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Structure and Reversible Pyran Formation in Molybdenum Pyranopterin Dithiolene Models of the Molybdenum Cofactor

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Supporting Information

ABSTRACT: The syntheses and X-ray structures of two molybdenum pyranopterin dithiolene complexes in biologically relevant Mo(4+) and Mo(5+) states are reported. Crystallography reveals that these complexes possess a pyran ring formed through a spontaneous cyclization reaction of a dithiolene side-chain hydroxyl group at a C=N bond of the pterin. NMR data on the Mo(4+) complex suggest that a reversible pyran ring cyclization occurs in solution. These results provide experimental evidence that the pyranopterin dithiolene ligand in molybdenum and tungsten enzymes could participate in catalysis through dynamic processes modulated by the protein.

Molybdenum and tungsten enzymes possess at their catalytic sites the oldest and perhaps most unusual ligand in biology.[1,2] This ligand consists of three components: a reduced pterin, a pyran ring fused to the 6,7-positions of the pterin ring system, and a dithiolene chelate that tethers the ligand to the metal (Mo or W, Figure 1).[3] Over a half-century

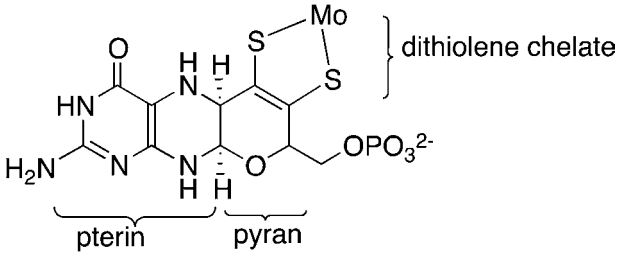

Figure 1. Pyranopterin dithiolene ligand unique to molybdenum and tungsten enzymes (where W replaces Mo). In certain bacteria the phosphate is replaced by several types of dinucleotides.

of research on Mo enzymes is a testament to their importance to life on this planet. Originally identified in mammals and plants,[4] recent additions to the Mo and W enzyme family are found in bacteria, where they are used for respiration in unusual environments,[5] thereby playing critical roles in ecological niches in the environment.

The gross structure of the special ligand in Mo and W enzymes,[6] known variously as molybdopterin or pyranopterin dithiolene, was observed by X-ray crystallography nearly 20 years ago,[7,8] yet several critical questions remain unanswered for this ligand. One question concerns the reduction state and redox activity of the pterin.[9] A second question is whether the pyran ring undergoes reversible scission and re-cyclization.[10,11] Another question is whether these two aspects, pterin redox and pyran ring reactivity, are involved in the catalytic reaction of the enzymes.[10,11] The molybdenum-bound pyranopterin dithiolene is recognized as the most redox-rich cofactor in all of biology since all three parts—the Mo atom, the pterin, and the dithiolene—can potentially participate in electron transfer. Our research has been motivated by these questions about this intriguing ligand, and we sought to answer them using model chemistry. Previously we reported our methodology for building a pterin-substituted dithiolene ligand on Mo.[12] Here we report the synthesis and X-ray structures of the first synthetic pyranopterin molybdenum complexes with evidence for facile and reversible pyran ring formation in this model system.

Our previous work showed that a pterin dithiolene ligand could be formed by reaction of a molybdenum tetrasulfide reagent with a pterinyl arylalkyne.[12] That strategy was employed in the work reported here using a pterinyl alkyne bearing a hydroxyl group in the correct position for pyran ring formation (Scheme 1). This alkyne precursor to the dithiolene is very similar to the structure of the Mo cofactor degradation product known as Form A.[1,13] The reaction of TEA[MoTp*-(S)(S_4)] with the pterinyl alkyne precursor BMOPP yields the dithiolene complex [TEA][Mo(4+)Tp*(S)(S_2BMOPP)] (**1**).[14] Hydrolysis of the Mo=S group to Mo=O is accomplished by treatment with PPh_3 in acetonitrile with 0.2% H_2O to produce TEA[Mo(4+)Tp*(O)(S_2BMOPP)] (**2**). Oxidation of **2** with iodine produces the Mo(5+) complex **3**.[14] The formulations of all three complexes are confirmed by ESI-MS, FT-IR, electronic absorption, and ^{1}H NMR (for **3**) spectroscopies which have been used to characterize the products (see Supporting Information). The oxo-Mo(4+) and (5+) complexes **2** and **3** crystallized and have been structurally characterized by X-ray diffraction.[15]

The crystal structures of **2** and **3** are shown in Figure 2. Both structures clearly show that the alkyne α-OH has added across a C=N bond of the pterin pyrazine, forming a pyran ring through a spontaneous cyclization reaction (Scheme 1). The description of the pterin in both structures as dihydropterin is consistent with bond distances in the pyrazine ring, where C17−N7 bond distances are typical for a C=N bond while C22−N10 distances are longer, as expected for saturated bonds that result from −OH addition. Dithiolene Mo−S, S−C, and

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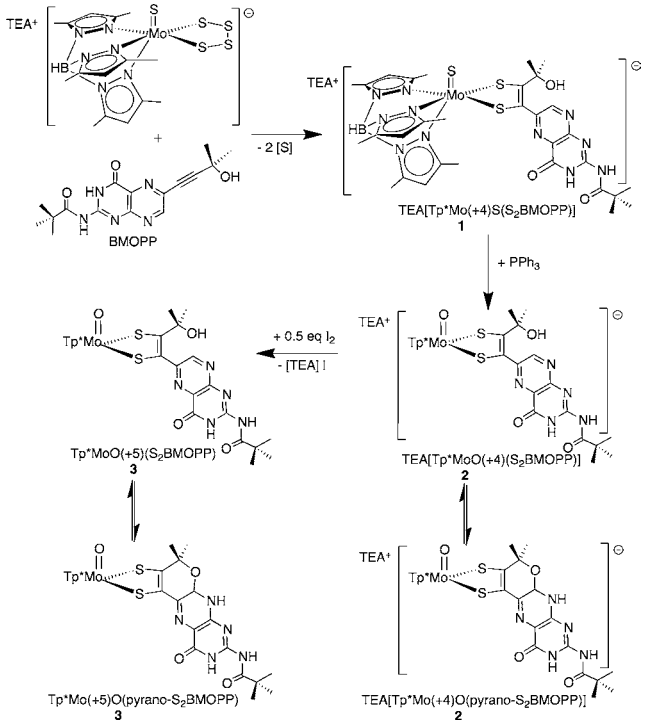
Scheme 1. Synthesis of Molybdenum Pterin Dithiolene Complexes 1, 2, and 3[a]



[a]Tp* = tris(3,5-dimethylpyrazolyl)hydroborate, TEA = tetraethylammonium.

C−C bond distances are in the range of those observed in Mo(5+) and Mo(4+) complexes,[16,17] with a very slight asymmetry observed within the chelate ring. The complexes are chiral in regard to whether the pterin extends to the right or left of the dithiolene chelate. In the case of **2**, only one enantiomer occupies the non-centrosymmetric $P2_12_12_1$ cell, in contrast to the centrosymmetric $P2_1/c$ unit cell of **3**, where both enantiomers are present. We note that the right orientation of the pterin dithiolene in **2** and one enantiomer of **3** is the same as observed for the molybdenum cofactor in the sulfite oxidase family of enzymes, while the left-oriented pterin dithiolene of the other enantiomer in **3** is the same as in members of the xanthine dehydrogenase family.[4,19] The bridgehead carbon atom C22 has an *R*-absolute configuration in all molybdoenzymes. In complex **2**, C22 has *S*-chirality, opposite the configuration observed in the enzymes, whereas in **3**, the right-oriented pterin dithiolene has *R*-chirality at C22 and the left-oriented enantiomer has *S*-absolute configuration at C22.

The two complexes exhibit different dithiolene fold angles between planes defined by Mo−S1−S2 and S1−S2−C16−C24. The dithiolene chelate on the Mo(4+) complex **2** is nearly planar with a slight fold angle of 5°, while the Mo(5+) complex **3** shows a distinctly bent dithiolene having a 26° fold angle (Figure 3). These geometries nicely illustrate the previously reported correlation of dithiolene fold angle and metal oxidation state, where d^2 ions such as Mo(4+) are predicted to adopt planar chelate conformations and dithiolene folding increases with decreasing d-orbital populations.[20]

The observation of pyranopterin dithiolenes in the solid-state structures of **2** and **3** immediately suggests the question: at what point does pyran cyclization occur? In the first study of a synthetic pyranopterin, Pfleiderer et al. documented its behavior as a highly fluxional system,[21] in which two different

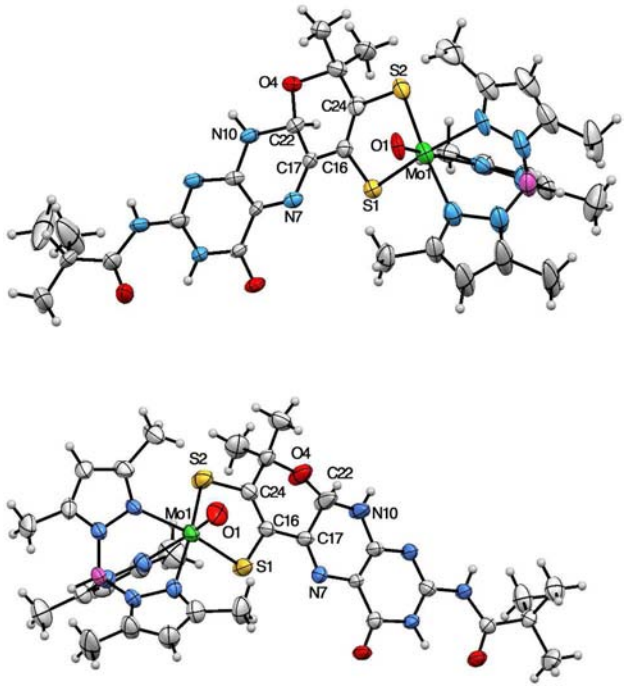


Figure 2. Molecular diagram and labeling scheme for TEA[MoTp*(O)(pyrano-S_2BMOPP)] (**2**, top) and MoTp*(O)(pyrano-S_2BMOPP) (**3**, bottom) with 20% and 50% probability ellipsoids, respectively. H-atoms are depicted with arbitrary radii. Countercation TEA^+ in **2**, second symmetry unique molecule in **3**, and disordered solvent molecules are omitted for clarity. Selected bond distances (Å) for **2**: Mo1−O1 1.645(7), Mo1−S1 2.353(3), Mo1−S2 2.387(3), S1−C16 1.800(11), S2−C24 1.768(10), C16−C24 1.373(12), N7−C17 1.316(11), C16−C17 1.342(13), C17−C22 1.479(13), N10−C22 1.512(12), O4−C22 1.354(10). For representative molecule in **3**: Mo1−O1 1.691(3), Mo1−S1 2.3580(12), Mo1−S2 2.3762(13), S1−C16 1.750(4), S2−C24 1.752(4), C16−C24 1.350(5), N7−C17 1.269(5), C16−C17 1.439(5), C17−C22 1.518(6), N10−C22 1.428(5), O4−C22 1.339(6).

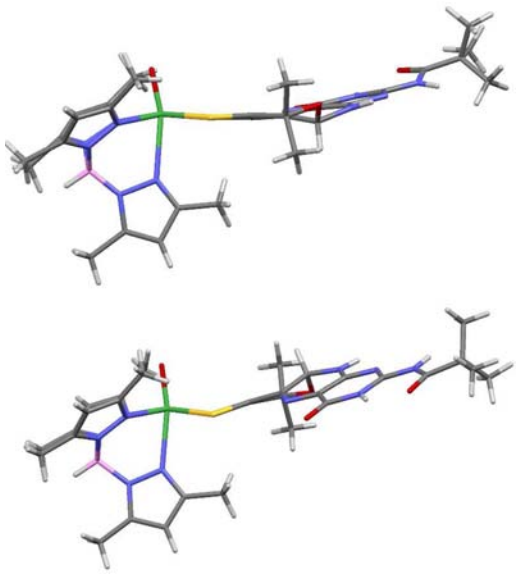


Figure 3. Comparison of the two fold angles observed in the Mo(4+) complex **2** (5°, top) and Mo(5+) complex **3** (26°, bottom).

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hydroxyl addition processes yielded both pyrano- and furano-pterin cyclized species in a dynamic process, producing an equilibrium 3:1 mixture. On this basis, one might expect that all three complexes **1**−**3** would similarly exhibit reversible pyran ring formation.

To further explore the reversibility of pyranopterin formation in our model system, we are investigating the behavior of the diamagnetic Mo(4+) complex **2** by NMR. ^{1}H NMR is useful for identifying the solution structures of the pterin dithiolene ligand since the pyrazine proton (H22) on C22 (see Figure 2) is a reporter of the pterin structure. In the uncyclized, "open" form of **2** depicted in Scheme 1, this proton is expected to appear at a downfield chemical shift of 9−10 ppm, a resonance characteristic of pyrazine heterocycle subsitutents on chelated dithiolenes.[16] Following pyran cyclization, this proton would be expected to be shifted upfield between 5 and 6 ppm.[21] ^{1}H NMR spectra obtained for **2** indicate that pyran cyclization is strongly solvent dependent. In $CDCl_3$, a resonance integrating to one proton is observed at 9.54 ppm and has been assigned to H22 when the pterin dithiolene is in the "open" form (Figure S4). In deuterated acetonitrile the 9.54 ppm resonance is observed, but its integration is reduced to only ∼10% that expected for one proton, while a new, somewhat broad resonance is observed at 6.35 ppm (Figure S5). We tentatively assign the 6.35 ppm resonance to H22 in the pyranopterin form of the complex on the basis of the similarity of its chemical shift to those of reported pyranopterins.[21] When small amounts of d^4-MeOH (10−30%) are added to a sample of **2** in $CDCl_3$, the 9.54 ppm resonance intensity sharply decreases and the entire spectrum broadens (Figure S6). We interpret these results as being consistent with a reversible pyranopterin cyclization, where the open form is the major species in chloroform, the pyranopterin form is the major species in acetonitrile, and equilibrium mixtures of cyclized and non-cyclized forms exists in the presence of methanol.

Reversible pyran cyclization has been proposed as a possible mechanism for modulating the electronic environment of the dithiolene in molybdoenzymes.[10,11,22] Cited in support of the proposed reversible pyran reactivity for the molybdenum pyranopterin dithiolene cofactor are several examples of bis-pterin dithiolene enzymes whose X-ray structures show one dithiolene ligand in the pyranopterin form and the other as an uncyclized pterin dithiolene ligand.[23−25]

The results from our pterin dithiolene model system are significant in the context of the proposed reversible pyran formation in Mo enzymes. Pyranopterin forms of **2** and **3** are favored when these complexes crystallize from solution, but a non-cyclized form is observed in solution by ^{1}H NMR in the case of **2**. The results also indicate that spontaneous pyranopterin formation and scission depends on the solvent environment.

The pyranopterin dithiolene conformation in the molybdoenzymes could be similarly controlled by the protein environment around the pyrazine ring of the pterin. Recent computational work used pterin structural metrics from 103 protein structures to analyze the range of observed pterin conformations.[22] These researchers concluded that in bis-pterin dithiolene enzymes, one pterin conformation is more tetrahydro-like while the other has a geometry more similar to a dihydropterin, but overall, a continuum of pterin conformations exist. Their conclusions were supported by the hydrogen-bonding patterns around the pyrazine.

We have presented here the first synthetic molybdenum pyranopterin dithiolene complexes of molybdenum in the biologically relevevant Mo(4+) and Mo(5+) states. The pyran ring is formed through a spontaneous cyclization reaction where a hydroxyl group on the side chain of the dithiolene adds across a pyrazine C═N bond. It is notable in our work that metal chelation at an appropriately substituted pterin dithiolene is sufficient to induce pyran cyclization, in contrast to other synthetic models where pyran formation required chemical assistance.[26] Our model system provides, in addition, the first chemical evidence for reversible pyran ring cyclization on a pterin dithiolene chelated to Mo in two biologically relevant oxidation states. These results are significant for their implication that the pyranopterin dithiolene ligand in molybdenum and tungsten enzymes may also be a dynamic system. Reversible pyran cyclization may have a role in the catalytic reaction of these enzymes, as it adds yet more versatility to a ligand already rich in redox possibilities.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, ESI-MS and FT-IR spectral data for **1**−**3**, ^{1}H NMR data for **2**, and crystallographic information for **2** and **3** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(14) Abbreviations used: Tp* = tris(3,5-dimethylpyrazolyl)hydroborate, TEA = tetraethylammonium.
(15) The asymmetric unit of **3** contains two unique molecules, and only one is shown in Figure 2. The two molecules do not differ significantly in bond distances, though they exhibit different dithiolene fold angles of 26° and 19°.
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