Promotion of oxygen atom transfer in Mo and W enzymes by bicyclic forms of the pterin cofactor†

Jonathan P. McNamara,[a] John A. Joule,[a] Ian H. Hillier*[a] and C. David Garner[b]

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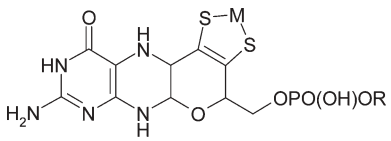
Density functional theory calculations suggest that bicyclic structures of the "molybdopterin" in DMSO reductases may have an important catalytic role in oxygen atom transfer reactions.

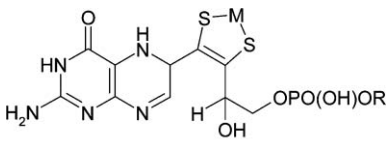
Molybdenum and tungsten are the only 4d (Mo) and 5d (W) transition metals required for the normal metabolism of biological systems. These metals act as the catalytic centre of a wide variety of enzymes, virtually all of which catalyse the oxygen atom transfer (OAT) to, or from, the substrate. Mo-enzymes include nitrate and dimethyl sulfoxide reductases (DMSORs) and sulfite, xanthine, and aldehyde oxidases.[1] The structures of many Mo- and W-enzymes have been investigated by protein crystallography.[2] In each case the metal is co-ordinated by the dithiolene group of "molybdopterin" (MPT, **1**). MPT is unique to these enzymes and comprises a pterin, the pyrazine ring of which is fused to a pyran ring that bears the dithiolene group. In the DMSOR class of enzymes, the metal is bound by two MPTs; in other enzymes, the metal is bound by one MPT.[1] In this communication we show that bicyclic forms of the pterin may facilitate OAT.



1
(M = Mo or W; R = H or nucleoside)

The OAT reactions catalysed by the Mo- and W-enzymes are metal-centred[1–4] and spectroscopic studies have shown that catalysis involves the metal cycling between the M(IV) and M(VI) oxidation states: the net reaction being $M(IV) + XO + 2H^+ \rightarrow M(VI)O + X + H_2O$.[1] Many related OAT chemical reactions of Mo and W complexes have been observed.[4]

The role of MPT in the Mo- and W-enzymes has been considered from various perspectives. Beyond providing the dithiolene group to the co-ordinate metal, it has been suggested that MPT could facilitate outer-sphere electron transfer and/or proton diffusion to (or from) the metal centre.[5] Also, the nature of MPT in the active form of the Mo- and W-enzymes is of considerable interest. The information obtained from the structural studies of the Mo- and W-enzymes[2] is generally interpreted in terms of the pyrazine ring of MPT (**1**) being in the fully reduced, tetrahydro-form. However, opening the pyran ring of (**1**) yields a dihydropterin (*e.g.* **2**) with unsaturation in the pyrazine ring.[5,6] The possible significance of a ring-opened form of the MPT in the function of the Mo- and W-enzymes has been reinforced by information obtained recently in two studies of Mo-enzymes: (i) both tricyclic and a bicyclic form of MPT were identified in a crystallographic study of *Escherichia coli* nitrate reductase A (1Q16) and the two forms were postulated to be in equilibrium;[7] (ii) the electrocatalytic activity observed for *Rhodobacter capsulatus* xanthine dehydrogenase, expressed in *E. coli*, has been interpreted in terms of a pterin-associated oxidative switch that involves a bicyclic form of MPT.[8]



2
(M = Mo or W; R = H or nucleoside)

Several tautomeric forms of bicyclic MPT can be envisaged, including some in which there is conjugation between the metallo-dithiolene and pyrazine rings. The possible occurrence of these has been supported by the results of *ab initio* calculations.[6] We here report the results of further calculations that have probed the possible role of bicyclic forms of MPT in the catalytic action of the Mo-DMSORs.

There have been several experimental[4,9] and electronic structural[10,11] studies of OAT from a sulfoxide to the Mo atom of a $[Mo^{IV}(OR')(S_2C_2R_2)_2]^-$ complex which are effective chemical analogues of the catalytic centre of the Mo-DMSORs.[4] There is general agreement that the reaction pathway for such OAT reactions involves *two* transition states.[9–11] We have used density functional theory to investigate the reduction of DMSO to DMS by $[Mo^{IV}(OMe)(S_2C_2H_2)_2]^-$ and found that the second transition state (TS2), involving the breaking of the S=O bond in DMSO and the formation of the Mo=O bond, is the rate determining step, the two barriers being 47.6 and 72.1 kJ mol^{-1}.

We have investigated the effect that various forms of bicyclic MPT have on the catalysis of OAT from DMSO to a $[Mo^{IV}(OMe)(S_2C_2H_2)(S_2C_2HR)]$ complex, to produce DMS and the corresponding $[Mo^{VI}O(OMe)(S_2C_2H_2)(S_2C_2HR)]$ complex. These studies have been accomplished for the MoS_2C_2HR group involving the bicyclic tautomers shown in Fig. 1. It is relevant to note that the pyrazine ring of T1 corresponds to that observed for

† Electronic supplementary information (ESI) available: Absolute energies, important structural parameters and Cartesian coordinates at the B3LYP level of theory. See http://www.rsc.org/suppdata/cc/b4/b415480k/

*Ian.Hillier@man.ac.uk

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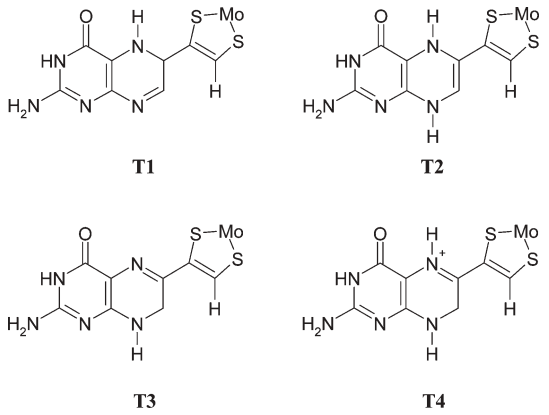

Fig. 1 Bicyclic tautomers of a simplified form of MPT.

the bicyclic dihydropterin tautomer in the crystal structure of *E. coli* nitrate reductase A.[7]

We have performed density functional calculations using the GAUSSIAN 98[12] suite of programs, employing the B3LYP functional[13] with the Los Alamos effective core potential (LANL2DZ)[14] on Mo and a 6–31G(d) basis on all other atoms. Stationary points were characterised as minima or transition states on the potential energy surface by calculation of harmonic vibrational frequencies. The results obtained are summarised in Table 1. The stationary structures for T4 are displayed in Fig. 2.

These calculations have shown that, for a $[Mo^{IV}(OMe)(S_2C_2H_2)(S_2C_2HR)]$ complex, the length of the C–C bond between the metallo-dithiolene and the pyrazine rings of the S_2C_2HR moiety is influenced by the form of the bicyclic MPT. The length of this bond varies as: T4 (1.414 Å) < T3 (1.460 Å) < T2 (1.469 Å) < T1 (1.515 Å). Thus, the extent of the conjugation between the metallo-dithiolene and the pyrazine rings varies as T4 > T3 > T2 > T1. Also, and of significance for the reactivity of these Mo^{IV} centres, the activation energy (TS2) for the OAT reaction varies with the nature of the bicyclic MPT (Table 1). The lowest activation energy, 52.4 kJ mol^{-1}, is found

Table 1 Relative internal energies (in kJ mol^{-1}) on the potential energy surface for OAT from DMSO to $[Mo^{IV}(OMe)(S_2C_2H_2)(S_2C_2HR)]$ (MoS_2C_2HR = T1–T4, Fig. 1)

	Tautomer[b]			
Structure[a]	T1	T2	T3	T4
R	0.0	0.0	0.0	0.0
TS2	78.3	75.9	77.8	52.4
P	−89.1	−87.8	−92.0	−77.2

[a] Reactant (R), transition state 2 (TS2), and product (P). [b] Relative to the isolated species $[Mo^{IV}(OMe)(S_2C_2H_2)(S_2C_2HR)]$ (MoS_2C_2HR = T1, T2, T3, or T4) and DMSO.

for T4; this is *ca.* 24 kJ mol^{-1} lower than that calculated for each of the neutral bicyclic pterins T1, T2, and T3. Thus, the increased conjugation between the metallo-dithiolene and pyrazine rings transmits the effect of the protonation of N5 to the metal centre and this facilitates conversion of $Me_2S{=}O + Mo^{IV}$ to $Me_2S + O{=}Mo^{VI}$.

This interpretation is consistent with the dimensions determined for the TS2 transition state; thus, the length of the Mo=O bond is shortest in T4 (1.903 Å) and longest in T1 (1.935 Å).

Since the catalytic centres of the Mo-DMSORs involve the metal coordinated by *two* MPTs,[1–3] we have augmented the above studies by an investigation of the energy profile of OAT from DMSO to the $[Mo^{IV}(OMe)(S_2C_2HR)_2]$ complex with MoS_2C_2HR = T4. The inclusion of the second protonated bicyclic form of MPT results in a further and significant lowering of the activation energy barrier, TS2, to just 26.2 kJ mol^{-1}.

Enemark and Garner[5] have noted that all of the pterin of the Mo- and W-enzymes is involved in extensive hydrogen bonding interactions within the protein matrix and that these interactions could provide routes for easy proton diffusion to (or from) the pterin moiety. Thus, the protonated form T4 could well be present. The results of the calculations reported herein provide new insights into the manner in which protonation of the pterin, specifically at N5, can facilitate catalysis of OAT reactions at a Mo (or W) centre of the oxotransferase enzymes.

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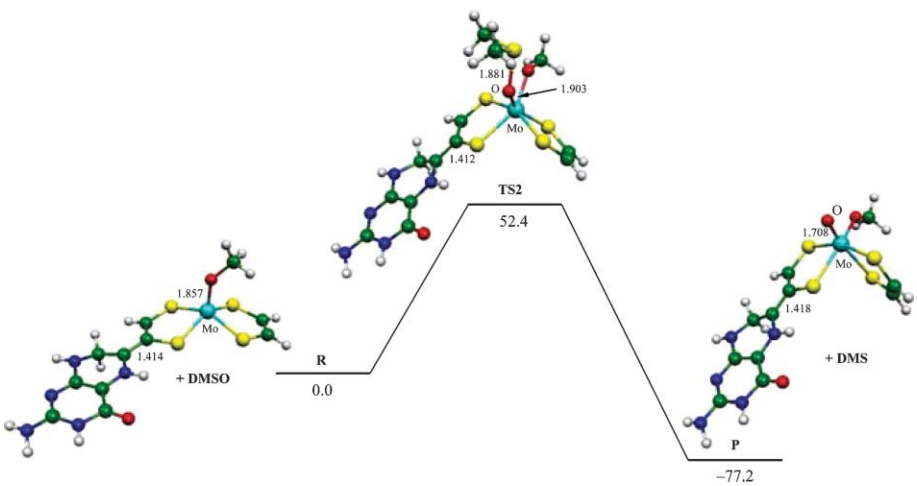

Fig. 2 Optimised structures (distances in Å) and relative energies (in kJ mol^{-1}) of stationary points along the pathway of OAT from DMSO to MPT (T4).

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Jonathan P. McNamara,[a] John A. Joule,[a] Ian H. Hillier*[a] and C. David Garner[b]
[a]*School of Chemistry, University of Manchester, Manchester, UK M13 9PL. E-mail: Ian.Hillier@man.ac.uk; Fax: +44 (0) 161 275 4734; Tel: +44 (0) 161 275 4686*
[b]*School of Chemistry, University of Nottingham, University Park, Nottingham, UK NG7 2RD*

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