCl and 1.0 mM NaCl buffer. The data indicated the Cu K-edge signal is too poor to observe the edge jump.

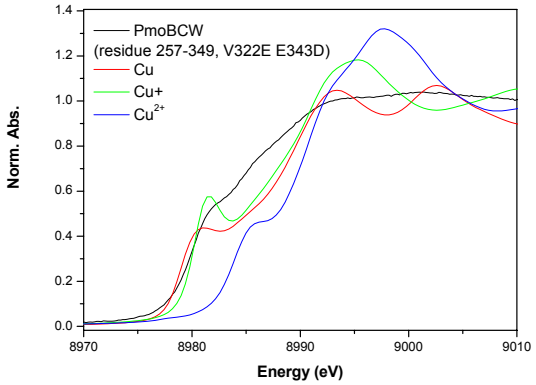
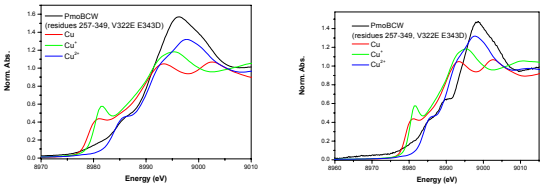



Figure 1. The Cu Kα-edge spectrum of PmoBCW (residue 257-349, V322E E343D) was added 50 eqivalent of $CuSO_4$ in the presence 100 eq. DTT and dialyzed against solution I. To compare with the various oxidative states of authetic copper foil standard, we found the incorporated copper ions appeared as $Cu^+$ ions.



(a) (b)

Figure 2. The Cu Kα-edge spectrum of PmoBCW (residue 257-349, V322E E343D) was added 50 eqivalent of $CuSO_4$ in the presence 100 eq. DTT and dialyzed against solution I plus 100 eqivalent ferric cyanide. The XAS measurements were carried out at 10 K and the samples were prepared as (a) powder and (b) solution form.

The experimental outcome has performed the $Cu^+$ ions exhibited much higher affinity toward the *C*-terminal water exposed domain. Even in the presence of strong oxidant such as ferricyanides, some of the incorporated cupric ions are still appeared as reduced form. Those copper ions with high redox potential presumably served as kinetic barrier for electron transfer with respect to methane oxidation. The scenario is highly consistent with the results we presented in E-cluster studies of pMMO.