Modulation of the Molybdenum Coordination Sphere of *E. coli* Trimethylamine *N*-oxide reductase and role of the nucleotides in the bis-MGD molybdenum cofactor

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The well-studied enterobacterium *Escherichia coli* present in the human gut is able to reduce TMAO to trimethylamine (TMA) during anaerobic respiration. The TMAO reductase TorA is a monomeric, bis-molybdopterin guanine dinucleotide (bis-MGD) cofactor-containing enzyme belonging to the dimethylsulfoxide (DMSO) reductase family of molybdoenzymes. We report on a system for the *in vitro* reconstitution of TorA with molybdenum cofactors (Moco) from different sources. Higher TMAO reductase activities for TorA were obtained when using Moco-sources containing a sulfido ligand at the molybdenum atom. For the first time, we were able to isolate functional bis-MGD from *Rhodobacter capsulatus* formate dehydrogenase (FDH), which remained intact in its isolated state and after insertion into apo-TorA yielded a highly active enzyme. Combined characterizations of the reconstituted TorA enzymes by spectroscopic techniques and emphasized that TMAO reductase activity can be modified by changes in the Mo-coordination sphere. The combination of these results together with studies on amino acid exchanges at the active site led us to propose a novel model for substrate binding to the molybdenum atom of TorA.

Further,for long, the role of the GMP nucleotides of the bis-molybdopterin guanine dinucleotide (bis-MGD) cofactor of the DMSO reductase family had been a subject of discussion. The recent characterization of the bis-molybdopterin (bis-Mo-MPT) cofactor present in the *E. coli* YdhV protein, which differs from bis-MGD solely by the absence of these nucleotides, enabled us to study the role of the nucleotides of bis-MGD and bis-MPT cofactor for Moco insertion and activity of molybdoenzymes. Using the established *E. coli* TMAO reductase TorA as a model enzyme for cofactor insertion, we were able to show that the GMP nucleotides of bis-MGD are crucial for the insertion of the bis-MGD cofactor into apo-TorA.