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Electronic State of the Dimethyl Sulfoxide Reductase Active Site

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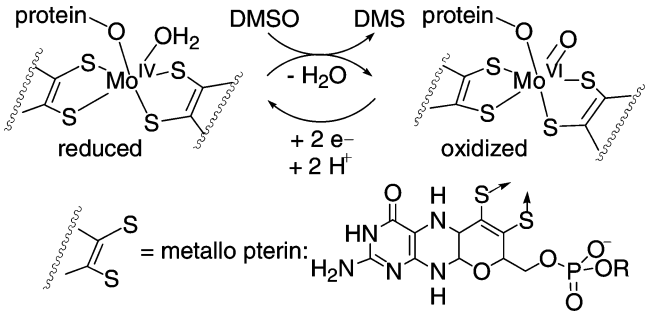
Computations suggest that in contrast with small models the active site geometry of reduced dimethyl sulfoxide reductase might prefer a triplet over a singlet electronic state.

Dimethyl sulfoxide (DMSO) reductase is the prototype of the largest family of mononuclear molybdoenzymes, which play a major role in carbon, nitrogen, and sulfur cycles.[1] The active site is composed of a molybdenum ion bound to two macrocyclic metallopterin chelating ligands and to a protein side chain (Ser147). In catalysis of oxygen atom transfer (OAT) reactions, desoxomolybdenum(IV) and oxomolybdenum(VI) are involved.[2]



Structurally characterized small model complexes[3] as well as computed models[4−6] show remarkable geometrical differences for the reduced and oxidized states, i.e., tetragonal-pyramidal 5-fold-coordinated or trigonal-prismatic 6-fold-coordinated Mo(IV) but a distorted octahedral arrangement of six donor atoms in Mo(VI) complexes (compare **1S**, **3S**, and **4S** in Figure 1). In the latter, the good π-donating oxo ligand favors the octahedral over the prismatic coordination environment.[7] Crystal structures of reduced and oxidized DMSO reductases (DMSORs),[8−10] however, show distorted geometries as compared to model complexes. The geometrical peculiarities of the active site have been interpreted as an entatic state:[11] The distortions of both the desoxo and oxo forms toward the transition-state geometry diminish the reorganization involved and activation needed.[4] The enzyme's catalytic effect is due to the reduced barrier.

Model computations on the DMSOR mechanism have been reported:[4−6] on the singlet potential energy surface (PES), which, to the best of our knowledge, has exclusively been considered so far, the DMSO adduct **3** resides in a shallow minimum and the barrier for OAT is only slightly higher than that for the DMSO release to give 5-fold-coordinated complex **1**; both **3** and water adduct **2** are endothermic, and the formation of oxo complex **4** is strongly exothermic (by 31 kcal mol^{-1}; compare Scheme 1b and Table 1). For stationary points along the nitrate reduction path by dissimilatory nitrate reductase (NR) models, low-lying triplet states have been found (0.1−9.4 kcal mol^{-1} above singlet states depending on the models chosen and methods used).[12] Because dissimilatory NR also belongs to the DMSOR family, we computed[13]the reaction path for DMSO reduction by $[Mo(Me_2C_2S_2)_2(OMe)]^-$ (**1**) as a model for the DMSOR active site on the singlet as well as on the triplet PES.

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(13) Computations have been performed with *Gaussian 03*.[14] Relative energies reported correspond to B3LYP/SDDp//B3LYP/ Lanl2DZp* + ZPE. Restricted and unrestricted formalisms were applied for singlet (**S**) and triplet (**T**) states, respectively. S^2 values between 2.010 and 2.034 resulted for the latter, indicating pure triplet states without significant spin contaminations. See the Supporting Information for a more detailed description of the computational methods and for additional benchmark calculations.

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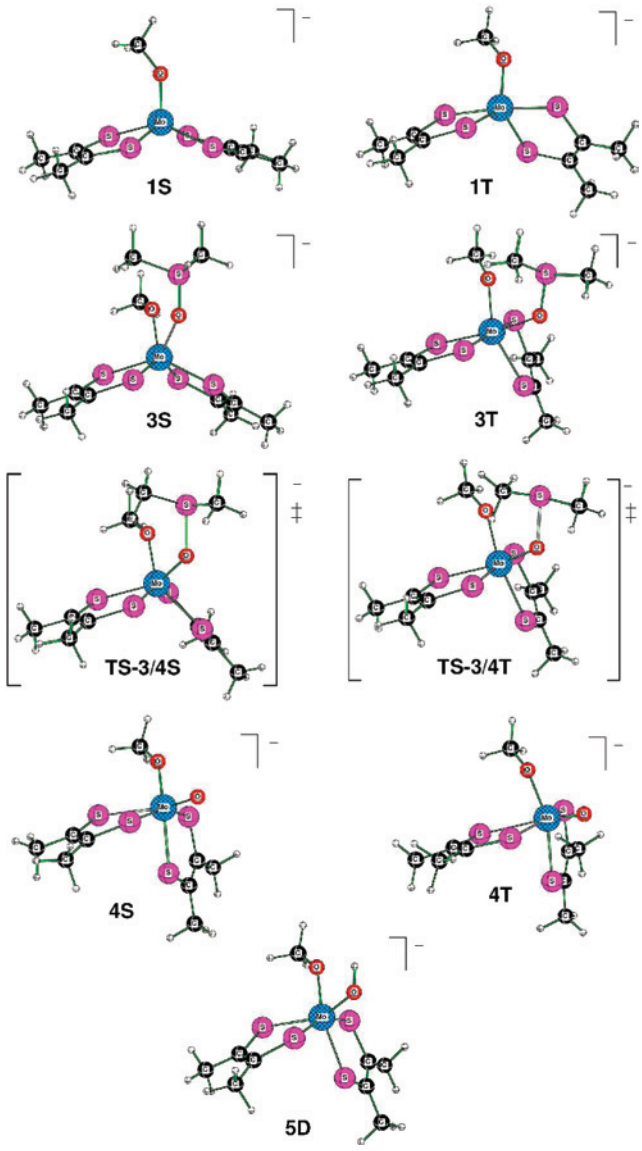


Figure 1. Optimized structures of some molybdenum complexes (**S** = singlet, **T** = triplet, and **D** = duplet) as models for the DMSOR actitve site (see the Supporting Information for a more complete version).

Some optimized geometries are shown in Figure 1; geometrical details are included in the Supporting Information (Figure S1).

The singlet results are in accordance with earlier reports.[4] The triplet states are higher in energy throughout (see Scheme 1b), although for the DMSO complex only by 1.6 kcal mol^{-1} (see Table 1). Qualitative differences of the triplet PES are as follows: both the water adduct **2T** and the DMSO adduct **3T** are bound relative to **1T** (by 2.8 and 2.1 kcal mol^{-1}, respectively), and the activation energy for OAT starting with complex **3** is considerably larger (14.9 vs 3.3 kcal mol^{-1}). However, when the OAT barriers relative to the lowest points on the two paths are considered, the values are not as different (14.9 kcal mol^{-1} for **TS-3/4T** vs **3T** and 9.9 kcal mol^{-1} for **TS-3/4S** vs **1S**). The barrier for DMSO addition to **1T** is very small (0.9 kcal mol^{-1} only), while on the singlet PES, it is almost as large as the OAT barrier. At the geometries optimized for the triplet states, however, the singlet energies are higher with the exception of the OAT transition state **TS-3/4**. Hence, the singlet surface might be shifted above the triplet surface by appropriate geometrical distortions.

Scheme 1. (a) Energies of Singlet States at the Triplet Geometries (S//T) Relative to Singlet States along the DMSO Reduction Path; (b) Singlet and Triplet Reaction Paths for DMSO Reduction by Model Complex **1**

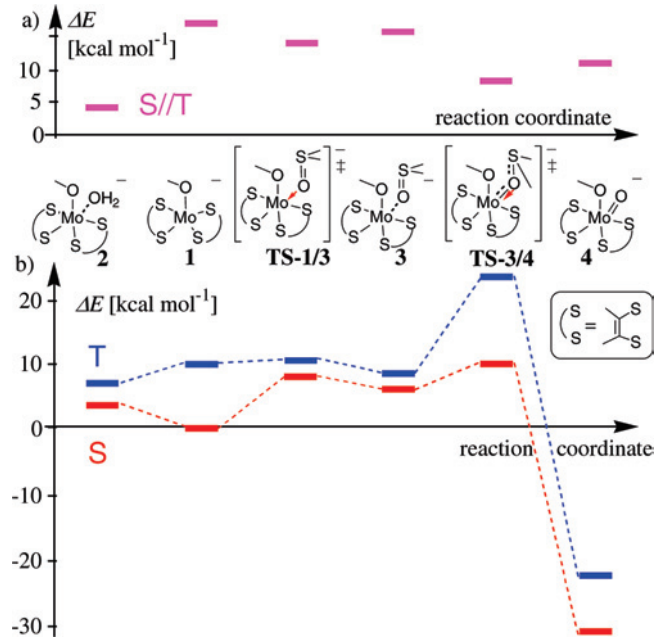


Table 1. Relative Energies [kcal mol^{-1}] for Stationary Points along the Reaction Path of DMSO Reduction by **1**[a]

	1	**2**	**TS-1/3**	**3**	**TS-3/4**	**4**
S[b]	0.0	3.2	8.7	6.6	9.9	−31.4
T vs S[c]	10.3	4.3	2.5	1.6	13.2	8.7[d]
S//T vs T[e]	7.0	0.6	11.6	14.7	−5.0	2.8

[a] Computed at the B3LYP/SDDp//B3LYP/Lanl2DZp* + ZPE level. [b] Relative energies of singlet electronic states, small molecules (H_2O, DMS, or DMSO) were added as appropriate. [c] Triplet relative to corresponding singlet electronic states. [d] The triplet energies of Mo(VI) complexes but not of Mo(IV) complexes are likely to be too low (Table 2). [e] Singlet energies at the geometries optimized for the triplet states relative to the optimized triplet states.

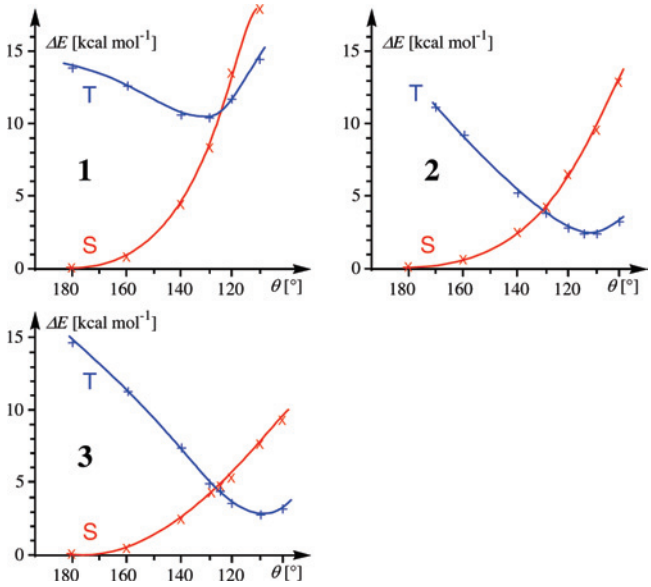
Table 2. Triplet vs Singlet energies [kcal mol^{-1}] Computed at Various Levels for Molybdenum Model Complexes[a]

	B3LYP	B98	QCISD (T)	QCISD	CCSD (T)	CCSD
$[Mo(SH)_4OH]^-$	22.8	22.4	21.3	18.5	20.7	17.0
$[Mo(SH)_4(OH)(OH_2)]^-$	16.7	16.5	15.5	12.0	14.4	10.0
$[Mo(SH)_4(OH)(OSH_2)]^-$	18.1	17.7	16.3	13.1		
$[Mo(SH)_4(O)(OH)]^-$	27.5	27.7			35.8	27.4

[a] SDD effective core potential basis supplemented by d-type polarization functions on O and S atoms (see ref 13).

The geometrical differences between triplet and singlet states are most pronounced for the reduced complexes: The triplet states have enedithiolato ligands considerably twisted relative to each other and larger differences in Mo−S distances (as was reported for reduced DMSOR active sites;[8,9] compare Figure 1). Scheme 2 shows singlet and triplet energies for an increasingly twisted orientation of the two bidentate ligands in **1**−**3**:[15] the triplet states become stabilized, while the singlet states become destabilized. Triplet stabilization is more pronounced along the series no ligand < DMSO < water ligand. The triplet states of **1**−**3** are favored for S−S−S−S dihedral angles θ smaller than ca. 125, 132, and 128°, respectively.

Scheme 2. Energies of Triplet (**T**) and Singlet (**S**) States of Complexes **1**−**3** as a Function of S−S−S−S Dihedral Angle θ



Benchmark calculations[16] demonstrate that the triplet states of **1**−**3** are not given artificially low in energy by our method. Because model complexes **1**−**4** are too big to be treated with highly correlated ab initio methods, we computed energies of singlet and triplet states for tetrathiolato complexes of Mo(IV) and Mo(VI) complexes (see Table 2), which we also used before.[17] For Mo(IV) complexes, B3LYP and B98 density functionals give slightly larger numbers than both CCSD and QCISD (both with and without triple corrections). Density functional theory gives lower triplet energies than the ab initio methods only for the molybdenum(VI) oxo complex, but not for Mo(IV) complexes. Therefore, the relative energies computed for triplet states of Mo(IV) complexes appear to be quite accurate.

Computationally, we showed for model complexes that the electronic states flip by dithiolene ligand distortions.[18] We therefore suggest that the protein environment might enforce ligand orientations that lead to triplet ground states in the reduced form.[19] This favors substrate binding. For dissimilatory NR, no protein structure of the reduced form was reported. The extremely small computed[12] triplet singlet splitting of 0.1 kcal mol^{-1} for the model complex **1** suggests that a triplet ground state might be generated by appropiately oriented ligands even more easily than in the DMSOR case. The profiles shown in Scheme 1 illustrate that the OAT on the triplet PES requires more activation than that on the singlet PES. At the OAT transition-state geometry **TS-3/4T** optimized for the triplet state, the singlet has a lower energy. Therefore, we believe that (probably early) in the course of OAT the electronic state switches from triplet to singlet. Effective spin−orbit coupling facilitates spin inversion at the triplet−singlet intersection, which avoids following the high-energy PES.[20]

Regeneration of the reduced active site requires uptake of two electrons (and two protons) involving an intermediate Mo(V) doublet state. The optimized geometry of the corresponding hydroxo model complex **5D** looks like an average of the **4S** and **2T** geometries. The coordination geometry of the molybdenum center in **5D** is much more similar to **2T** than to the alternative **2S** (Figure S1 in the Supporting Information). The second reduction step might directly lead to the triplet state of the Mo(IV) form. For the active site's restricted geometry, this might be easier than singlet-state formation. At least reorganization is further minimized among **4S**, **5D**, and **2T**.

Enforcing a triplet ground state for the reduced form of the enzyme might be a more general principle in members of the DMSOR family of molybdoenzymes and also for tungsten enzymes, where the orientation of two large macrocyclic metallopterin ligands is largely determined by the protein environment. We hope to stimulate experimental investigations for a molybdoenzyme active site triplet state. *Rhodobacter* DMSOR without any further transition-metal centers[8–10] would be a good candidate to start with.

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Supporting Information Available: Benchmark studies, computational details, frontier molecular orbitals, and a reference listing. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The S^1−S^2−S^3−S^4 dihedral angle θ was kept fixed, and the remaining geometrical parameters were optimized. S^1 and S^2 belong to the same dithiolene ligand; S^2 and S^3 as well as S^1 and S^4 are trans to each other with S^2−Mo−S^3 > S^1−Mo−S^4.

(16) B3LYP was observed before to favor triplet states too much as compared to ab initio methods. For example, see: Graham, D. C.; Beran, G. J. O.; Head-Gordon, M.; Christian, G.; Stranger, R.; Yates, B. F. *J. Phys. Chem. A* **2005**, *109*, 6762–6772.

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(18) Unfortunately, there are no good quality structural data available for the active site in the enzyme, on which additional computations could be based. Crystals that were used for X-ray structure determinations are now believed to have contained mixtures of active and inactive enzyme but were interpreted for a single species (see ref 10). Resonance Raman and EXAFS spectroscopies provide evidence that the X-ray bond differences are incorrect. (a) George, G. N.; Hilton, J.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1996**, *118*, 1113–1117. (b) George, G. N.; Hilton, J.; Temple, C.; Prince, R. C.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1999**, *121*, 1256–1266. (c) Baugh, P. E.; Garner, C. D.; Charnock, J. M.; Collison, D.; Davies, E. S.; McAlpine, A. S.; Bailey, S.; Lane, I.; Hanson, G. R.; McEwan, A. G. *J. Biol. Inorg. Chem.* **1997**, *2*, 634–643.

(19) The ligand twist significantly reduces singlet−triplet splittings (see Scheme 2), and further distortions might be supportive.

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