Comparative Electrochemistry of *cis*-$MoOS^{2+}$, MoO_2^{2+}, and MoS_2^{2+} Centres in Complexes of the Type *cis*-$[MoY_2L_2]$ (Y = O and/or S, HL = 1-hydroxypiperidine). Comments on Active and Desulpho-forms of Xanthine Oxidase and Xanthine Dehydrogenase

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Cyclic voltammetry has been used to characterise the electrochemical reductions of the complexes *cis*-$[MoY_2(C_5H_{10}NO)_2]$ (Y = O and/or S, $C_5H_{10}NOH$ = 1-hydroxypiperidine) in dimethylformamide. The complexes reduce at −2.50 (dioxo), −1.94 (oxothio), and −1.59 V (dithio), referenced to the ferrocenium–ferrocene couple. For the first time, electrochemical comparisons have been made within an homologous series of molybdenum(VI) complexes. The results are especially relevant to the chemical differences between the molybdenum centres of intact and desulpho xanthine oxidase and xanthine dehydrogenase.

The nature of the co-ordination environment of molybdenum in molybdoenzymes has been the subject of considerable investigation in recent years.[1] In particular, molybdenum *K*-edge extended *X*-ray absorption fine structure (EXAFS) studies have provided direct evidence concerning the immediate co-ordination environments about molybdenum in nitrogenase,[2] sulphite oxidase,[3,4] xanthine oxidase,[5] and xanthine dehydrogenase.[4] On this basis, the hypothesis [6] that the cyanolysable sulphur, present in the active forms of xanthine oxidase and xanthine dehydrogenase, is a terminal thio-group ligated to molybdenum has received considerable support. The centre thus postulated for the enzymes in their oxidised, active forms is a *cis*-oxo,thio $MoOS^{2+}$ moiety and a *cis*-dioxo MoO_2^{2+} moiety in their oxidised, desulpho-forms.

E.s.r. spectroscopy has also proved to be useful in elucidating the nature of oxomolybdoenzymes. Thus, a number of molybdenum(V) species may be detected under appropriate conditions, and these probably correspond to the products of oxidation of molybdenum(IV) species, in turn produced by the interaction of substrates with Mo^{VI} in the resting, oxidised enzyme. For xanthine oxidase, Bray [7] has proposed a mechanism involving the $MoOS^{2+}$ group, consistent with the known e.s.r. properties of the various states of this enzyme.

However, the *cis*-oxothiomolybdenum(VI) entity remained unknown until the work of Wieghardt and co-workers,[8] who reported the preparation of *cis*-$[MoOS(C_5H_{10}NO)_2]$ ($C_5H_{10}NOH$ = 1-hydroxypiperidine). The subsequent structural and spectroscopic characterisation of this centre [9] lends further support to the inferred presence of a $MoOS^{2+}$ centre in the oxidised, active enzymes.

It seemed pertinent, therefore, to investigate the electrochemical properties of *cis*-$[MoOS(C_5H_{10}NO)_2]$, along with its *cis*-dioxo- and *cis*-dithio-analogues, for comparison with data obtained on the enzymes.[10–14]

Experimental

Cyclic voltammograms were recorded using a Hi-Tek Instruments Ltd potentiostat type DT2101 and a Chemical Electronics (Birtley) waveform generator type 01, connected to a Philips PM 8043 X-Y recorder. All cyclic voltammetric measurements were made in *ca.* 0.2 mol dm^{-3} solutions of $[NBu^n_4][BF_4]$ in deaerated dimethylformamide (dmf), using a vitreous carbon working electrode and a silver–solvent pseudo-reference electrode, under an atmosphere of dinitrogen.

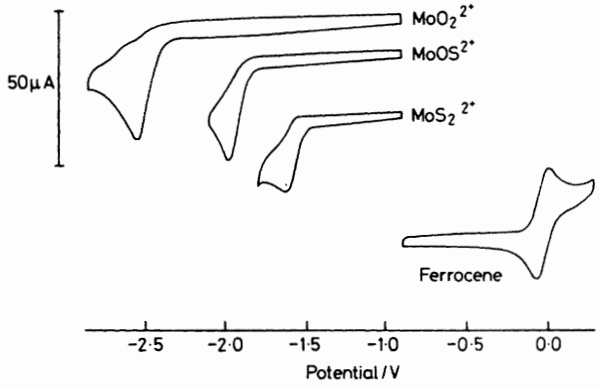

Figure 1. Cyclic voltammograms of the complexes *cis*-$[MoY_2(C_5H_{10}NO)_2]$ (Y = O and/or S) in solution in 0.2 mol dm^{-3} $[NBu^n_4][BF_4]$ in dmf, at 298 K, at a vitreous carbon electrode; scan rate = 0.3 V s^{-1}

All potentials were measured with respect to the $[Fe(\eta^5\text{-}C_5H_5)_2]^+$–$[Fe(\eta^5\text{-}C_5H_5)_2]$ couple as an internal reference. Solution concentrations were in the range 2.0—2.2 mmol dm^{-3}.

Controlled-potential electrolyses were conducted at potentials slightly more negative than the observed reductions under conditions identical to those for cyclic voltammetry, except that a platinum-gauze working electrode was employed, and the solvent was deaerated tetrahydrofuran. Solution concentrations were in the range 5.0—6.5 mmol dm^{-3}.

Results and Discussion

The electrochemical behaviour of the three molybdenum(VI) complexes is discussed in terms of their relative reduction potentials, evidence for the formation of mononuclear molybdenum(V) intermediates, the fate of these intermediates, insofar as we have determined it, and the bearing this study has on the redox chemistry of the active and desulpho-forms of xanthine oxidase and xanthine dehydrogenase.

Cyclic voltammograms recorded at a scan-rate, v, of 0.3 V s^{-1} at 298 K in the dmf electrolyte are shown in Figure 1. The peak potential, E_p, and peak current, i_p, ratios are given in the Table. The trend in E_p for the complexes is clear: the progressive substitution of Mo=S for Mo=O results in easier

Table. Reduction potentials for *cis*-$[MoY_2(C_5H_{10}NO)_2]$ (Y = O and/or S) complexes

Complex	Potential/mV [a]	Peak current (i_p) ratio [b]
$[MoO_2(C_5H_{10}NO)_2]$	−2 500	1.83
$[MoOS(C_5H_{10}NO)_2]$	−1 940	0.71
$[MoS_2(C_5H_{10}NO)_2]$	−1 590	0.78

[a] *vs.* $[Fe(\eta^5\text{-}C_5H_5)_2]^+$–$[Fe(\eta^5\text{-}C_5H_5)_2]$, in dmf. [b] i_p of complex × $[Fe(\eta^5\text{-}C_5H_5)_2]/i_p$ of ferrocene × [complex]. The numbers of electrons transferred in the redox processes were estimated as 1.272 × (peak current ratio); thus, we have assumed that the diffusion coefficient of each complex is similar to that of $[Fe(\eta^5\text{-}C_5H_5)_2]$ and applied the correction factor for comparing the number of electrons transferred in a reversible *vs.* an irreversible system.

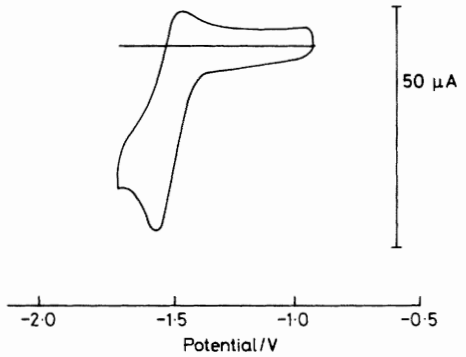

Figure 2. Cyclic voltammogram of *cis*-$[MoS_2(C_5H_{10}NO)_2]$ in solution in 0.2 mol dm^{-3} $[NBu^n_4][BF_4]$ in dmf, at 298 K, at a vitreous carbon electrode; scan rate 1 V s^{-1}

reduction. These potentials can be taken to parallel the thermodynamic trend (E_p + 30 mV ≈ E°), insofar as both the $MoOS^{2+}$ and MoS_2^{2+} species show reversible electron-transfer behaviour under modified conditions (see below). The trend in E_p we have observed is consistent with earlier studies on molybdenum(v) complexes with dimeric $Mo_2O_{4-x}S_x^{2+}$ cores [15–17] and monomeric MoO^{3+} species when sulphur replaces oxygen as the ligand atom.[18]

The primary reduction step of MoS_2^{2+} and $MoOS^{2+}$ complexes is the reversible transfer of a single electron to give the corresponding unstable molybdenum(v) intermediate. Thus, *cis*-$[MoS_2(C_5H_{10}NO)_2]$ showed reversible reduction to the monoanion at scan rates ≥ 1 V s^{-1} at 298 K, as shown by Figure 2. Although *cis*-$[MoOS(C_5H_{10}NO)_2]$ displayed irreversible reduction characteristics at scan rates up to 30 V s^{-1} at 298 K, cyclic voltammetry performed at low temperatures (using a propanone–CO_2 cooling bath) revealed reversible electron-transfer behaviour, as shown by the cyclic voltammogram obtained at 0.3 V s^{-1} and *ca.* 195 K (Figure 3). These reversible processes are represented by equation (1) (Y = O or S).

$$cis\text{-}[Mo^{VI}YS(C_5H_{10}NO)_2] \underset{-e}{\overset{+e}{\rightleftharpoons}} cis\text{-}[Mo^{V}YS(C_5H_{10}NO)_2]^- \quad (1)$$

In contrast to the reversible behaviour of the sulphur-ligated species, the dioxo-complex, *cis*-$[MoO_2(C_5H_{10}NO)_2]$, reduced irreversibly even at low temperatures and fast scan rates. Superficially, we can conclude that substitution of one (or two) oxygen atom(s) by sulphur stabilises the molybdenum(v) anions, *i.e.* this stability increases in the order $Mo^VO_2^+ < Mo^VOS^+ < Mo^VS_2^+$. This conclusion parallels

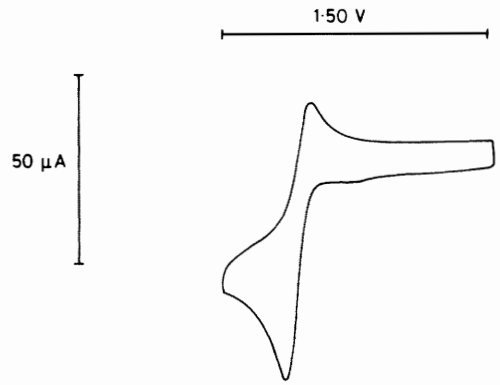

Figure 3. Cyclic voltammogram of *cis*-$[MoOS(C_5H_{10}NO)_2]$ in solution in 0.2 mol dm^{-3} $[NBu^n_4][BF_4]$ in dmf, at *ca.* 195 K, at a vitreous carbon electrode; scan rate = 0.3 V s^{-1}; peak-to-peak separation is 130 mV

earlier observations by Schultz and co-workers [15,16] on dimeric systems possessing $Mo_2O_{4-x}S_x^{2+}$ cores.

The fate of the molybdenum(v) anions has been briefly investigated, by variable scan-rate cyclic voltammetry, controlled-potential electrolysis, and e.s.r. spectroscopy. The peak-current functions, $i_p/v^{\frac{1}{2}}$, of each complex deviate towards two-electron stoicheiometries at $v < 0.3$ V s^{-1} and 298 K. Evidently, the products of chemical reaction of the anions are electroactive at potentials > E_p. Controlled-potential electrolysis of *cis*-$[MoOS(C_5H_{10}NO)_2]$ consumes 2 F per mol and yields an e.s.r.-silent, lemon-yellow catholyte. Under similar conditions, *cis*-$[MoS_2(C_5H_{10}NO)_2]$ shows current *versus* charge-passed behaviour consistent with the formation of a molybdenum(IV) intermediate which attacks the molybdenum(VI) starting material to give an e.s.r.-silent, orange catholyte, in an overall 1 F per mol process. Presumably, the product of this electrolysis is a molybdenum(v) dimer.

Data obtained from early studies of the redox chemistry of xanthine oxidase [10] and dehydrogenase [12] suggest that the Mo^{VI}–Mo^V couple, and also the Mo^V–Mo^{IV} couple, shift towards more negative values, on conversion of the active enzymes into their desulpho-forms. The magnitude of shifts in redox potentials was found to be between 40 and 125 mV. However, further investigations of the redox properties of xanthine oxidase, at room temperature and as a function of pH, by Barber and Siegel [13] and Porras and Palmer [14] appear to demonstrate a minimal effect of desulphurisation on the redox potential of the Mo^{VI}–Mo^V couple. The Mo^V–Mo^{IV} couple appears to be somewhat more sensitive than the Mo^{VI}–Mo^V couple, to the loss of sulphur; however, definitive comments are restricted since the interpretations of the pH dependence of the redox-potential data from the two studies [13,14] were not concordant. In the light of their results, Barber and Siegel [13] concluded that 'the conversion of a terminal sulphur to a terminal oxygen ligand has little effect on the intrinsic electron affinities of the various molybdenum oxidation states.'

This conclusion is in stark contrast to our results which show redox-potential changes of *ca.* 350—550 mV, on replacement of S by O in the *cis*-$[MoY_2(C_5H_{10}NO)_2]$ series. The similarity [13,14] of reduction potentials for the Mo^{VI}–Mo^V couple in the active and desulpho-forms of xanthine oxidase may be compatible with a change from a $MoOS^{2+}$ to a MoO_2^{2+} centre; however, the electrochemical data presented here indicate that, for this to be true, the inherent differences of the $MoOS^{2+}$ and MoO_2^{2+} centres must be modified by other effects in the enzymes. One possibility is that, in contrast

to these *cis*-$[MoY_2(C_5H_{10}NO)_2]$ complexes, delocalisation of electronic charge density, added to the molybdenum, could occur in the enzymes *via* the co-ligand system of the molybdenum cofactor.[19] This would buffer the influence of thio-*versus* oxo-substitution on the metal-centred orbitals involved in the redox processes. Furthermore, we note that the redox-potential data presented here should be compared with pH-*independent* data for the Mo^{VI}–Mo^{V} couple in both forms of xanthine oxidase. The latter are inaccessible, since it is clear [13,14] that protonation accompanies this electronation. However, *if* the oxidised centres of active and desulpho-forms of xanthine oxidase involve $MoOS^{2+}$ and MoO_2^{2+} centres, respectively, *and* these differ in their electron affinity to the extent observed in the *cis*-$[MoY_2(C_5H_{10}NO)_2]$ (Y_2 = O_2 or OS) complexes, we must conclude that, for the coupled proton–electron transfer in the enzymes, *the* MoO_2^{2+} *centre is more basic than is the* $MoOS^{2+}$ *centre by several* (*ca.* 9) pK_a *units*. Therefore, further studies of these biochemical and related chemical systems are required, in order to clarify these and other important aspects of the chemical properties of the molybdenum centres of active and desulpho-xanthine oxidase.

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