Isolation, Purification and Characterization of Hemerythrin from *Methylococcus capsulatus* (Bath)

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Recently we reported a quantitative proteomic analysis of the metabolic regulation by copper ions in *M. capsulatus* (Bath) [1].As part of this study, we uncovered a hemerythrin in *M. capsulatus* (Bath) (McHr), which, like pMMO, was also over-produced in the cells .At a Cu concentration of 30 μM in the growth medium, the expression of the hemerythrin was stimulated four fold relative to its level when the cells were grown in the absence of added Cu ions. The structure and mechanism of eukaryotic hemerythrin have also been well studied and reviewed [2]. While hemerythrin is thought to be present only in marine invertebrates in most of the current literature, several bacterial hemerythrin genes have recently been implicated by genomic sequencing [3]. Until our quantitative proteomics study [1], no native or *bona fide* bacterial hemerythrin has been identified, isolated and characterized. Accordingly, we have decided to isolate and purify the McHr after completion of the proteomics study. In comparison with known primary sequences from the marine invertebrate *Themiste dyscritum* , the diiron center chelating residues were all conserved despite only just 29% overall sequence identity. In this study, we present the isolation and purification of McHr from cells of *M. capsulatus* (Bath) enriched in pMMO, as well as characterization of different states of the McHr *via* UV-Visible, circular dichroism, resonance Raman, electron paramagnetic resonance spectroscopy and X-ray absorption spectroscopy at NSRRC 17C beamlight.

Atomic absorption gave two irons per protein. Circular dichroism revealed the standard α -helix characteristics. UV-visible, EPR and resonance Raman suggested the existence of the oxo-bridge between two iron atoms. NEXAS , UV-visible and EPR exhibit three different states, deoxyMcHr (Fe(II),Fe(II)), oxyMcHr (Fe(III),Fe(III)) and metMcHr (Fe(III),Fe(III),as shown in Figure 1, which is similar with the hemerythrin from eukaryote. Finally, sequence alignment together with CD implicated a four-helix bundle and active site structure. Combined with above all pieces of evidence ,the structure and property of hemerythrin from *M. capsulatus* (Bath) is basically similar with the hemerythrin from marine invetebrate. Besides, we discovered the rate of auto-oxidation of metHr of *M. capsulatus* (Bath) ($t_{½}$~50 min) was much faster than that of hemerythrin from marine invertebrates ($t_{1/2}$ ~20 hours).

Based on these findings, we implied that in the cellular environment, the consumption of O_2 by pMMO must be significantly faster than the rate of auto-oxidation of the oxy-McHr, based on the turnover frequency of the pMMO. In addition, the cell is sufficiently reducing so that the steady concentration of the met-McHr cannot be high. Thus, there is a major difference in the importance of the auto-oxidation in the cellular milieu versus a protein solution in buffer. Auto-oxidation of hemerythrin is primarily an artifact of studying hemerythrin in buffer.

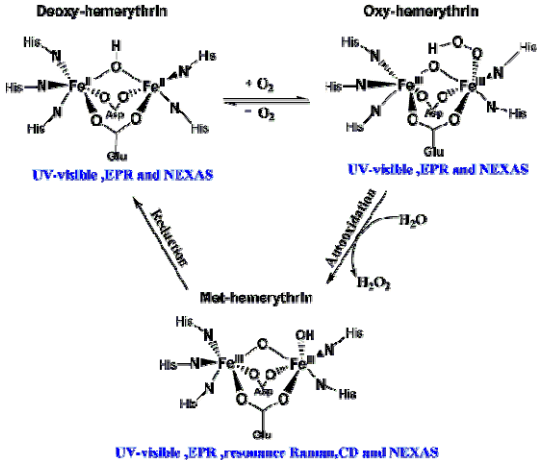

Figure 1. Three different states of McHr revealed by several spectroscopies

References:

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