Application of CW and pulsed EPR, MoSophe and DFT calculations in unravelling the electronic structure of the molybdenum(V) centre in dimethylsulfoxide reductase

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Dimethylsulfoxide reductase, a bacterial molybdenum oxotransferase, belongs to the Type-III Clade of the dimethylsulfoxide (DMSO) reductase family of molybdenum enzymes and catalyses the conversion of DMSO to dimethylsulfide (DMS) with an accompanying two electron transfer. The molybdenum cofactor within DMSO reductase contains an organic component known as molybdopterin (MGD) which is a modified pterin providing an ene-dithiolene side chain responsible for ligating the Mo. The active site of the DMSO reductase contains two MGD ligands, a single oxo group and the amino acid ligand Serine in a trigonal prismatic geometry.

A continuous wave (CW) EPR spectrum attributable to the Low-g Type-I Mo(V) species and a sulfur centered radical were observed upon dithionite reduction of dimethylsulfoxide reductase from the photosynthetic bacterium *Rhodobacter capsulatus* (Lan *et al.,* 2007a&b), of the naturally abundant and ^{95}Mo enriched Low-g Type-I Mo(V) CW EPR spectrum reveals that while the magnitudes of the principal components of the g and A matrices resemble the Slow Mo(V) center found in desulfo xanthine oxidase, their orientation is quite different and the largest ^{95}Mo hyperfine component is associated with the smallest g value rather than the largest g value. The coordination sphere of the Low-g Type-I Mo(V) species consists of an an ene-dithiolene (P-MGD), Ser-147 and a protonated oxo group, which form the base of a square pyramid. In conjunction with the results obtained from a multifrequency CW EPR and density functional theory (ORCA) of a series of thiomolybdenyl complexes (Drew *et al.*, 2007a&b), the g and triclinic A(^{95}Mo) matrices are consistent with the unpaired electron located in a $|d_{x^2-y^2}>$ ground state molecular orbital in which the x and y axes are located between Mo-ligand bonds. In addition to the Low-g Type-I Mo(V) species, the CW EPR spectrum exhibits an orthorhombic signal (g_z = 2.0545, g_y = 2.0182, g_x = 1.999) with small ^{95}Mo ($A_2 = 5.0\text{x}10^{-4}$ cm^{-1}) hyperfine coupling on the g_y resonance. Both 3-pulse ESEEM and HYSCORE spectra revealed the presence of one or more weakly coupled protons and isotropic hyperfine coupling ($A_{iso}(^{14}N)$ = 6.7 MHz) to a single nitrogen nucleus. The CW- and pulsed-EPR results are consistent with an unpaired electron centered on sulfur atom (S1) of Q-MGD which is delocalized onto the pyranopterin ring system. These results implicate sulfur centered radicals in the stabilization of the charge on the molybdenum ion in DMSO reductase and/or electron transfer between the native electron donor DorC and the Mo center via the Q-MGD.

Analysis of the continuous wave and pulsed electron paramagnetic resonance spectra and EPR potentiometric titration experiments reveal that the Mo(V) High-g Unsplit Type-2 species is the intermediate species formed during the catalytic reduction of DMSO reductase from Rhodobacter capsulatus. The spin Hamiltonian parameters for the Mo(V) High-g Unsplit Type-2 species obtained from naturally abundant and ^{95}Mo enriched DMSO reductase reveal that the unpaired electron is present in a $|d_{z^2}>$ ground state molecular orbital and that the geometry of the active site Mo centre is trigonal prismatic. Since the tigonal prismatic geometry of the Mo centre is retained upon reduction of the resting (Mo(VI)), to Mo(V) and Mo(IV) the active site within DMSO reductase is an example of an entatic state. The Mo(V) High-g Split species, previously proposed to be catalytically relevant in the reductive direction, has been shown to be involved in the oxidative half reaction.

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