Crystal structure and mechanism of action of the xanthine oxidase-related aldehyde oxidoreductase from Desulfovibrio gigas

M. J. Romão* and R. Huber†

*Instituto de Tecnologia Química e Biológica, Apt. 127, 2780-Oeiras and Instituto Superior Técnico, Dep. Química, 1096 Lisboa Codex, Portugal and †Max-Planck-Institut für Biochemie, am Klopferspitz 18a, D-82152 Martinsried, Germany

The crystal structure of the aldehyde oxidoreductase (Mop) from the sulphate-reducing bacterium Desulfovibrio gigas has been analysed and refined to 1.8 Å (0.18 nm) resolution in its native 'desulpho' form, as well as in 'resulphurated', oxidized, reduced and alcohol-bound forms [1,2], allowing a detailed look at several structural aspects relevant to catalysis. In analogy with eukaryotic xanthine oxidases, xanthine dehydrogenases and aldehyde oxidases, Mop is a homodimer of two 100 kDa subunits (2 × 907 amino acids) [3] and it contains, per subunit, a molybdopterin cytosine dinucleotide (MCD) cofactor as well as two different kinds of [2Fe-2S] clusters [4], but lacks the flavin and its domain which is present in most molybdenum hydroxylases. It represents the first structure of a member of the xanthine oxidase family of enzymes, with the redox-active cofactors found in discrete domains within a single polypeptide chain. The molecule is roughly globular with an approximate diameter of 75 Å and folds into four distinct domains (Fe/S_a, Fe/S_b, Mo1 and Mo2): the first two (Fe/S_a and Fe/S_b) bind the two iron-sulphur clusters, whereas the larger domains (Mo1 and Mo2) bind the MCD cofactor in extended conformation by a network of hydrogen-bonding interactions: Mo1 contributes with two single molybdopterin-binding segments and Mo2 binds the other side of the pterin system and provides all of the dinucleotide-binding segments. These two large domains also surround the molybdenum catalytic site and define, at their interface, a 15 Å deep tunnel, wide open at the surface and constricted in the middle, which leads substrate molecules into the buried molybdenum catalytic site. The metal-binding site is surrounded by residues from both subdomains Mo1 and Mo2, which interact with the cofactor by a number of hydrogen-bonding interactions.

The molybdenum atom adopts a distorted square-pyramidal co-ordination geometry with the metal approx. 0.5 Å displaced out of the equatorial plane. The pterin cofactor binds to the molybdenum via its dithiolene, defining one side of the equatorial plane of the co-ordination sphere. Trans to the dithiolene are two oxygen ligands: one, at a shorter distance, assigned to a Mo=O bond, and one longer Mo-O bond (~2.4 Å) which is assigned to a bound water molecule on the basis of the bond length as well as hydrogen-bonding interactions. The apical position is a Mo=O bond in the 'desulpho' form of Mop, replaced by Mo=S in crystals that have been resulphurated. This apical Mo=S ligand is in the vicinity of His-653 with the imidazole ring approx. 3.2 Å away from the sulphur atom. There is no protein ligand to the molybdenum atom, although Glu-869 is quite close, at 3.5 Å, and trans to the apical position, and it may play a role in the catalytic cycle, co-ordinating to the metal by a minor change in the carboxylate position.

The redox-active cofactors of Mop are inserted in the protein matrix in close proximity.
Molecular representation of the three cofactors of Mop, MCD and both close contacting centres, Fe/S_a and Fe/S_b, which is exposed to solvent through Cys-60.

For the sake of clarity, residues contacting the dinucleotide part are omitted. Two structural propan-2-ol molecules point to the direction of the tunnel which leads into the metal site.

Hypothetical structures for the reductive half-cycle of the hydroxylation reaction of Mop and xanthine oxidase.

I, the Michaelis complex with aldehyde substrate close to $\text{Mo}^{5+}$; II, the enzyme-carboxylic acid product complex ($\text{Mo}^{5+}$); III, after product dissociation, intermediate with Glu-869 bound to the metal.
to each other, and define an electron-transfer pathway (Figure 1). Whereas the first Fe/S centre b is quite exposed to the solvent via its Cys-60, Fe/S centre a is buried approx. 15 Å below the molecular surface. The closest distance between the iron atoms of the two Fe/S centres is about 12 Å, and the molybdenum atom lies 15 Å away from the nearest iron atom of centre a. The molybdenum site is also buried but accessible to the protein surface through a deep tunnel as described.

The bound inhibitory propan-2-ol in the inner compartment of the substrate-binding tunnel is a model for the Michaelis complex of the reaction of Mop with aldehydes (RCH=O) (Figure 2). The reaction is proposed to proceed by transfer of the molybdenum-bound water molecule as OH−, after proton transfer to Glu-869, to the carbonyl group of the substrate, concertedly with hydride transfer to the sulphido group to generate: Mo" + =O, -SH, −−−RCOO−. The dissociation of the carboxylic product may be facilitated by transient binding of Glu-869 to the molybdenum atom. The metal-bound water is then replenished from a chain of structural internal water molecules. A second alcohol-binding site, located in a wider part of the tunnel (Figure 1), may explain the strong substrate inhibition observed in Mop [5] as well as in xanthine oxidase [6]. This is also the putative binding site of large inhibitors of xanthine oxidase [7].

This work was supported by PRAXIS 2/2.1/BIO/05/95.


Received 2 April 1997

Biosynthesis and processing of the molybdenum cofactors
K. V. Rajagopalan
Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, U.S.A.

Structural studies in our laboratory on the cofactor associated with liver sulphite oxidase revealed the presence of a unique pterin component that has been termed molybdopterin (MPT; see Figure 1 for the proposed structure) [1,2] and subsequently led to the identification of several dinucleotide forms [3–6], including molybdopterin guanine dinucleotide (MGD, Figure 1), the component of the cofactor present in all Escherichia coli molybdoenzymes. The unusual features of the 6-alkyl side chain of the pterin, with four carbons, a terminal phosphate ester and the unique dithiolene group critical for metal ligation, suggested that the assembly of such a cofactor might involve heretofore undescribed enzymes and chemical pathways. Indeed studies on MPT biosynthesis have shown that the pathway involves several novel reactions as described below. Much of the progress in this area has been achieved through use of MPT mutants of E. coli. These mutants, long referred to as chl mutants (chlorate-resistant), are now renamed mo mutants, more accurately reflecting the role of the individual genes in molybdenum cofactor biosynthesis [7]. A summary of the current information on the biosynthetic pathway in E. coli is presented in Figure 1.

Early steps in MPT biosynthesis
The biosynthetic pathways of folate and riboflavin in plants and micro-organisms and of bioperten in animals all utilize GTP as the initial precursor of the pterin ring [8,9]. In order to