

Computer graphic study on models of the molybdenum cofactor of xanthine oxidase

Gerd Folkers, Michael Krug and Susanne Trumpp

Pharmaceutical Institute of the University of Tübingen, Auf der Morgenstelle 8, 7400 Tübingen, F.R.G.

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SUMMARY

Within the scope of our molecular modeling studies on xanthine oxidase (XOD) inhibition by purine analogs we were interested to build up a three-dimensional model of the molybdenum active site. Spectroscopic data indicated that a Mo (VI) atom which is coordinated to sulfur, oxygen and/or nitrogen is clearly involved in substrate binding. In the present study, those data and X-ray crystallography data were used to reconstruct molybdenum-organic complexes from models proposed in the literature. The computer graphic-assisted modeling and evaluation of the model complexes show that the description of the molybdenum center needs further refinement.

INTRODUCTION

The enzyme xanthine oxidase (XOD) catalyzes the oxidation of hypoxanthine and xanthine to uric acid by means of four redox-active centers: molybdenum, FAD and two sulfur-iron complexes. It is not yet known how these redox centers interact in the electron transfer processes. However, ESR investigations on XOD clearly demonstrate that the molybdenum located in the active center is directly involved in substrate binding by charge transfer interactions [1]. Molybdenum (VI) is thought to initiate the hydroxylation step by activating a ring carbon atom in α -position to a Mo(VI)-coordinated nitrogen [2]. The α -carbon with the positive partial charge is attacked by a nucleophilic thiolate group. This enables the proton to transfer to another Mo ligand. Simultaneous reduction of the molybdenum might take place by electron transfer via the nitrogen coordinate bond. Hydrolytic cleavage of the α -carbon-sulfur bond finally leads to the hydroxylated heterocycle. Recent EXAFS studies show that the proton at the carbon is transferred to an oxygen or sulfur ligand of the Mo (VI) center [3]. The molybdenum cofactor of XOD has been isolated in 1980 [4] and the complete structure has been proposed as shown in Fig. 1 [5].

The authors proposed that the molybdenum is attached to the pyrimidone-oxygen, the nitrogen

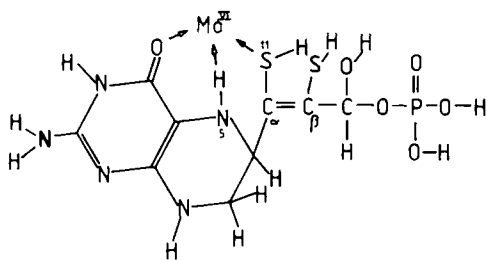


Fig. 1. The molybdenum cofactor according to Refs. 4, 5, 6.

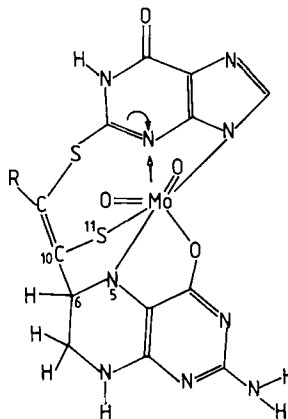


Fig. 2. The molybdenum (VI) dioxo-hypoxanthine complex as proposed by Robins and coworkers[7].

at N-5 and the sulfur at the carbon of the β -mercapto thioenolate side chain [6]. Based on these results, Robins and coworkers [7] constructed a model of the molybdenum (VI) substrate complex (Fig. 2).

Within the scope of our molecular modeling studies on XOD inhibitors [8] we were interested to build up those molybdenum substrate complexes three-dimensionally by means of computer graphic methods. It was our aim to investigate the steric and electronic requirements of substrate and/or inhibitor interaction at the XOD active site.

METHODS

We used the molecular modeling software SYBYL [9] for the construction of the interaction complexes. The complexes were built up from fragment structures from the Cambridge Structural Database (CSD). The CSD was also used to retrieve bond distances and valence angles by statistical analysis of suitable molybdenum-dioxo complexes.

The partial structures were optimized by quantum chemical methods (MOPAC [10]). The calculations were run on a DEC micro VAX II. Modeling was done on an Evans & Sutherland PS 350 computer graphics system connected to the micro VAX II via Ethernet.

RESULTS AND DISCUSSION

It can be seen from the EXAFS data of Cramer and Rajagopalan [3] that in the Mo (VI)-O₂ complex the molybdenum is double bonded to either two oxygens or one oxygen and one sulfur (Fig. 3). The short Mo-O distance of about 1.7 Å proves the existence of a special molybdenum-dioxo fragment. Molybdenum-dioxo complexes almost exclusively form octahedrons with a characteristic *cis*-dioxo structure. This typical behaviour allows a quite realistic prediction of the ligand orientation [11]. Within an octahedral ligand field, the d-orbitals split into two sets t and e. While e takes part in the formation of six d²sp³ hybrid orbitals the d_{xy}, d_{xz} and d_{yz} orbitals (set t) form 1.5 fold pⁿdⁿ bonds to the *cis*-orientated terminal oxygens. This *cis* configuration can be explained in terms of maximum utilization of the three dⁿ orbitals (Fig. 4).

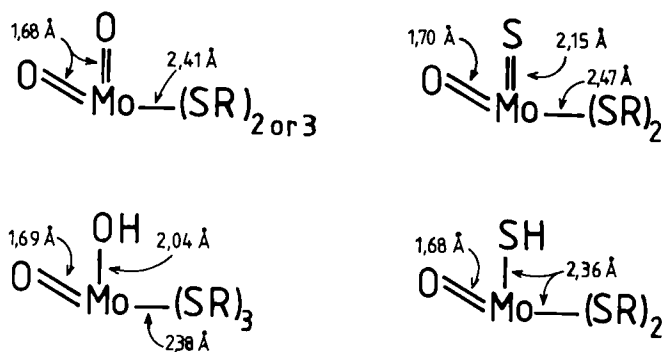


Fig. 3. Structural representation of the EXAFS data (Ref. 3).

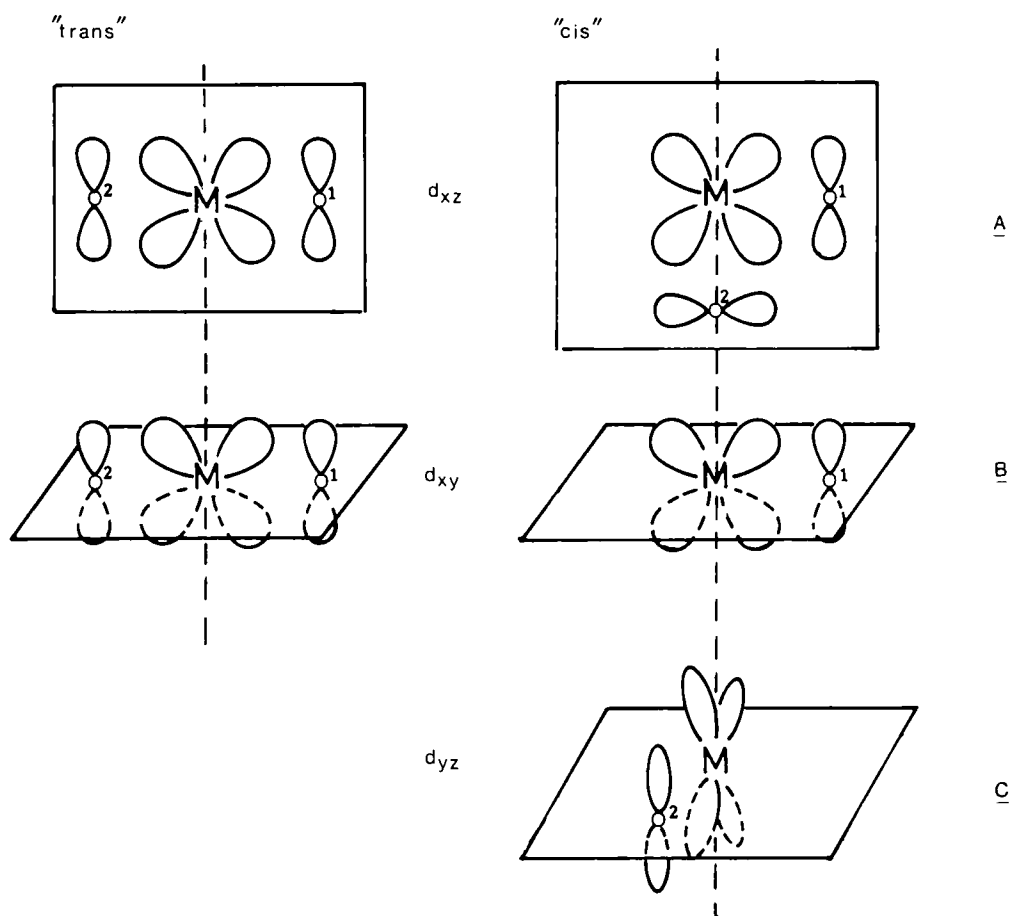


Fig. 4. Schematic description of two sp-type oxygens (O-1 and O-2) overlapping the molybdenum (M) d^{π} orbitals. In the 'trans' configuration overlap of the oxygen p-orbitals must take place at the same molybdenum d-orbitals (d_{xy} (A) and d_{xy} (B)), whereas in the 'cis' configuration the molybdenum d_{yz} orbital (C) can be overlapped additionally by the second (*cis*) oxygen. This leads to a 1.5 fold π -bond for the *cis* configuration by sharing the d_{xz} orbital (A) and having one exclusive of each other (B and C).

The schematism in Fig. 4 shows that a second *trans*-orientated oxygen would be forced to share the same $d\pi$ orbitals as the first one. This would allow for a single $p\pi d\pi$ bond at most, without participation of the third $d\pi$ orbital in the YZ-plane.

However, the short Mo-O distances require overlap integrals which are larger than those of a common double bond. In a *cis* configuration, all three $d\pi$ orbitals can be used by the two oxygens, sharing one and each having exclusive use of another [12] and providing for maximum overlap with all $d\pi$ orbitals. The electrostatic interaction of the two *cis*-terminal oxygens increases their valence angle with molybdenum to roughly 105° distorting the octahedron. Further distortion is caused by the concentration of bonding at the *cis*-terminal oxygen site which leads to a significant bond lengthening for the atoms situated *trans* to the terminal oxygens. It has been established as a general principle that, in mixed-ligand complexes, the weaker bonding donors are therefore *trans* to the terminal oxygen [11]. A statistical analysis of MoO₂-complexes with nitrogen ligands showed an average bond length of 2.4 Å for a neutral nitrogen ligand *trans* to a terminal oxygen.

Application of these facts leads to a geometrical hypothesis on the basic structure of the Mo-cofactor-substrate interaction complex (Fig. 5).

It should be evident from the above-mentioned properties of MoO₂-complexes that the nitrogen N-5 as well as the nitrogen of the attacking substrate (N-subst) have to occupy the *trans* positions to the terminal oxygens. Taking this into account, the geometry of the first ligand shell is fixed. Conformation analysis of the cofactor and docking of the substrate will show if the geometric requirements can be accomplished.

It can be shown by NMR experiments that the tetrahydropyrazine moiety preferably exists in a half-chair conformation [13]. This leads to four different possibilities for the orientation of the side chain at the chiral center C-6. However, an optimal bond distance between sulfur S-11 and the molybdenum center drastically reduces the degrees of freedom for the side chain and for the chiral center. The evaluation of crystallographic data (CSD) shows that a distance of 2.35–2.55 Å is most often found for the sulfur-molybdenum complex bond. When we used this distance inter-

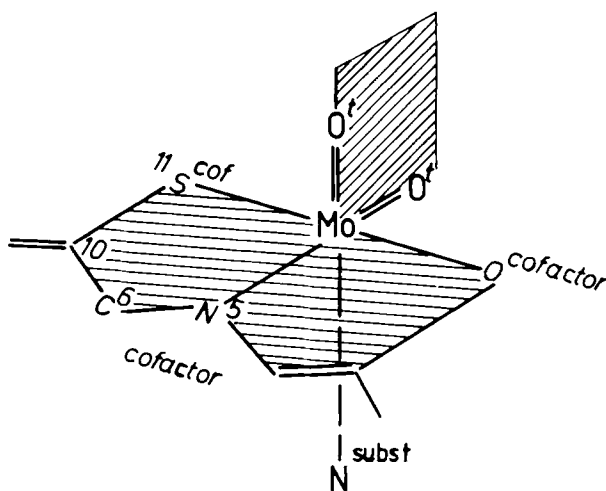


Fig. 5. The environment of the Mo(VI)center as predicted from the known regularities of molybdenum-dioxo complexes.

val as a constraint within the SEARCH subroutine of the SYBYL program, only two valid conformations were found. Both conformations exhibited an *S*-equatorial chiral center and a torsion angle (N-5, C-6, C-10, S-11) of 300° for the first and 360° for the second conformation. Fig. 6 shows the 'molybdenum active site' put together based on the above facts.

It can be seen that this is the only possibility to get a valid orientation of the ligands; for example, an anionic interaction of N-5 with Mo would cause a *trans*-terminal oxygen position of the pyrimidon oxygen. However, because the N-heterocyclic substrate must occupy the second *trans*-terminal oxygen position, there remains no way to bring the chelating sulfur atom into an interaction distance to the molybdenum center.

Docking of the substrate hypoxanthine is the last step in the construction of the MoO_2 octahedral complex. For an attack at C-2, as proposed, the β -sulfur has to be brought into an 1.8 \AA interaction distance simultaneously to the formation of an activating molybdenum nitrogen bond. Because of geometry constraints only an activation via N-9 can be done in this model (Fig. 7). This requires the hypoxanthine to be in the N-7-H tautomeric state. We have simulated the electrophilic attack at N-9 of the hypoxanthine by protonation. Quantum chemical calculation of the charge distribution shows that a positive partial charge occurs at C-2 and at C-8 (Fig. 8).

Therefore, in our opinion, the introduction of a seventh ligand (arrow in Fig. 2) is, besides the problematic geometry, not needed for the activation of the substrate. The molybdenum nitrogen distance of 2.4 \AA has been derived from crystallographic data of MoO_2 complexes with nitrogen heterocyclic ligands.

However, the direction of the nucleophilic attack at C-2 seems to be rather unfavorable. The perpendicular geometry of the attacking sulfur atom and the sp^2 ring carbon would require large

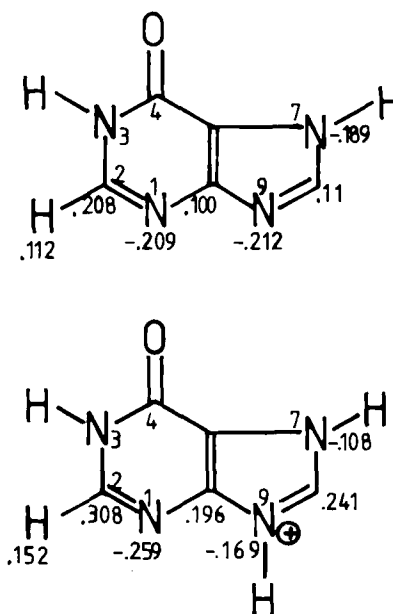


Fig. 8. Atom charges calculated by the MNDO method for the hypoxanthine-7-H tautomer (top) and its protonated form (bottom).

TABLE I
VALENCE ANGLES OF THE MODEL COMPLEX COMPARED WITH MoO₂ COMPLEXES RETRIEVED FROM CSD

Valence angle	Model	Comparative complexes		
		Min	Max	\bar{X}
O ^{cof} -Mo-S ^{cof}	122.6	156.0	158.0	157.0
N ^{cof} -Mo-O ^{cof}	73.4	73.6	114.0	85.3
N ^{cof} -Mo-S ^{cof}	83.4	72.9	88.8	84.0
N ^{cof} -Mo-O _i ¹	88.6	87.1	102.0	93.5
N ^{cof} -Mo-O _i ²	166.3	151.7	164.8	160.4
N ^{sub} -Mo-O _i ²	165.7	151.7	164.8	160.4
S ^{cof} -Mo-N ^{sub}	67.3	76.0	120.0	94.5
O ^{cof} -Mo-N ^{sub}	68.0	74.0	114.0	88.0
N ^{sub} -Mo-O _i ¹	65.0	87.0	102.0	93.5
N ^{cof} -Mo-N ^{sub}	103.0	76.0	103.0	83.5

conformational changes for a bond to be formed in the plane of the heterocycle. These large movements are not possible as can also be seen by the representation of the ligand van der Waals volumes (Fig. 9). Additionally, the black circular gaps in the spheres of the terminal oxygens and the substrate nitrogen indicate non-bonded interactions which further diminish the stability of the complex.

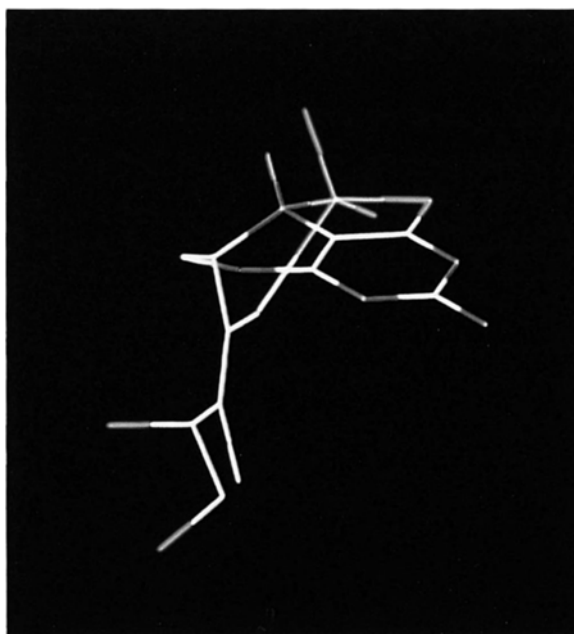


Fig. 6. The molybdenum-dioxo active site (color coding by atom type).

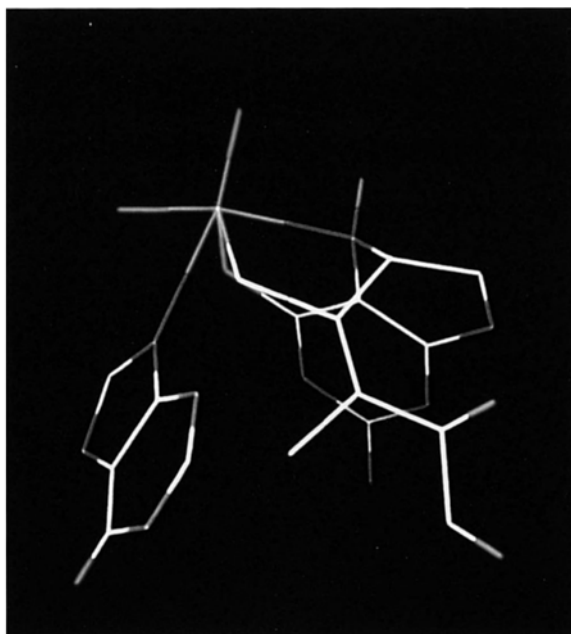


Fig. 7. The molybdenum-dioxo complex with the docked hypoxanthine. (Color is coded by atom type, hydrogens are omitted.)

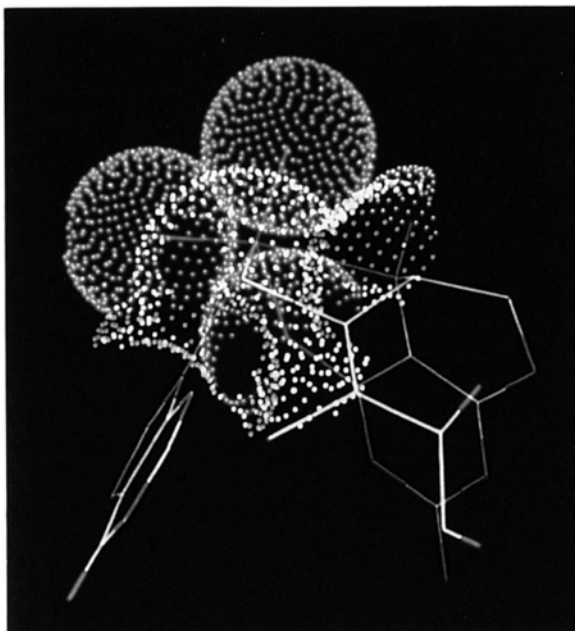


Fig. 9. Representation of the van der Waals volumes of the model complex calculated for a sphere of enclosure of 3 Å around the molybdenum center (color coding by atom type).

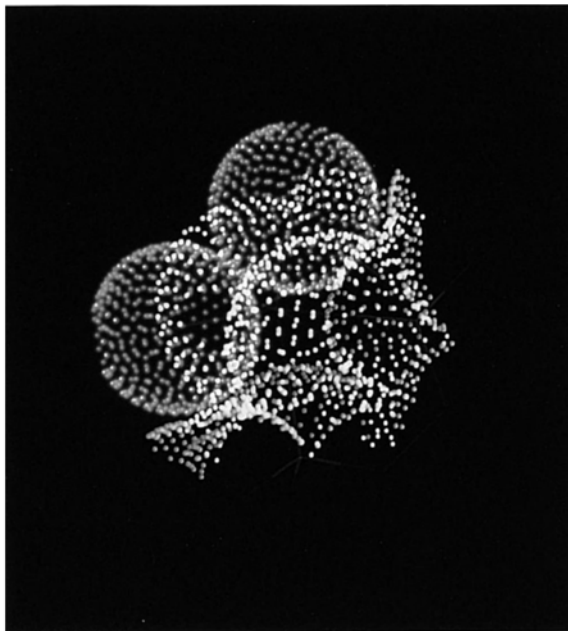


Fig. 11. Representation of the van der Waals volumes of the complex shown in Fig. 10. Volumes are calculated for a sphere of enclosure of 3 Å radius around the Mo and are color coded by atom type.

Table 1 summarizes the geometry data of the model complex in terms of valence angles. The underlined values indicate the largest differences between the model and the geometry of the MoO_2 complexes found in the CSD. Figs. 10 and 11 show comparatively a molybdenum-dioxo complex

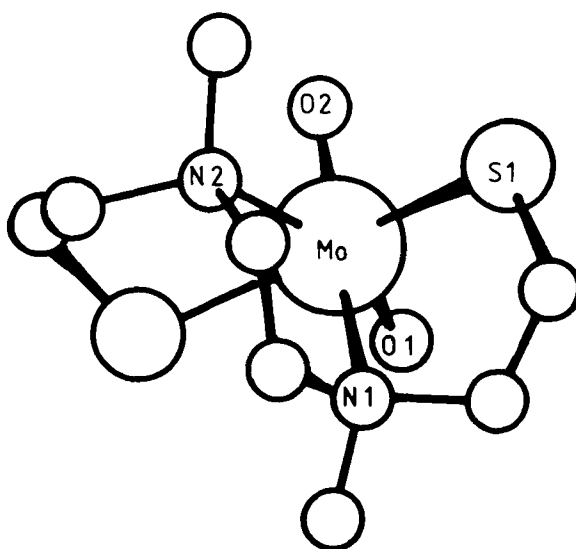


Fig. 10. Ball-and-stick representation of one of the comparative complexes retrieved from CSD.

[14] with sulfur and nitrogen ligands in ball-and-stick (Fig. 10) and van der Waals volume (Fig. 11) representation respectively.

CONCLUSION

Although the molybdenum-purine analogue interaction complexes proposed seem to be a good starting point, we were able to point out the difficulties of their three-dimensional construction. The 35° deviation of the sulfur group (Table 1) from the chelating plane of the cofactor as well as the steric interactions in the docking of the substrate make this model somewhat unlikely. Further investigations are in progress to parameterize a force field which can handle molybdenum-dioxo complexes for ligand optimizations while bound to the metal center.

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