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Oxo-molybdenum enzymes: new insights from metal and ligand hyperfine coupling

Anthony G. Wedd
Department of Chemistry, La Trobe University, Bundoora, Victoria, 3083, Australia and
Jack T. Spence
Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322–0300, USA.

Abstract - Molybdenum-95, hydrogen-1, oxygen-17 and sulfur-33 hyperfine coupling constants are available via electron spin resonance for a number of oxo-molybdenum enzymes, and for xanthine oxidase in particular. The enzyme data is compared with complementary information obtained for a series of model complexes featuring quadridentate N_2S_2 ligands. The comparisons indicate that $Mo^{V}OS(OR)$ and $Mo^{V}O(SH)(OR)$ (OR = bound product) are the centres responsible for the "Very Rapid" and "Rapid" ESR signals of xanthine oxidase. A $Mo^{V}O(OH)$ centre appears to be involved in the "Slow" ESR signal of xanthine oxidase and in the low pH forms of sulfite oxidase and the nitrate reductases.

BACKGROUND

There are two classes of molybdenum enzymes: the nitrogenases and the oxo-molybdenum or hydroxylase enzymes (ref. 1). The latter catalyse two-electron redox reactions which may include a primary oxo transfer step, the source of the oxygen atom being water. Most information is available for xanthine oxidase/dehydrogenase (XO/XD), sulfite oxidase (SO) and the nitrate reductases (NR). Each enzyme features a pterin unit (the Mo cofactor) closely associated with the Mo site (ref. 2).

The enzymes cycle through oxidation states VI, V and IV during turnover. The most direct structural information comes from EXAFS data (ref. 3) which indicates the presence of $[Mo^{VI}O_2(SR)_n]$ (n=2,3) units at the oxidised Mo sites in certain SO and NR enzymes and in the inactive "desulfo" form of XD. $[Mo^{VI}OS(SR)_2]$ units are detected in active XO or XD.

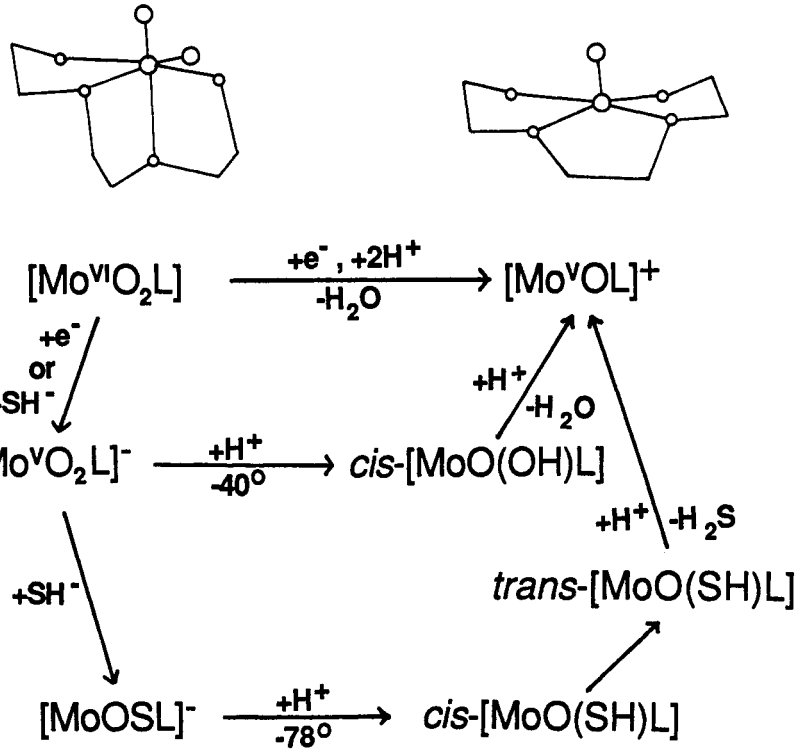
ENZYME ESR SIGNALS

ESR signals characteristic of Mo^{V} appear upon reduction of the enzymes (ref. 4). In particular, the Very Rapid ($t_{1/2}$, ~10ms) and Rapid ($t_{1/2}$ ~25ms) signals are associated with turnover of active xanthine oxidase by xanthine. The Slow signal appears upon reduction of desulfo xanthine oxidase by dithionite. Bray's prolific work (ref. 4,5) has provided a wealth of ESR information at 9 & 35 GHz. This includes ^{1}H, ^{13}C, ^{17}O, ^{33}S, ^{95}Mo and ^{97}Mo hyperfine coupling data for atoms associated with the catalysis.

Our own work on the enzymes has concentrated on examination of the xanthine oxidase ESR signals at low frequencies (2-9 GHz). Advantages include reduced linewidths allowing better resolution of hyperfine structure and a useful redistribution of spectral information: to first order, hyperfine interactions are independent of frequency whereas g-value resolution is frequency dependent (ref. 6). Importantly, the effects upon the spectra of non-coincidence of the coordinate axes of the **g** and various hyperfine coupling matrices is minimised at low frequency. This is particularly helpful in the simulation of spectra from low symmetry enzyme sites. Adequate simulations are obtained for low frequency spectra featuring both magnetic and non-magnetic nuclei. Then, non-coincident angles are refined at higher frequencies. A parameter set can be accepted only when it reproduces spectra at all frequencies examined.

Recently we have defined the ^{95}Mo hyperfine matrices for the Rapid type 1, Rapid type 2 and Slow signals for ^{95}Mo-enriched XO (refs. 6-8), while George and Bray have provided those for the Very Rapid and Rapid type 1 signals, as well as their ^{97}Mo quadrupole coupling matrices (ref. 5). Interestingly, the Rapid type 1 spectrum of ^{95}Mo-enriched XO was first published more than 20 years ago (ref. 9). That it has taken until now to define the ^{95}Mo hyperfine matrix demonstrates the complexity of simulating ESR signals from low symmetry Mo^{V} centres.

Reaction Scheme



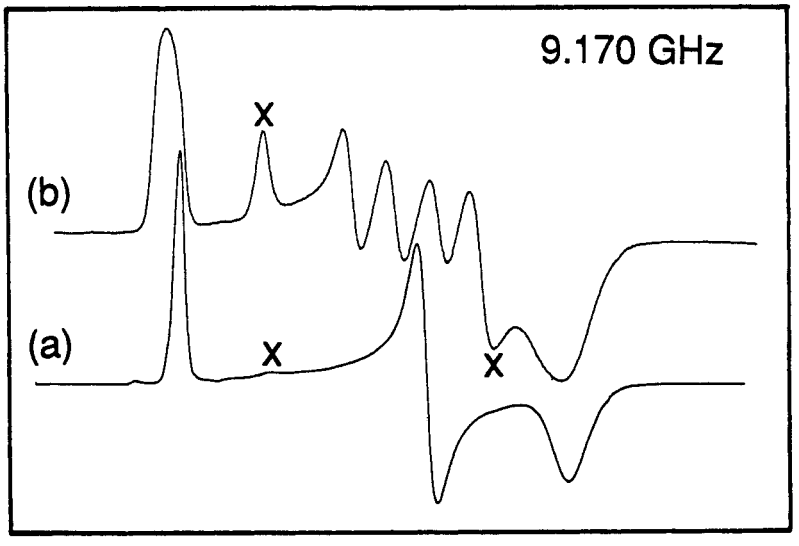
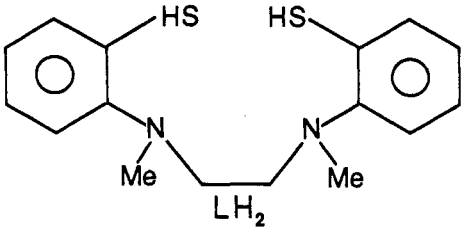


Figure 1. ESR spectra in thf/MeCN (10:1 v:v) at 77K (97.2 atom % ^{98}Mo)
(a) $[^{98}MoOSL]^-$
(b) $[^{98}MoO^{33}SL]^-$ (99.25 atom % ^{33}S)

X marks resonances containing contributions from contaminating $[^{98}MoO_2L]^-$.

SYNTHESIS OF MODEL COMPOUNDS

When the vast majority of *cis*-$Mo^{VI}O_2$ complexes are reduced, H_2O is eliminated producing $Mo^{V}O$ or $Mo^{IV}O$ species, often coupled with condensation to binuclear centres. For a number of quadridentate ligands, in-built steric barriers disfavour condensation as well as the ligand conformation change necessary for the conversion of *cis*-$Mo^{VI}O_2$ to $Mo^{V}O$ species (refs. 10-12; see Reaction Scheme). A number of intermediates can be intercepted along the reaction pathway. Very clean chemistry is seen for LH_2 = N,N'-dimethyl-N,N'-bis(2-mercaptophenyl)ethylenediamine (12).



Reversible one-electron reduction of *cis*-[$Mo^{VI}O_2L$] at room temperature produces a highly anisotropic ESR spectrum characteristic of [$Mo^{V}O_2L$]$^-$. At -40° in the presence of H_2O, *cis*-[$Mo^{V}O(OH)L$] can be trapped: that the ESR signal intensity is maximised in the presence of 1.5M H_2O emphasises the importance of the steric barrier.

Structural assignment of *cis*-[$Mo^{V}O(OH)L$] rests upon observation of hyperfine coupling to a single ^1H and two inequivalent ^{17}O atoms. Similar properties are observed for the structurally-characterised [$Mo^{V}O(OSiMe_3)L$] (refs. 7,12).

Hydrosulfide also reduces [$Mo^{VI}O_2L$] to [$Mo^{V}O_2L$]$^-$ which then undergoes a slow substitution reaction to form [$Mo^{V}OSL$]$^-$. This can be protonated at low temperature to *cis*-[$Mo^{V}O(SH)L$], which converts to the *trans*-isomer upon warming (Reaction Scheme). Structural assignments are made on the basis of ^1H and ^{17}O hyperfine coupling (ref. 12) and the isolation of Ph_4P[MoOSL] and *trans*-[MoO(SH)L] in substance (ref. 13), although X-ray quality crystals have not been grown to date. Interestingly, X-ray absorption spectroscopy at the Mo and S K-edges provide no evidence for a short MoS bond in Ph_4P[MoOSL] and the exact nature of the material remains uncertain. This observation may be related to the fact that [MoOSL]$^-$ produces [MoO(SH)L] and not [MoS(OH)L] upon protonation.

Isotope substitution of these species with ^2H, ^{17}O, ^{33}S, ^{95}Mo and ^{98}Mo has been carried out to provide high quality ESR data for comparison with the enzymes. Use of the non-magnetic isotope ^{98}Mo allows ligand hyperfine coupling to be observed unambiguously, while use of ^{95}Mo ensures the metal hyperfine coupling is observed in the absence of the quadrupole effects of the ^{97}Mo nucleus (natural abundances: ^{95}Mo, $I = {}^5/_2$, 15.72 atom %; ^{97}Mo, $I = {}^5/_2$; 9.46 atom %). The latter are important both in XO and model compound spectra (ref. 5).

ENZYME AND MODEL COMPOUND COMPARISONS

Only the most striking analogies apparent from existing data are canvassed in this brief review.

Comparisons of the average coupling constants $a(^1H)$ and $a(^{17}O)$ are given below (units: $x10^{-4}$ cm^{-1}):-

	$a(^1H)$	$a(^{17}O)$
XO Rapid type 1	11.7-12.8	6.5
[$Mo^{V}O(SH)L$]	9.7	2.0
[$Mo^{V}O(OH)L$]	14.8	2.3, 7.5
XO Slow	13.7-14.9	~9

The values observed for the enzyme signals vary somewhat with substrate, buffer and added anion. A dependence upon the particular quadridentate ligand occurs for the synthetic species.

$a(^1H)$ is larger for the Slow signal than for the strongly coupled proton of the Rapid signal and this is mirrored in the values for [MoO(OH)L] and [MoO(SH)L].

At least two oxygen atoms are coupled to the Slow signal (ref. 14) and the more strongly coupled one has a coupling constant similar to that of the hydroxyl oxygen of [MoO(OH)L]. While a coupling constant close to $2x10^{-4}$ cm^{-1} appears to be diagnostic of an apical oxo ligand in $Mo^{V}O$ species, the available data for an OR group *cis* to oxo suggests that the magnitide of the oxygen coupling varies significantly with the detailed environment of the oxygen atom (ref. 7). The observed average value of $6.5x10^{-4}$ cm^{-1} for the single O atom coupled to the Rapid type 1 signal is too large to be attributed to an apical oxo ligand as present in [MoO(SH)L] and bound product OR is a more likely candidate.

The a(^{1}H) and a(^{17}O) couplings are consistent with [Mo^{V}O(SH)(OR)] and [Mo^{V}O(OH)] centres being responsible for the Rapid type 1 and Slow signals of XO. These assignments are reinforced by the observed ^{95}Mo hyperfine matrices (ref. 6-8) where the relative magnitudes of the hyperfine components and misalignment angles are similar and one of the principal axes of **g** and **A** coincide in each case.

The relationship between the Very Rapid and Rapid type 1 signals of functional XO is of considerable interest. We have previously assigned a Mo^{V}OS(OR) centre to the Very Rapid signal on the basis of the anisotropy of **g** (with g_1 being greater than that for the free electron), the absence of ^{1}H coupling and the presence of strong ^{17}O coupling. This assignment is made less plausible by the observation that **g** for [MoOSL]$^{-}$ is much more highly rhombic than that for the Very Rapid centre (ref. 12,15). However, we have noticed that the rhombicity of **g** (essentially determined by the high field g-value, g_3) in [MoO_2L]$^{-}$ and [MoOSL]$^{-}$ is very dependent on the exact nature of the ligand L and experimental conditions. For example, the presence of Li^{+} in solutions of [MoO_2L]$^{-}$ significantly affects g_3, apparently by ion-pairing effects.

The close relationship between the Very Rapid signal and [MoOSL]$^{-}$ is best illustrated by preliminary ^{33}S substitution experiments (Figure 1; ref. 15). [MoOSL]$^{-}$ shows strong anisotropic coupling to ^{33}S ($I = {}^{3}/_{2}$) which largely collapses in [MoO(SH)L] (units, $x10^{-4}$ cm^{-1}).

	A_1	A_2	A_3	angles
Rapid type 1	3.2	3.3	3.3	0,0,0
[MoO(SH)L]	~2.8	<3.2	<3.2	?
[MoOSL]$^{-}$	<3.4	~22	<4.1	?
Very Rapid	2.8	25.6	6.4	40,0,10

The values quoted for the model compounds are estimated directly from the spectra: the apparent change in g-values and asymmetric line shapes in [MoO^{33}SL]$^{-}$ (fig. 1) are symptomatic of misalignment of **g** and **A** (^{33}S) principal axes, as seen in the Very Rapid Signal.

The distinctive pattern of ^{33}S coupling in the Very Rapid signal is reproduced so convincingly by [MoOSL]$^{-}$ that there can be little doubt that a Mo^{V}OS centre is responsible for that signal.

In summary, the comparisons presented here identify the following relationships for the characteristic ESR signals of XO:

ESR Signal	Assigned Centre
Very Rapid	Mo^{V}OS(OR)
Rapid	Mo^{V}O(SH)(OR)
Slow	Mo^{V}O(OH)

[Mo^{V}O(OH)] centres also appear to be present in SO (low pH form) and NR (low pH forms of *E. coli* and *C. vulgaris*; signal A from spinach). In addition, the ESR signal of [W^{V}O(OH)L] closely resembles that of the tungsten form of SO.

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