High-Resolution Structure of Bovine Milk Xanthine Oxidoreductase and Inhibitor Complexes

Xanthine oxidoreductase catalyzes the hydroxylation of hypoxanthine and xanthine, the last two steps in the formation of urate in purine catabolism in man. The enzyme is a target of drugs not only against gout [1], but also against oxygen radical-induced tissue damage [2]. Allopurinol has been used as a widely used drug effective against gout and hyperuricemia since it was introduced more than 30 years ago. Bovine milk xanthine oxidase is an archetypal enzyme, which was originally described as aldehyde oxidase in 1902 [3] and has been the benchmark of the metallo-flavoproteins [4,5]. The enzyme from mammalian sources, including man, is synthesized as the dehydrogenase form but it can be readily converted to the oxidase form by oxidation of sulfhydryl residues or by proteolysis. The dehydrogenase shows a preference for NAD^+ reduction, while the oxidase fails to react with NAD^+ and exclusively uses oxygen molecule as its substrate leading to the formation of superoxide anion and hydrogen peroxide (Fig. 1). The enzyme has been implicated in diseases characterized by oxygen radical-induced tissue damage, such as postischemic reperfusion injury [2]. The crystal structures of xanthine oxidoreductase in the two forms, dehydrogenase and oxidase, have been solved after successful crystallization of both forms of the enzyme, to clarify the structure-based mechanism of conversion [6,7]. The experiment was car out at beamline **BL40B2**.

The active form of the enzyme is a homodimer of molecular mass 290 kDa, with each of the monomers acting independently in catalysis. Each subunit molecule is composed of an N-terminal 20 kDa domain containing two iron sulfur centers, a central 40 kDa FAD domain, and a C-terminal 85 kDa molybdopterin-binding domain with the four redox centers aligned in an almost linear fashion (Fig. 2). The hydroxylation of xanthine takes place at the molybdopterin center (Mo-pt) and the electrons thus introduced are rapidly transferred to the other linearly aligned redox centers as illustrated in Fig. 1. The reaction catalysed by the Mo hydroxylases is distinct from those of other biological hydroxylation systems like P450 in that an oxygen atom is incorporated into the product from water rather than from an O_2 molecule [5]. In

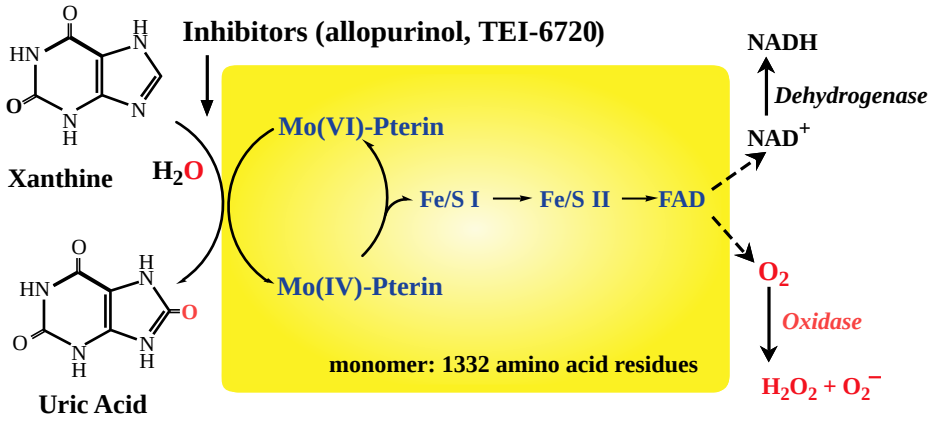

Fig. 1. Reaction scheme of xanthine dehydrogenase and xanthine oxidase. The enzyme is a dimer (MW 290,000) having two identical subunits of 1332 amino acids.

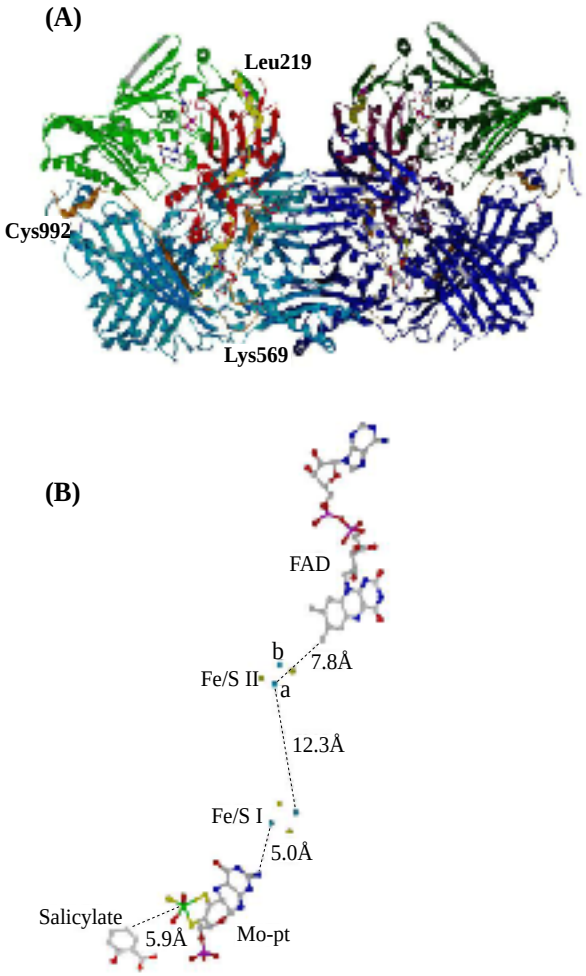

Fig. 2. ***(A)*** *Crystal structure of the xanthine dehydrogenase dimer divided into the three major domains. The enzyme contains salicylate, a competitive inhibitor. From N- to C-terminus, the domains are: iron-sulfur-center domain (residues 1- 165; red), FAD domain (residues 226 - 531; green), and Mo-pt domain (residues 590 - 1332; blue). Cofactors are also included.* ***(B)*** *The arrangement of the cofactors and salicylate in one subunit of the enzyme are presented. The Mo ion is in green, the iron ions are in light blue, and the sulfur atoms in yellow.*

the crystal structure of the active enzyme, we assigned a double-bonded sulfur atom, a double-bonded oxygen atom (=O), and an oxygen atom with a single bond (OH) as ligands to the Mo ion. It should be noted that the protein environments of two oxygen ligands are distinct in the high resolution crystal structure and the OH ligand is considered to be labile oxygen based on the structure of the alloxanthine enzyme complex (to be published). In addition to the crystal structure of the complex of the enzyme and alloxanthine, the oxidative product of allopurinol, we solved the crystal structure of the complex of the enzyme and TEI-6720, a strong candidate for a new anti-gout drug. In the crystal structure of the enzyme TEI-6720 complex, numerous hydrogen bonds and hydrophobic interactions were observed between the protein and this extremely potent inhibitor (Fig. 3), and some of them seemed to contribute to strong binding in a similar way to substrate recognition [8].

The FAD active site is the part of the enzyme that shows the largest difference between xanthine dehydrogenase and oxidase [1,2]. Cleavage of a surface-exposed loop of XDH causes major structural rearrangement of a loop close to the flavin ring (Fig. 4). This movement partially blocks NAD substrate access to the FAD cofactor and changes the electrostatic environment of the active site, accounting for the switch of substrate specificity observed for the conversion between the two forms.

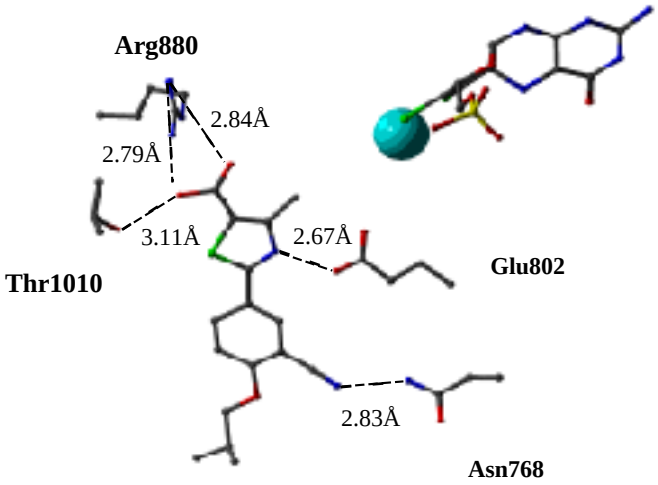

Fig. 3. Active site structure at the molybdenum center of TEI-6720 inhibitor bound form. The molybdenum atom is shown in cyan.

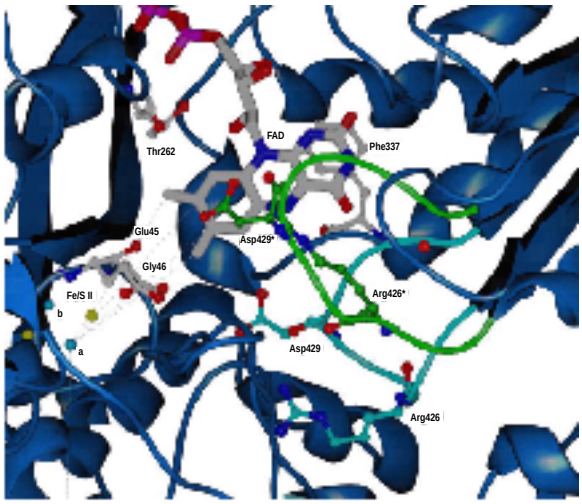

Fig. 4. Ribbon diagram of the FAD and Fe/S II active sites. The active site loop from Asp 426 to Asp 434 which changes its conformation during the XDH to XO transition is shown in light blue for XDH and in green for XO. The positions of residues Asp 429 and Arg 426 are indicated; their side chains show dramatic shifts in position and are major contributors to the change in electrostatic charge at the flavin site.

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References

[1] G.B. Elion, Science **244** (1989) 41.
[2] J.M. McCord, N. Engl. J. Med. **312** (1985) 159.
[3] F. Schardinger, Z. Untersuch. Nahrungs Genussmittel **5** (1902) 1113.
[4] T. Nishino, J. Biochem.(Tokyo) **116** (1994) 1.
[5] R. Hille and T. Nishino, FASEB J. **9** (1995) 995.
[6] B.T. Eger *et al.*, Acta Cryst. **D 56** (2000) 1656.
[7] C. Enroth *et al.*, Proc. Natl. Acad. Sci. USA (2000) 10723.
[8] K. Okamoto *et al.*, in preparation.