Recent Advances in Pterin-Based Enzymes

Robert J. Pafford, IV Literature Seminar September 26, 1996

The essential role of molybdenum in a variety of biological processes has been known for over 60 years.[1] However, the notion that tungsten has a functional role in biological systems was only substantiated recently. It was not until the 1980's that the first naturally occurring tungsten-containing protein was purified and characterized.[2]

Except for nitrogenase, all tungsten and molybdenum enzymes contain a mononuclear metal center. These enzymes are known as oxotransferases because they transfer an oxygen atom to or from a substrate. Furthermore, these contain pterin-based ligands, the structure of which was first elucidated by Rajagopalan. The cofactor is comprised of the pterin ligand and a tungsten or molybdenum center (Figure 1).[3]

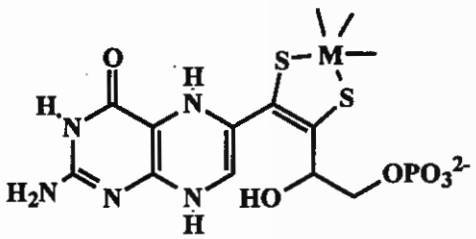


Figure 1

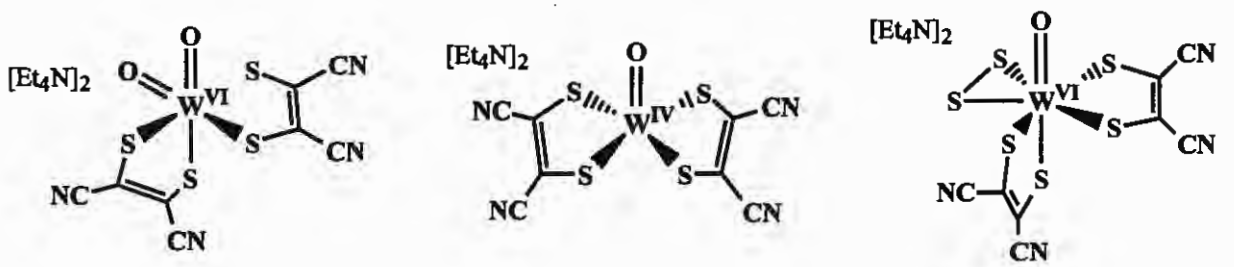
Two examples of oxotransferases are DMSO reductase and aldehyde ferrodoxin oxidoreductase (AOR). DMSO reductase contains molybdenum and converts dimethyl sulfoxide to dimethyl sulfide. AOR contains tungsten and converts aliphatic aldehydes to their corresponding carboxylic acids. AOR was isolated from the hyperthermophilic archaeon *Pyrococcus furiosus*. When exposed to oxygen, the aldehyde oxidoreductase is converted to a red-colored tungsten protein (or RTP). This tungsten-containing iron-sulfur protein was determined to be an inactive form.[4]

Various characterization techniques have been utilized to probe the active site of these enzymes. EPR, XANES, and EXAFS experiments have been performed on these enzymes and active site structures have been proposed for these data.[5,6,7] Moreover, single crystal X-ray diffraction has elucidated the structures of both DMSO reductase from *Rhodobacter sphaeroides*[8] and aldehyde ferrodoxin oxidoreductase from *P. furiosus*.[9]

The crystal structure of DMSO reductase reveals a mono-oxo molybdenum cofactor containing two molybdopterin guanine dinucleotides that asymmetrically coordinate the molybdenum through their dithiolene groups.[8] One of the pterins exhibits different coordination modes to the molybdenum between the oxidized and reduced states. This change in pterin coordination between the Mo(VI) and Mo(IV) forms suggests a mechanism for substrate binding and reduction by this enzyme.

The crystal structure of AOR shows the enzyme consists of two identical subunits, each contains an Fe_4S_4 cluster and a molybdopterin-based tungsten cofactor.[9] Each AOR subunit unexpectedly contained two pterin ligands that coordinate a tungsten by a total of four sulfur ligands.

Recently, the complexes $[Et_4N]_2[W^{VI}O_2(mnt)_2]$, $[Et_4N]_2[W^{IV}O(mnt)_2]$, and $[Et_4N]_2[W^{VI}O(S_2)(mnt)_2]$ (mnt^{2-} = 1,2-dicyanoethylenedithiolate) have been synthesized.[10] These are possible structural models for the tungsten cofactor of inactive red tungsten protein (RTP) and the active aldehyde oxidoreductase (AOR) (Figure 2).



inactive RTP model reduced AOR model oxidized AOR model

Figure 2

Many catalytic models for the active site of oxo-transfer enzymes have been synthesized.[11] To avoid possible dimerization of a $Mo^{IV}O$ complex with a $Mo^{VI}O_2$ complex, sterically hindered $Mo^{VI}O_2$ complexes have been prepared. An example is MoO_2(*t*BuL-NS)$_2$ (*t*BuL-NS = *bis*(4-*tert*-butylphenyl)-2-pyridylmethanethiolate(1-)).[11e] Dimerization was not seen for this complex in THF. With use of ^{18}O-labeled complexes and substrates, it was demonstrated that reactions in this system proceeded by oxygen atom transfer (Figure 3).

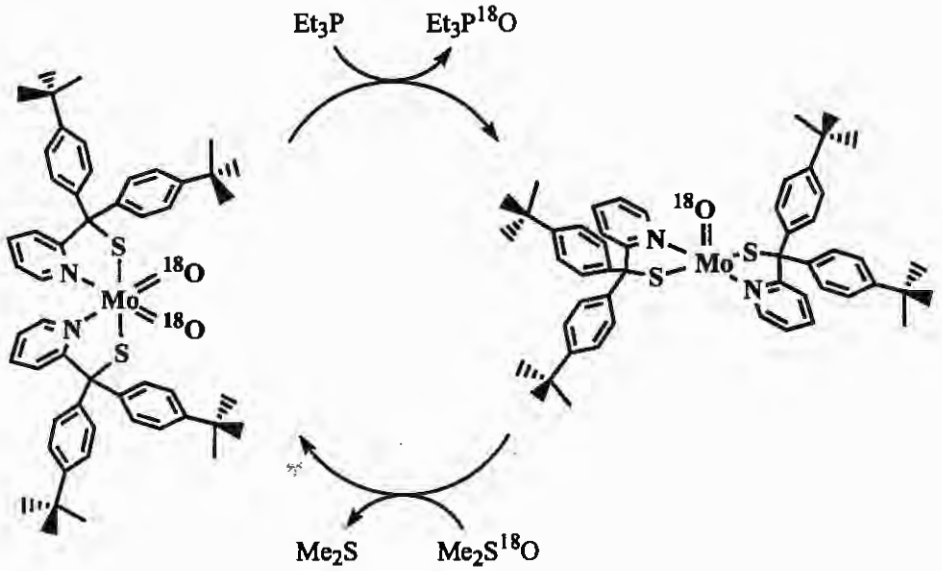


Figure 3

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