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COMMENTARY

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The molybdenum site in the xanthine oxidase-related aldehyde oxidoreductase from *Desulfovibrio gigas* and a catalytic mechanism for this class of enzymes

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Abstract The crystal structure analysis of the aldehyde oxidoreductase from *Desulfovibrio gigas* was exceptionally revealing with regard to the ligands and structure of the molybdenum site and the mechanism of the hydroxylation reaction catalyzed. The metal is pentacoordinated by two sulfurs of the *cis*–dithiolene group of the molybdopterin cofactor and by facially arranged sulfido, oxo and water ligands. The latter is in hydrogen-bonding contact with the carboxylate group of Glu 869 and the hydroxyl group of an isopropanol molecule, a substrate analogue inhibitor. This steric arrangement strongly suggests a mechanism for the reductive half-cycle of the reaction with Glu 869 as the base, the metal-bound water as the source of the transferred hydroxyl group, and the sulfido group as the hydride acceptor. The geometry and the proposed mechanism are in agreement with density functional calculations on a model of the molybdenum site. In the oxidative half-reaction, electrons are withdrawn from Mo^{IV} through the rigidly held pterin ring system, via the iron-sulfur clusters, to the protein surface.

Key words Molybdoenzymes · Molybdopterin · Protein crystallography · Xanthine oxidase

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In contrast to other transition metals which catalyze one-electron transfer reactions, molybdenum is able to carry out controlled two-electron or oxo-transfer processes, whereby the metal shuttles between oxidation states VI and IV in the catalytic cycle.

The chemistry of molybdenum in aqueous solution, in oxidation states lower than VI, is characterized by aggregation and cluster formation. Models for the enzymatic systems, where cluster formation cannot occur, are therefore rare but do exist for complexes of molybdenum with bulky, multidentate ligands, which prevent dinucleation. Such complexes catalyze, for example, the oxo transfer between tertiary phosphines and sulfoxides and cycle between $Mo^{VI}O_2$ and $Mo^{IV}O$ (for reviews see [1]) and have been structurally and functionally characterized in detail.

It has been clearly shown by single turnover experiments for xanthine oxidase that a tightly bound oxygen is incorporated into the urate product [2, 3], which was interpreted as a transfer of the oxo ligand of the molybdenum center. This catalytically active oxygen was shown to be ultimately derived from the solvent [4].

The aldehyde oxidoreductase from *Desulfovibrio (D.) gigas* (MOP) is an enzyme closely related to the xanthine oxidase family of molybdenum enzymes, and its high-resolution crystallographic analysis [5, 6] provides sufficient structural detail to suggest an enzymatic mechanism consistent with the structural and functional data.

In MOP, the molybdenum atom is coordinated by the dithiolene side chain of a molybdopterin cytosine dinucleotide. The tricyclic pterin-pyran system of the molybdopterin is deeply buried in the protein matrix, suggesting that protein folding and cofactor assembly are concerted events. The protein is composed of four domains from consecutive polypeptide chain segments, which do not interpenetrate and are likely to fold independently. Domain assembly and cofactor insertion may therefore be the final step in folding.

In its very intricate interaction with the protein, all hydrogen bonds of the cofactor are established, mostly

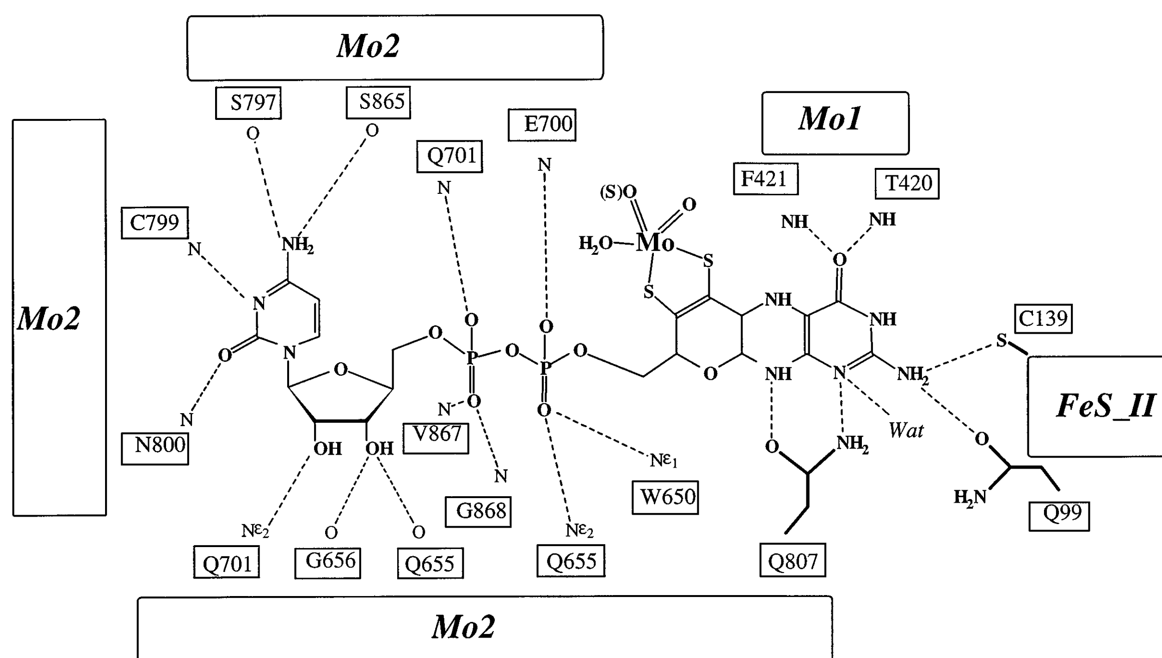

Fig. 1 Molybdopterin cytosine dinucleotide of Mop and representation of the hydrogen-bonding interactions with surrounding residues: fourteen interactions with domain Mo2, two with domain Mo1, and two with the closest iron domain FeS_II

with domain 4 (Mo2) and a few with domain 3 (Mo1), clamping these structural units together (Fig. 1). The two domains define, at their interface, a deep hydrophobic tunnel leading to the molybdenum site.

Chemical characterization of the isolated cofactor has documented a dihydropterin with an open side chain [7]. The ring closure in situ to yield a pterin-pyran system is facilitated by the nucleophilic attack of the side-chain hydroxyl group, generating formally a tetrahydropterin species as found in all crystal structures of molybdopterin-containing enzymes known to date [5, 8–11]. An oxidized planar pterin moiety would be incompatible with its protein-binding site. The tetrahydropterin-pyran system is apparently rigid and redox inert, as expected for a mediator of electron transfer in the course of catalysis (to be discussed later).

In MOP and xanthine oxidase, one molybdopterin cofactor coordinates the molybdenum (a molybdopterin cytosine dinucleotide in MOP), while there are two cofactors in all other structurally defined molybdopterin enzymes: DMSO reductases (from *Rhodobacter capsulatus* [9] and *R. sphaeroides* [10] (two molybdopterin guanine dinucleotides), formate dehydrogenase from *Escherichia coli* [11] (two molybdopterin guanine dinucleotides) and a tungsten aldehyde oxidoreductase from *Pyrococcus furiosus* [8].

A number of high-resolution (1.75–1.9 Å) data were collected for MOP under different experimental conditions, and the structures were refined [6]. The quality of these data has allowed conclusions to be drawn about important aspects of the molybdenum catalytic site under reductive and oxidative conditions, in native and resulfurated states, and in inhibited forms of the enzyme. Resulfuration experiments of MOP applying drastic conditions and very high sulfide concentrations led to full incorporation of sulfur by replacement of an oxo ligand at the apex of the tetragonal pyramid. However, this replacement did not stimulate enzymatic activity. Despite considerable uncertainty in the experimental bond lengths between molybdenum and its ligands, the variation between the large number of data sets is small enough to identify one equatorial oxo ligand by its shorter distance to the metal (1.7 Å) and the absence of any protein hydrogen bond partners, and one water ligand at a distance of 2.2 Å, which is longer than expected for an Mo-OH bond (1.9 Å in molybdenum complexes). The molybdenum site structure is thus defined as: Mo = O, $-OH_2$, = S. The second coordination sphere is provided by the carboxylate group of Glu 869 (a residue highly conserved in the xanthine oxidase family) hydrogen bonded to the water ligand and by a isopropanol molecule (an inhibitor of Mop [12]) from the crystallization medium, which is hydrogen bonded to the water ligand and to an interior ordered water molecule. The water ligand also establishes hydrogen bonds to Glu 869, to the isopropanol molecule and to the amide from Gly 697 (Fig. 2).

EXAFS data provide experimental evidence for four of the molybdenum ligands, two dithiolene sulfur atoms, a sulfido sulfur and an oxo group and their distances to the metal [13]. However, the indications of a fifth ligand at a wider distance were then inconclusive. Quantum chemical methods employing gradient-corrected density functional calculations [14] allowed us to obtain exact geometrics and energies of the isolated molybdenum coordination site, which confirm the as-

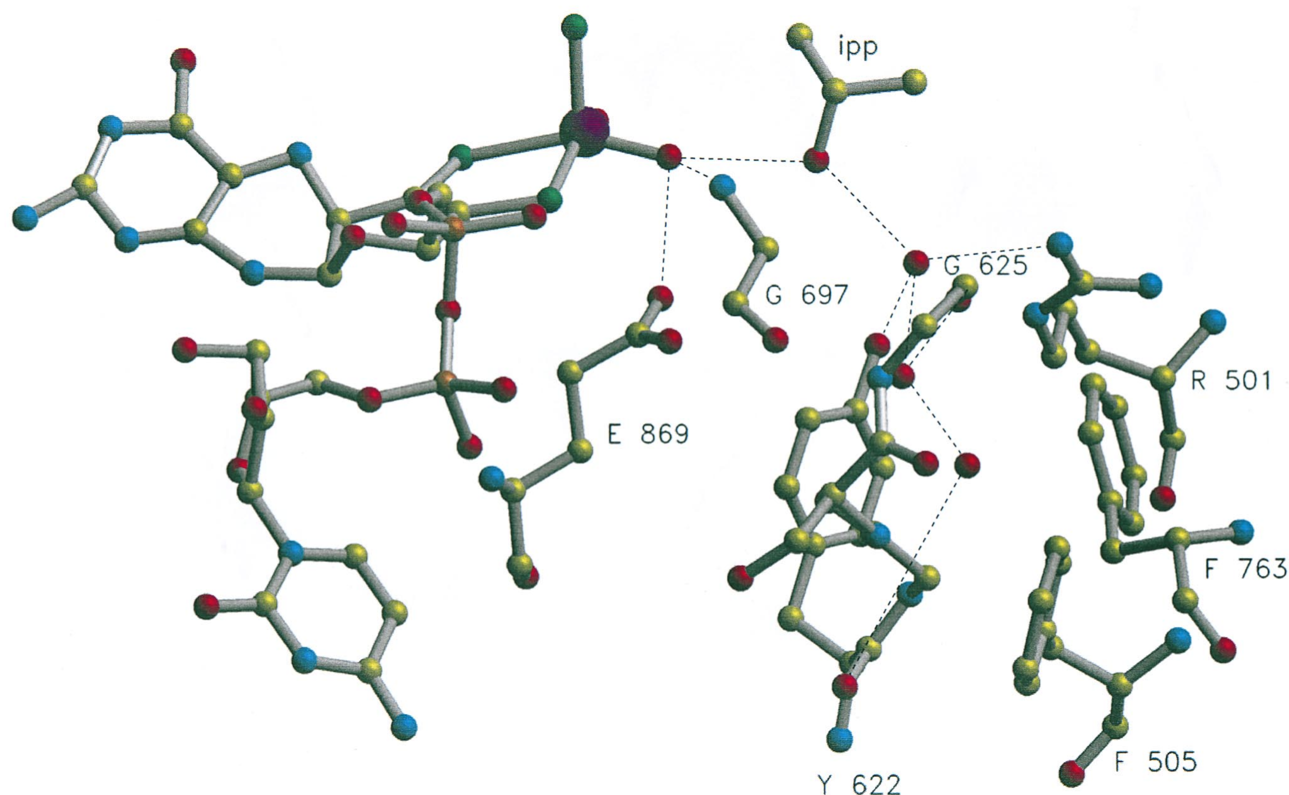

Fig. 2 Representation of the molybdenum catalytic site of Mop (resulfurated form) and surrounding residues within hydrogen-bonding distance, including one isopropanol and the three buried water molecules

signments from the crystal structure and exactly define the geometry. Alternative isoenergetic arrangements shown by these calculations are excluded by the protein matrix in the protein cofactor complex. The calculations also demonstrated the possibility of hydride transfer and binding to the metal as an alternative to the sulfido group as a hydride acceptor.

A mechanism for the reductive half-reaction of the oxygen atom transfer catalyzed by xanthine oxidase-related enzymes may be proposed on the basis of our X-ray structural data, where we assume the isopropanol inhibitor positioning relatively close to the molybdenum as a model for the Michaelis complex. As depicted in Fig. 3, the main aspects of this mechanism are: (a) The coordinated water molecule is activated by the neighboring Glu 869, and represents the labile oxygen transferred onto the carbonyl carbon atom of the substrate. This nucleophilic attack is facilitated by hydrogen-bonding interactions of the carbonyl oxygen with water 137W and with the water ligand, which polarize the carbonyl double bond. (b) The resulting carboxylic acid product, generated after hydride transfer to the sulfido group, replaces the coordinated water at the molybdenum. (c) Finally, the product is released from the reduced molybdenum center, which may be facilitated by the transient binding of the proximal Glu 869 to the metal. (d) A water molecule present in the active site cavity reoccupies the vacant coordination position.

The proposed mechanism focuses on the molybdenum site, but one has to consider the role of the molybdopterin cofactor and its possible involvement in the catalytic process. Several roles may be attributed to the pterin-pyran dithiolene system: (1) Structurally, the cofactor anchors the molybdenum center in the core of the protein matrix. (2) It mediates the electron flow in and out of the molybdenum center, which shuttles between different redox states. The relative proximity of the Fe-S centers (closest distance Fe,S_I–Fe,S_II = 12 Å) facilitates an electron transfer route from the molybdenum site to the closest center Fe/S II and from this to the exposed center Fe/S I. (3) The cofactor, via its dithiolene moiety, modulates the redox potential of the molybdenum centre. Isolated pterin systems are redox active and may accept up to eight electrons to reach the fully reduced tetrahydro state. In the protein complex, however, it is tightly clamped and unlikely to change its “redox state” during catalytic turnover. The dithiolene substructure of molybdopterin, however, seems to change conformation, as the oxidized state of the enzyme has a partial S-S bond character (ca. 3.0 Å), while in the reduced crystals this distance is considerably enlarged (to ca. 3.5 Å). This effect has also been observed in model compounds of molybdenum [15]. It indicates a response of the dithiolene moiety to different Mo oxidation states in order to achieve optimal local structure, and it is tempting

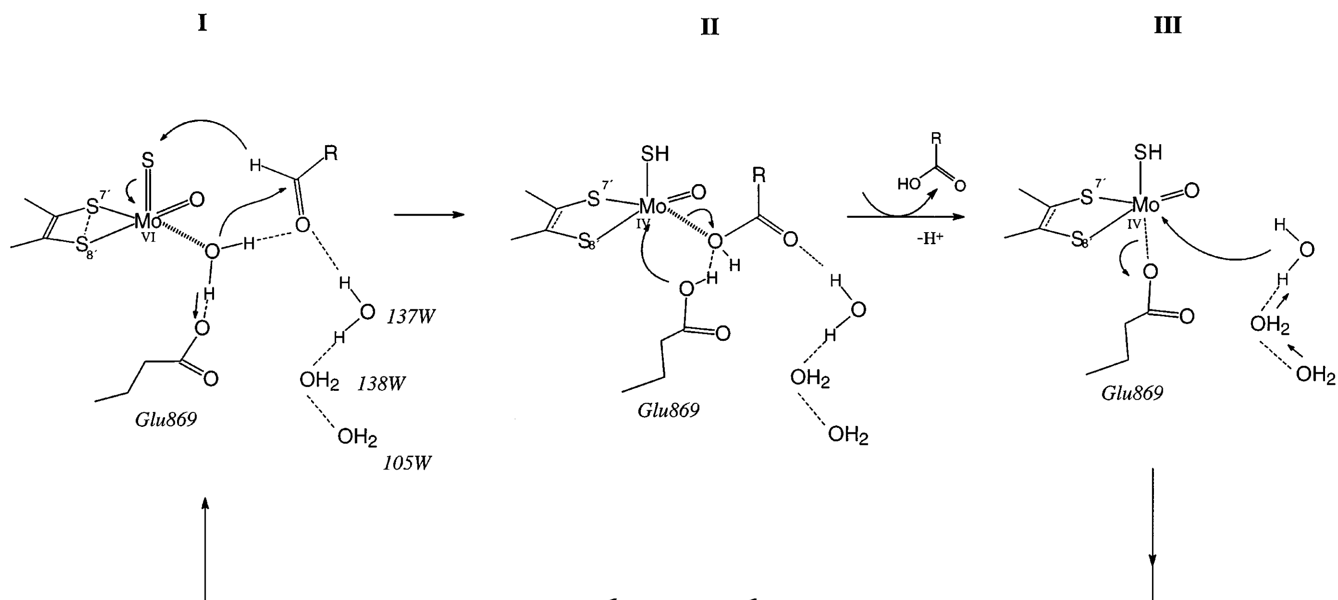

Fig. 3 Proposed mechanism for the reductive half-cycle of the hydroxylation reaction of Mop and xanthine oxidase: **I** the Michaelis complex with an aldehyde substrate close to Mo^{VI}, **II** the enzyme/carboxylic acid product complex (Mo^{IV}), and **III** intermediate, after product dissociation, with Glu 869 bound to the metal

to interpret this effect as evidence for dithiolene-facilitated electron transfer. Reduction of the enzyme also involves a change in the puckering of the molybdenum-dithiolene cycle.

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References

1. Enemark JH, Young CG (1993) Adv Inorg Chem 40:1–8
2. Hille R, Sprecher H (1987) J Biol Chem 262:10914
3. Howes BD, Bray RC, Richards RL, Turner NA, Bennett B, Lowe DJ (1996) Biochemistry 35:1432
4. Murray KN, Watson JG, Chaykin S (1966) J Biol Chem 241:4798
5. Romão MJ, Archer M, Moura I, Moura JJG, LeGall J, Engh R, Schneider M, Hof P, Huber R (1995) Science 167:1170–1176
6. Huber R, Duarte R, Moura I, Moura JJG, LeGall J, Liu M, Hille R, Romão MJ (1996) Proc Natl Acad Sci USA 93:8846–8851
7. Rajagopalan KV (1991) Adv Enzymol Relat Areas Mol Biol 64:215–290
8. Chan MK, Mukund S, Kletzin A, Adams MWW, Rees DC (1995) Science 267:1463–1469
9. Schneider F, Löwe J, Huber R, Schindelin H, Kisker C, Knäblein J (1996) J Mol Biol 263:53–69
10. Schindelin H, Kisker C, Hilton J, Rajagopalan KV, Rees DC (1996) Science 272:1615–1621
11. Boyington JC, Gladyshev VN, Khangulov SV, Stadtman TC, Sun PD (1997) Science 275:1305–1308
12. Barata BAS, LeGall J, Moura JJG (1993) Biochemistry 32:11559
13. Cramer SP, Moura JJG, Xavier AV, LeGall J (1984) J Inorg Biochem 20:275
14. Voitjuk AA, Albert K, Köstlmeier S, Nasluzov VA, Neyman KM, Hof P, Huber R, Romão MJ, Rösch N (1997) J Am Chem Soc 119:3159–3160
15. Stiefel EI, Miller KF, Bruce AE, Corbin JL, Berg JM, Hodgson KO (1980) J Am Chem Soc 102:3624