Structural and Biochemical Studies of Molybdenum Cofactor Biosynthesis

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The molybdenum cofactor (Moco) is the essential component of a diverse group of redox enzymes, which catalyze important transformations in the global C-, N- and O-cycles. Moco consists of a mononuclear molybdenum coordinated by the dithiolene moiety of a family of tricyclic pyranopterin structures. Moco-biosynthesis is an evolutionarily conserved pathway comprising several unusual reactions and is initiated by the conversion of GTP through the action of MoaA and MoaC into the tetracyclic pterin derivative precursor Z. The MoaA enzyme is a member of the Radical SAM superfamily and its crystal structure revealed an N- and a C-terminal Fe_4-S_4 cluster both featuring a Cys_3 ligation. The N-terminal cluster binds S-adenosylmethionine (SAM), and reduction of this cluster leads to homolytic cleavage of SAM and subsequent conversion of GTP. Following the crystal structure analysis of *Staphylococcus aureus* MoaA biochemical studies have defined the GTP binding site and provided insights into the catalytic mechanism. In the next step during Moco biosynthesis the dithiolene moiety is incorporated into precursor Z by molybdopterin synthase. Structural studies of this enzyme have defined its substrate-binding site and identified residues involved in the catalytic mechanism, which involves transfer of the sulfur atom from a C-terminal thiocarboxylate. Formation of the thiocarboxylate is carried out in an ATP-dependent process, which is the evolutionary ancestor of the activation reaction of ubiquitin and related protein modifiers. Subsequently the metal is incorporated via the newly created dithiolene group resulting in the formation of the mononucleotide form of the cofactor.