The Molybdenum Factor

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The molybdenum enzymes catalyze a variety of important metabolic conversions. More than a dozen molybdenum-containing enzymes are now known [1]. In molybdenum enzymes, Mo(VI) acts as a two-electron acceptor for the oxidation of organic and inorganic substrate molecules. Mo(IV) is then reoxidized by some intra- or intermolecular electron transfer process. Most of the knowledge concerning the ligands surrounding the molybdenum center has come from EXAFS and EPR data. The molybdenum enzymes can be placed into two categories: the nitrogenases, which possess the iron-molybdenum cofactor (FeMo-co), and the oxo-molybdenum enzymes, which possess the molybdenum cofactor (Mo-co). Two of the most widely studied oxo-molybdenum enzymes are sulfite oxidase and xanthine oxidase. Sulfite oxidase is found in the intermitochondrial space of mammalian livers; it is responsible for the physiologically vital oxidation of sulfite to sulfate. Xanthine oxidase can be isolated easily from cow's milk; it is capable of oxidizing a variety of aromatic heterocycles and aldehydes to their hydroxy derivatives.

The molybdenum cofactor, Mo-co, is defined as a prosthetic group that can be released from a molybdenum enzyme by denaturation techniques and that is capable of reconstituting the apoprotein of nitrate reductase. The oxidative instability of isolated Mo-co has made it difficult to identify. Studies by Johnson and Rajagopalan [2] on the degradation products of the cofactor led them to propose that the cofactor contains a pterin component with two vicinal thio groups on a 4-carbon phosphorylated side chain (Fig. 1a). The organic portion of the cofactor is referred to as molybdopterin. Since the degradation products of molybdopterin were unsaturated (at positions 6 and 7) in the pterin ring, it was also possible that molybdopterin was unsaturated; however, by comparing the spectral properties of molybdopterin with that of tetrahydrobiopterin, Rajagopalan *et al.* [3] found that the pterin ring is saturated (at positions 6,7). Furthermore, the stoichiometric reaction of two molecules of iodoacetamide with one of molybdopterin supported the presence of two vicinal SH groups in molybdopterin [3,4]. The stable, alkylated product (Fig. 1b) was characterized in its oxidized and reduced forms by ^{1}H NMR, FAB-MS, XPS, and visible spectroscopy.

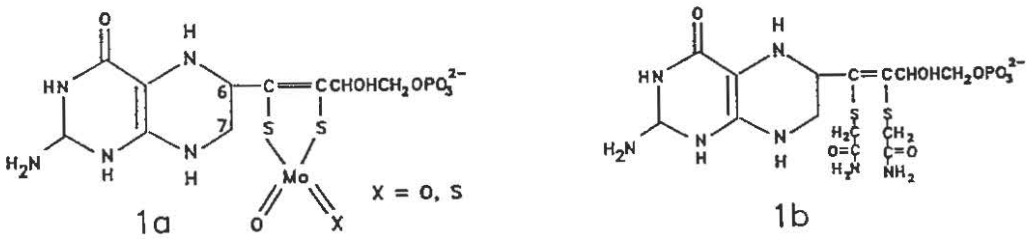

Fig. 1 (a) Proposed Structure of Mo-co; (b) Alkylated Molybdopterin

EXAFS and EPR spectroscopy of sulfite oxidase indicate that the molybdenum center is surrounded by two oxo groups and two to three sulfurs in the Mo(VI) state and by one oxo group and two to three sulfurs in the Mo(IV) state [5,6]. George *et al.* [7] recently reexamined the molybdenum center of sulfite oxidase as a function of pH and Cl^-. The strong oxo-edge absorption in the EXAFS spectra indicate that all species studied (Mo(IV),

Mo(V), Mo(VI)) contain the Mo=O group. EXAFS curve-fitting routines indicate that the number of oxo groups does not vary with pH and chloride concentration; however, the coordination number did vary with pH for the Mo(V) and Mo(IV) species, suggesting a gain of a chloride ligand at low pH and high chloride concentration.

Since it is readily available, xanthine oxidase is the most widely studied molybdenum enzyme. EXAFS and other studies establish that the Mo(VI) state is ligated by one oxo and one terminal sulfide ligand, as well as two thiol groups from molybdopterin [2,8,9,10]. The desulfo-inhibited Mo(V) form of the enzyme is prepared by removing the terminal sulfide ligand, reducing the enzyme to the Mo(V) species, and adding ethylene glycol [11]. Edmondson and D'Ardenne [12] used ENDOR spectroscopy to study the desulfo-inhibited Mo(V) form of xanthine oxidase. The ENDOR spectra were recorded at three g-values corresponding to absorption maxima in the EPR spectrum. The low temperature ENDOR spectra showed hyperfine splitting (Fig. 2), which was assigned to α protons of the cofactor. These findings suggest that molybdopterin contains a saturated side chain (cf. Fig. 1a).

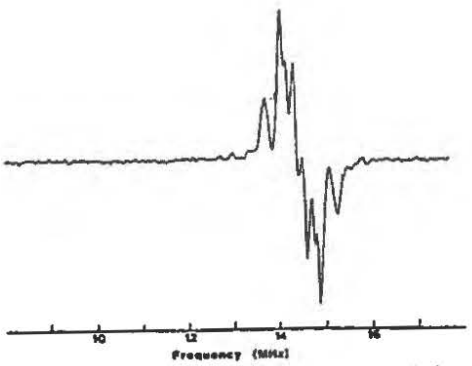

Fig. 2 ENDOR Spectrum of Desulfo-Inhibited Mo(V) Xanthine Oxidase

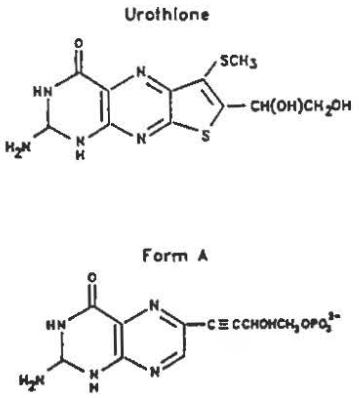

Fig. 3 Structure of Form A and Urothione

Much of the evidence for the structure of molybdopterin (the organic component) is based upon the oxidative degradation products, form A and urothione (Fig. 3). Taylor *et al.* [13] has been working toward the synthesis of molybdopterin. Recently, Taylor has completed a total synthesis of urothione [14]; its spectral properties are identical to urothione isolated from human urine. Taylor has also synthesized form A and compared its CD spectrum with that of the natural degradation product [15]. The CD spectrum of the (S) isomer matches that of the natural product.

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