Recent Efforts to Elucidate the Mechanism of Xanthine Oxidoreductase

Matthew Olsen Literature Seminar October 9th, 2007

Xanthine oxidoreductase (XO) is the final enzyme involved in the catabolism of all purines in humans. In birds, terrestrial reptiles, and insects, the scope of this catabolism extends to all nitrogenous compounds. Specifically, XO catalyses the $2e^-$ oxidation of hypoxanthine to xanthine and xanthine to uric acid.[1] Water is used as the source of oxygen atoms, and dioxygen serves as the oxidant (although NAD^+ can also function as the oxidant, depending on the form of the enzyme).[2]

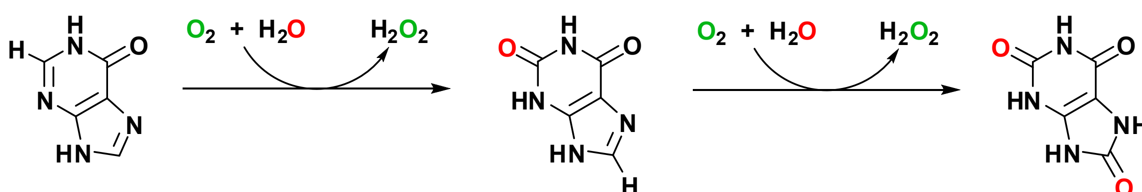

Figure 1: Oxidation of hypoxanthine to xanthine and then to uric acid.

Diseases that feature chronic elevations in blood uric acid levels (hyperuricemia) are often treated by inhibitors of XO, a prototypical compound being allopurinol.[1] For this reason, a detailed understanding of the mechanism by which XO operates is desirable.

The active site of XO features molybdenum ligated by a dithiolene, oxo ligands, and a sulfido ligand. The oxo and sulfido ligands cycle through varying protonation states during the catalysis. The mononuclear center shuttles between an oxidized state (Mo^{VI}), a reduced state (M^{IV}) and a short-lived paramagnetic state (Mo^{V}).[3] Recent research breakthroughs have provided critical insights into the interconversion of these states. The structure of XO is pH dependent,[4,5] and model compounds support these findings.[6] Solid state structures reveal a glutamate residue in proximity to the active site.[7] It is proposed that this residue deprotonates a hydroxyl ligand in the Mo^{VI} state, thus generating a potent Mo=O nucleophile.[5]

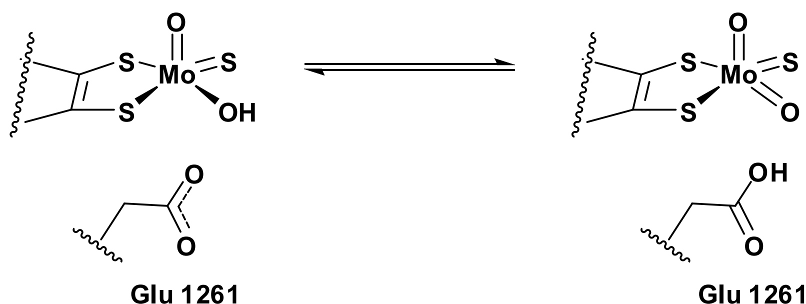

Figure 2: Deprotonation of the active site by Glu_{1261}.

XO is somewhat indiscriminant and can oxidize a range of small molecules such as purines, pyrimidines, pterins, and aldehydes.[2] Use of the 'slow' substrate FYX-051 allows for hydroxylation of the substrate to occur but prevents its release. X-ray diffraction of crystals of this inhibited XO display the Mo^{IV} intermediate containing Mo-O-substrate bonds.[2]

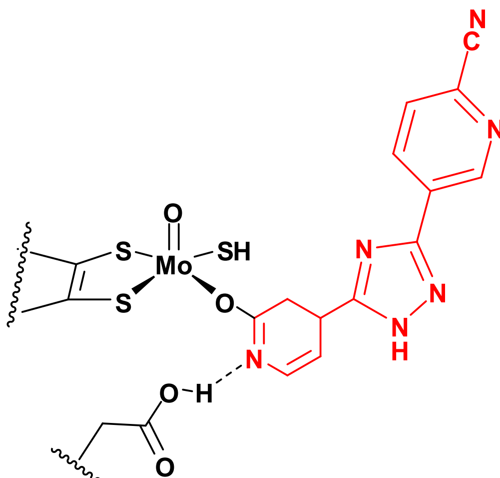

Figure 3: Mo^{IV} intermediate trapped the FYX-051 substrate (shown in red).

Following reduction to the Mo^{IV} intermediate, the mechanism of XO bifurcates.[8] One path involves displacement of the substrate by water, and $2e^-$ re-oxidation of the active site. However, depending on the substrate, varying amounts of $1e^-$ oxidized, EPR active intermediates are generated. These species have been characterized by EPR spectroscopy and have been probed with isotopically labeled water.[9] In particular, flash-freeze EPR experiments have revealed an EPR active species containing three coupling components that is proposed to be (pterin)Mo(O)(SH)(^{17}OH).[9] These studies confirm that the oxygen atom of water is incorporated into the hydroxyl ligand and imply the existence of a new intermediate in the catalytic cycle.[9] Model compounds have been used for comparison to justify a labeled hydroxyl ligand rather than a labeled oxo.[10]

References:

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