Enzyme reaction intermediates of molybdenum hydroxylases.

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Xanthine oxidoreductases have been isolated from a wide range of organisms, from bacteria to man, and all have similar molecular weights and compositions of their redox centers. They catalyze the hydroxylation of a variety of purine, pyrimidine, pterin, and aldehyde substrates. The enzyme isolated from milk is an archetypal enzyme which serves as benchmark for the whole class of complex metalloflavoproteins. The active form of the enzyme is a 290 kDa homodimer, of catalytically independent monomers. Each monomer contains one molybdopterin (MoPt) cofactor, two 2Fe/2S centers, and one FAD cofactor. Oxidation takes place at the MoPt center and the electrons are rapidly distributed through the 2Fe/2S centers to the FAD were reduction occurs. The mammalian enzyme is believed to have a primary in vivo activity of catalyzing the hydroxylation of hypoxanthine and xanthine, the final steps of urate formation. The enzyme is produced and predominantly exists in a dehydrogenase form (XDH). However, mammalian XDH can be converted to an oxidase form (XO), either by oxidation of sulfhydryl residues or by limited proteolysis of solvent exposed loops. Proteolysis of mammalian XDH cuts the enzyme into three tightly associated fragments of 20kDa (containing both 2Fe/2S centers), 40 kDa (containing the FAD center) and 85 kDa (containing the MoPt center). While XDH shows a preference for NAD reduction at the FAD reaction center, XO fails to react with NAD and exclusively uses dioxygen as its substrate, leading to the formation of superoxide anion and hydrogen peroxide. Despite allowing easy access to its FAD cofactor, XDH displays relatively low dioxygen reactivity. Stabilization of the flavin neutral semiquinone in the enzyme matrix has previously been invoked to explain this fact. The XO form is a target of drugs against gout and hyperuricemia and the conversion of XDH to XO has been implicated in diseases characterized by oxygen radical-induced tissue damage, such as postischemic reperfusion injury. We have previously published the first x-ray structures of both the XO (2.5 Å) and XDH (2.1 Å) forms of the bovine milk enzyme and identified the major changes that occur during the proteolytically induced transformation (Enroth et al., PNAS 97:10723-10728, 2000).

We have collected data of a complex of violapterin and reduced enzyme, which is thought to be a reaction intermediate. This structure is expected to help greatly in revealing the precise reaction mechanism of the enzyme. We have also collected data of the enzyme complexed to several inhibitor molecules, some of which are under development as anti-gout drugs. While several substrate-like inhibitors bind close to the position identified for allopurinol, larger drug leads occupy the entire long and narrow entrance cleft leading from the surface to the MoPt active site. This new position might shed light on substrate guiding mechanisms employed by XOR.

High resolution analysis of the new inhibitor PGD-045 bound human hematopoietic prostaglandin D synthase

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Prostaglandin (PG) D_2 is an allergic and inflammatory mediator produced by human mast cells and Th2 cells in a variety of human tissues. PGD_2 is formed from arachidonic acid by successive enzyme reactions: oxygenation of arachidonic acid to PGH_2 via PGG_2 by PG endoperoxide synthase, cyclooxygenase (COX), followed with isomerization of PGH_2 to PGD_2 by PGD synthase (PGDS). Hematopoietic PGDS (H-PGDS) is responsible for the biosynthesis of PGD_2 by mast cells and Th2 cells. Overproduction of PGD_2 exacerbates asthmatic reactions, such as enhancement of eosinophilia and accumulation of Th2 cytokines, as shown by an ovalbumin-challenged athma model in human L-PGDS- or H-PGDS-transgenic mice. On the other hand, these asthmatic reactions are weakened in gene-disrupted mice for the D type of prostanoid (DP) receptor specific for PGD_2. The DP receptor is constitutively expressed in human basophils and eosinophils, and is induced in pulmonary and airway epithelial cells by the allergic inflammation. Recently, PGD_2 was also identified as a ligand for an orphan receptor CRTH2, which is preferentially expressed in Th2 cells, eosinophils, and basophils and mediates chemotaxis of these cells for PGD_2. Therefore, PGD_2 produced by H-PGDS in human mast cells and Th2 cells is considered to accelerate allergic and inflammatory reactions by stimulating both DP and CRTH2 receptors in autocrine and paracrine manners. Thus, the human H-PGDS is a promising target for the anti-allergic drug design.

We obtained the complex crstal with inhibitor -045 with co-crystallization method by using hanging-drop vapour diffusion method. We collected the X-ray diffraction data under cryo-temperature using 1.0 A wavelength X-ray radiation at station BL40B2 of the SPring-8 synchrotron radiation source. The crystals diffracted beyond 2.3 A resolution at the synchrotron radiation source (Table 1). The structural refinement is now under way.

Table 1. Data collection statistics for complex crystal with inhibitor 045.

Beam source	BL40B2, SPring-8
Wavelength	1.00 A
Space group	$P2_1$
Cell dimensions	a = 48.25, b = 47.96, c = 184.81 A, β = 97.29 deg.
Resolution range	50 - 2.3 A
Measured reflections	77,224
Unique reflections	12,418
I/s	3.7
Completeness (outer shell)	78.7 (78.7)%
R_{merge} (outer shell)	13.9 (23.1)%