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Temperature dependent electrochemical investigations of molybdenum and tungsten oxobisdithiolene complexes†

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To achieve a better understanding why thermophilic and hyperthermophilic organisms use tungsten instead of molybdenum within the active sites of their molybdopterin dependent oxidases, electrochemical investigations of model complexes for the active sites of enzymes belonging to the DMSO reductase (molybdenum) and the aldehyde oxidoreductase (tungsten) family have been undertaken. Cyclic voltammetry and differential pulse voltammetry of four pairs of molybdenum and tungsten oxobisdithiolene compounds show huge differences in the response of their redox potentials to rising or decreasing temperatures, depending on the substituents at the dithiolene group. The mnt^{2-} compounds (**1a**, **1b**) respond with decreasing redox potentials $E_{1/2}$ to rising temperatures whereas all other compounds show positive gradients $\delta E/\delta T$. In every case the values for the gradients for the tungsten compounds are greater than those for the molybdenum compounds. Six of the investigated compounds are known in the literature and two compounds were newly synthesized. These two new compounds include the pyrane subunit of the native molybdopterin ligand and should therefore be even better models for the active site of the molybdopterin containing enzymes. The molybdenum/tungsten pair with these new ligands shows a remarkably small difference for the redox potentials of the transition $M^{IV} \leftrightarrow M^{V}$ of only 30 mV at 25 °C and the reversion of the usual order with higher potentials for the molybdenum than the tungsten compound at a temperature of 70 °C; a temperature that is in the range where usually tungsten containing enzymes instead of molybdenum containing ones are found.

Introduction

Some of the most interesting forms of life are those, which prosper in an environment that is life-hostile from a human point of view: the so-called Extremophiles. These organisms prefer habitats with high salt concentration, high or low pH value, high pressure or extreme temperature. The study of these organisms and their extremozymes constantly gains significance, since they are interesting not only from the scientific, but also from the industrial point of view.[1]

Thermophilic and hyperthermophilic microorganisms live in or next to hot and sulfur rich submarine vents or springs on the surface. In contrast to most though not all of the mesophilic organisms these unusual life forms use tungsten instead of molybdenum at the active sites of their molybdopterin containing oxidases which catalyze hydroxylation and oxo-transfer reactions that are two electron redox reactions (see reaction 1).

$$R + H_2O \leftrightarrow RO + 2\,H^+ + 2\,e^- \quad (1)$$

In Fig. 1 the different groups of molybdopterin containing enzymes following the Hille classification are shown. Despite its name molybdopterin[2] contains no molybdenum but is an organic compound also referred to as pyranopterindithiolate,[3] pterindithiolene[4] or pterin-ene-dithiolate (see Fig. 1).[5]

This ligand is believed to function not only as a modulator of the metal ion redox potential[5,6] but also to couple the metal into superexchange pathways for facilitating electron transfer regeneration of the active sites during the catalytical process.[7,8] The molybdopterin dependent enzymes in general are found in almost any known organism where they can have quite different tasks regarding the substrate and the direction of the above reaction 1 and also some structural differences (see Fig. 1).[9,10]

The reason for two different metals (molybdenum *vs.* tungsten) at the active sites is of great interest as its understanding might give some insight into the catalytic processes of these enzymes. It is discussed in the literature that because the thermophilic bacteria and hyperthermophilic archaebacteria belong to the oldest forms of life on this planet, the distribution of molybdenum and tungsten containing enzymes reflect the evolution of these enzymes.[11] Meaning: under the conditions of the early planet earth, that were presumably hot and sulfur rich, the molybdopterin enzymes were developed with tungsten utilized for their active sites and with the change of these conditions descending organisms switched from tungsten to molybdenum. But the question whether the change of metal happened due to environmental conditions because of supply-, stability- or redox potential-reasons is still unsolved.

The assumption that the molybdenum and tungsten enzymes in general simply catalyze different reactions can be refuted by two observations. First: by the existence of a molybdenum dependent formate dehydrogenase from *Escherichia coli*[12] and also a tungsten dependent formate dehydrogenase from *Chlostridium thermoaceticum* (optimum growth temperature T_{opt} is 55 °C).[13,14] And second: the *Methanobacterium thermoautotrophicum* (T_{opt} = 65 °C) expresses a molybdenum dependent formylmethanofurane dehydrogenase as well as a tungsten dependent formylmethanofurane DH depending on the environmental conditions.[15–18] For *in vitro* experiments these conditions were determined by the supply of only molybdenum or only tungsten. Interestingly, the two metals are not incorporated into the same enzyme but rather into two different though similar enzymes that are genetically separated and are expressed one or the other in response to the environment.[19,20]

Strictly inorganic arguments for the supply-determined change of metal follow the observation that in a sulfur and sulfide rich surrounding, molybdenum has the tendency to form only slightly soluble MoS_2 while tungsten under the same conditions

† Electronic supplementary information (ESI) available: Fig. S1: Temperature dependent plot of $E_{1/2}$ *vs.* Fc/Fc^+ of compound **4b**, sample 1. Typical course of the electrochemical measurements; indicated by the arrows. See http://www.rsc.org/suppdata/dt/b4/b414853c/

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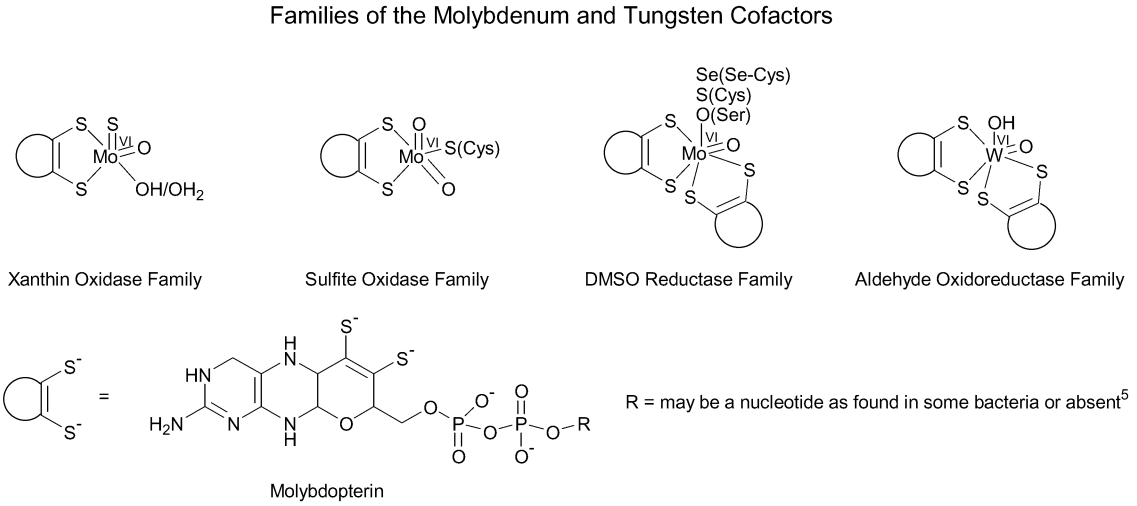

Fig. 1 Oxidized active sites of the molybdopterin dependent enzymes confirmed by EXAFS or X-ray structural analysis. The organization into families follows that of Hille[9] for molybdenum and that of Johnson *et al.*[10] for tungsten. For others than the aldehyde oxidoreductase family no structures are available for the tungsten enzymes.[3]

favours $WO_2S_2^{2-}$ and WOS_3^{2-} ions, which are soluble and can be easily utilized by the organisms.[21,22]

Arguments for the stability determined change rely on investigations of the LMCT energies of MoS_4^{2-} and WS_4^{2-}. They show that the ground state of the tungsten species is more stabilized than that of the molybdenum species, because the transfer of electron density from the sulfur to the metal requires more energy for the tungsten compound.[23] Furthermore model complexes for the active sites of the molybdopterin enzymes with tungsten show a higher thermal stability than their molybdenum counterparts due to stronger π–π-interactions between sulfur and metal.[21] Under the extreme conditions in the environment of the thermophilic and hyperthermophilic organisms this could be of some advantage. On the other hand, one has to take into account that the stability of an enzyme is usually determined by its protein structure while the active site is shielded very effectively from the environment.

Kinetic investigations of the oxidation of benzoin by $[MO_2(bdt)_2]^{2-}$ (M=Mo, W) at different temperatures revealed that the reactivity improvement at higher temperatures was greater for the tungsten compound than for the molybdenum compound.[24] Although within the temperature range of the experiment (RT → 100 °C) the molybdenum compounds reactivity was always better than that of the tungsten compound, this is an indication for a reactivity related change of metal at the enzymes active sites. Also based on a reactivity related change of metal is the suggestion that the tungsten enzymes might be better suited for higher temperatures than their molybdenum counterparts due to a different shift of their redox potentials with rising temperature. To test this assumption the temperature dependence of the redox properties of pairs of analogous known and new molybdenum and tungsten oxobisdithiolene compounds that model the active sites of the enzymes of the DMSO reductase family (molybdenum) or the aldehyde oxidoreductase family (tungsten) (see Fig. 1) were investigated by cyclic voltammetry or differential pulse voltammetry.

Experimental

Materials

All chemical reagents were purchased from Acros and used without further purification. Water and ethanol were deaerated with N_2. Solvents (*p.a.*) for the organic reactions were used as received. All reactions were carried out in an atmosphere of N_2 using standard Schlenk line techniques, and only the isolation procedures for the organic reactions were carried out in air.

3-Bromoflavanone,[25] $Na_3K[WO_2(CN)_4]\cdot 6H_2O$,[26] $Na_3K[MoO_2(CN)_4]\cdot 6H_2O$,[27] $[Et_4N]_2[MoO(mnt)_2]$[28] ($[Et_4N]^+$ was used as cation instead of $[Bu_4N]^+$), $[Et_4N]_2[WO(mnt)_2]$,[22] $[Et_4N]_2[MoO(bdt)_2]$,[29] $[Et_4N]_2[WO(bdt)_2]$,[30] $[Et_4N]_2[MoO(S_2C_2Me_2)_2]$[31] and $[Et_4N]_2[WO(S_2C_2Me_2)_2]$[32] were prepared according to literature procedures.

Syntheses

3-(*NN*-Diethyldithiocarbamate)-flavanone I. 3-Bromoflavanone (10.72 mmol; 3.25 g) dissolved in diethylether (80 mL) was added dropwise over a period of 15 min to a refluxing solution of sodium *N*,*N*-diethyldithiocarbamate (11.76 mmol; 2.65 g) in ethanol (10 mL). After the addition was complete the mixture was refluxed at 50 °C for another 10 min. The solvents were removed *in vacuo* and the residue extracted with water (70 mL) and dichloromethane (70 mL). The water layer was washed 3 times with CH_2Cl_2 (10 mL). The combined organic layers were washed 3 times with brine (10 mL), dried over $MgSO_4$ and the solvent removed *in vacuo*. The residue was recrystallized from ethanol giving a pale yellow crystalline solid. Yield: 2.45 g (6.59 mmol; 62%).

Elemental analysis: calcd. for $C_{20}H_{21}NO_2S_2$: C: 64.66%, H: 5.70%, N: 3.77%; expt: C: 65.18%, H: 6.21%, N: 3.85%; IR (KBr) [cm^{-1}]: 3061 (m, ν_{Ar-H}), 3033 (m, ν_{Ar-H}), 2980 (m, $\nu_{CHaliph.}$), 2934 (m, $\nu_{CHaliph.}$), 1689 (vs, ν_{CO}), 765 (vs), 698 (s), 315 (vs); ^{1}H-NMR (CD_2Cl_2, 400 MHz) [ppm]: δ = 1.25 (d), 3.72, 5.71 (d), 5.80 (d), 7.02 (s), 7.29 (s), 7.40–7.51 (m), 7.80(s).

2-(*N*,*N*-Diethylamino)-4,5-flavanyl-1,3-dithiolium hydrogensulfate II. Sulfuric acid (2.5 mL; 95%) was added dropwise over a period of 20 min to vigorously stirred solid **I** (5.32 mmol; 1.977 g) cooled by a water/ice-bath to 0 °C. After the addition was complete the mixture was heated to 60 °C for a period of 10 min and cooled again to 0 °C before 150 mL of ether were added. It was stirred vigorously for 30 min giving a greenish gumlike solid. The solvent was decanted and this procedure repeated twice. Acetone (40 mL) was added to the now honeylike residue and the mixture again stirred vigorously. The solvent was decanted and this procedure repeated six times until giving a microcrystalline white solid that was isolated by filtration, washed 3 times with acetone (10 mL) and dried *in vacuo*. Yield: 1.44 g (3.19 mmol, 60%).

Elemental analysis: calcd. for $C_{20}H_{21}NO_5S_3$: C: 53.19%, H: 4.69%; N: 3.10%; expt: C: 51.79%, H: 4.68%; N: 2.84%; IR (KBr) [cm^{-1}]: 3057 (m, ν_{Ar-H}), 3017 (m, ν_{Ar-H}), 2980 (m, $\nu_{CHaliph.}$), 2924 (m, $\nu_{CHaliph.}$), 1584 (m), 1540 (s), 1223 (vs), 1195 (vs),

703 (m), 590 (s), 467 (s); ^{1}H-NMR (CD_2Cl_2, 400 MHz) [ppm]: δ = 1.1–1.4 (m), 3.55–3.95 (m), 5.25 (s), 6.65–7.70 (m).

$[PPh_4]_2[Mo^{IV}O(fdt)_2]\cdot EtOH$ 4a. A solution of **II** (1.10 mmol; 0.495 g) in ethanol (8 mL) was added to a solution of $Na_3K[MoO_2(CN)_4]\cdot 6\ H_2O$ (0.55 mmol; 0.267 g) and NaOH (4.42 mmol; 0.177 g) in water (8 mL). The reaction mixture was heated to 65 °C for 3 h. After cooling the reaction mixture to 30 °C a solution of $[PPh_4]Cl$ (1.1 mmol; 0.431g) in ethanol (8 mL) was added dropwise giving a red–brown precipitate which was isolated by filtration, washed with water (3 times; 4 mL) and dried *in vacuo*. Yield: 378.6 mg (0.275 mmol, 50%).

Elemental analysis: calcd. for $C_{80}H_{64}O_4P_2S_4Mo$: C: 69.86%, H: 4.69%; expt: C: 69.19%, H: 4.62%; IR (KBr) [cm^{-1}]: 3054 (m, ν_{Ar-H}), 3021 (m, ν_{Ar-H}), 1598 (m), 1585 (m), 1483 (m), 1436 (s, ν_{P-Ph}), 1107 (s), 1029 (m), 1017 (shoulder, $\nu_{Mo=O}$), 996 (m), 722 (s, δ_{Ar-H}), 689 (s, δ_{Ar-H}), 527 (vs); F-IR (RbI) [cm^{-1}]: 392 (w, ν_{Mo-S}), 375 (vw, ν_{Mo-S}); UV-vis (CH_3CN solution, 10^{-4} M) [nm]: 557, 367, 274, 269; ^{1}H-NMR (CD_3CN, 500 MHz) [ppm]: δ = 1.32 (s), 2.14 (m), 5.38 (s), 7.70 (m), 7.75 (m), 7.93 (t).

$[PPh_4]_2[W^{IV}O(fdt)_2]\cdot EtOH$ 4b. A solution of **II** (1.99 mmol; 0.90 g) in ethanol (16 mL) was added to a solution of $Na_3K[WO_2(CN)_4]\cdot 6\ H_2O$ (1.01 mmol; 0.58 g) and NaOH (13.75 mmol; 0.55 g) in water (16 mL). The reaction mixture was heated to 65 °C for 3 h. After cooling the reaction mixture to 30 °C a solution of $[PPh_4]Cl$ (2.14 mmol; 0.85 g) in ethanol (16 mL) was added dropwise giving a dark brown precipitate which was isolated by filtration, washed with water (3 times; 8 mL) and dried *in vacuo*. Yield: 184.20 mg (0.048 mmol, 12%).

Elemental analysis: calcd. for $C_{80}H_{64}O_4P_2S_4W$: C: 65.66%, H: 4.41%; expt: C: 65.08%, H: 4.80%; IR (KBr) [cm^{-1}]: 3053 (m, ν_{Ar-H}), 3019 (m, ν_{Ar-H}), 1600 (m), 1585 (m), 1483 (m), 1436 (s, ν_{P-Ph}), 1107 (s), 1029 (m), 995 (m, $\nu_{W=O}$), 722 (s, δ_{Ar-H}), 689 (s, δ_{Ar-H}), 526 (vs); F-IR (RbI) [cm^{-1}]: 392 (w, ν_{W-S}), 363 (w, ν_{W-S}), 348 (vw, ν_{W-S}); UV-vis (CH_3CN solution, 10^{-4} M) [nm]: 560, 449, 360, 274, 269; ^{1}H-NMR (CD3CN, 500 MHz) [ppm]: δ = 1.27 (s), 2.10 (m), 5.39 (s), 7.67 (m), 7.72 (m), 7.90 (t).

Electrochemical measurements

Electrochemical measurements were performed with an AUTOLAB PGSTAT12 potentiostat/galvanostat with a glassy carbon working electrode, a platinum wire reference electrode and a platinum wire auxiliary electrode in acetonitrile solutions within a glovebox under argon atmosphere. Supporting electrolyte was 0.1 M Bu_4NPF_6 used as received from Fluka. Acetonitrile for the electrochemistry was predried over $CaCl_2$, dried by reflux over CaH_2 for two days and distilled onto freshly regenerated molecular sieve A3. Referenciation was done internally using the ferrocene/ferrocenium couple. Temperature during the measurements was controlled with a Julabo refrigerated/heating circulator FP50-MV. The solutions have been prepared within a glove box by dissolving 10^{-2} mol of the Bu_4NPF_6 electrolyte and exactly 10^{-4} mol of the compounds in the cases of **1ab**, **2ab**, **3ab** and due to poor solubility 0.5×10^{-5} mol in the cases of **4a** and **4b** in 100 mL of acetonitrile resulting in 10^{-3} M solutions and 0.5×10^{-4} M solutions respectively. For each measurement 6 mL of these solutions were transferred to the double wall electrochemistry cell. The temperature of the cell and the solution within was controlled with the FP50-MV sending water of the desired temperature through the double wall. The redox potentials of the couple $[MO(dt)_2]^{2-} \leftrightarrow [MO(dt)_2]^{-}$ were determined by cyclic voltammetry (scan rate = 100 mV s^{-1}) in the cases of **1ab**, **2a**, **3ab** and by differential pulse voltammetry (step potential = 2.55 mV; modulation amplitude = 25.02 mV; modulation time = 0.05 s) in the cases of **2b** and **4ab**. The samples were stirred prior to the measurements for at least 10 min at the desired temperature.

Other physical measurements

Elemental analyses were determined with a Heraeus CHN–O-Rapid elemental analyzer.

Infrared spectra were recorded as KBr pellets on a Mattson Genesis FT-MIR-spectrophotometer ATI from 4000 to 400 cm^{-1} and as RbI pellets on a Bruker FIR-spectrophotometer IFS 66 from 500 to 200 cm^{-1}. Absorption spectra were obtained on a Varian Cary 5 spectrophotometer as 10^{-4} M solutions in CH_3CN (Uvasol).

^{1}H-NMR spectra were recorded on a Bruker NMR-spectrometer AM 400 and on a Bruker Advance DRX 500 and referenced to the chemical shifts of the deuterated solvent.

Results and discussion

Choice and syntheses of compounds

The four pairs of analogous molybdenum and tungsten oxobis-dithiolene complexes that have been synthesized (see Fig. 2) were chosen for the different strength of non-innocence character of their dithiolene ligands; a characteristic that is also part of the natural molybdopterin leading to the consideration of the metal and the coordinated molybdopterin as one redox center.[33,34] This is because non-innocence means that the ligand itself is redox active (see Fig. 3) and the division of electrons between metal centre and ligand is flexible (Fig. 3). Mesomeric distribution of the intermediate unpaired electron as well as electron withdrawing groups at the dithiolene enhance its non-innocence character and hence the covalency of the metal sulfur bond.[35,36] In this group of ligand systems the non-innocence decreases therefore from mnt^{2-} over bdt^{2-} to $S_2C_2Me_2^{2-}$ with the fdt^{2-} ligand with the highest mesomeric potential presumably in the range of the mnt^{2-} ligand.

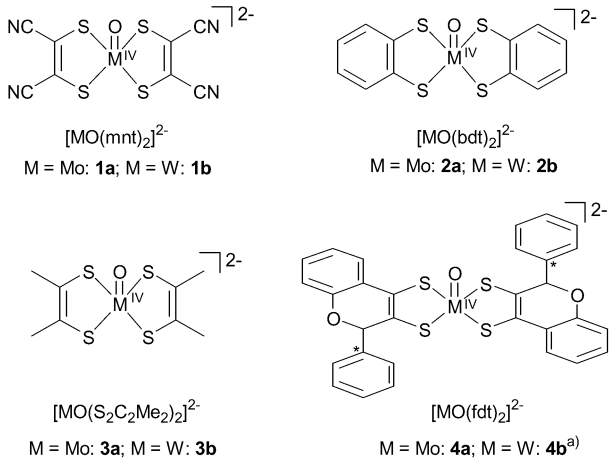

Fig. 2 Synthesized dithiolene complexes. (a) The complexes **4a** and **4b** were synthesized as a mixture of *cis*- and *trans*-isomers; here only the *trans*-isomer is shown.

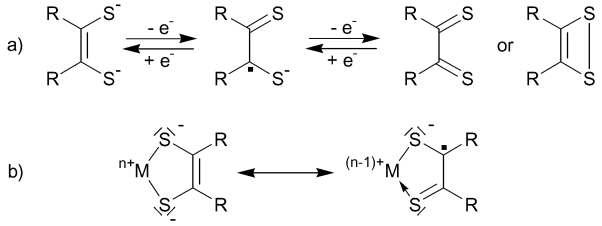

Fig. 3 (a) non-innocence of a dithiolene ligand; (b) redox interaction between metal center and dithiolene ligand.

It is remarkable that the preparation methods obviously depend strongly on the ligands used resulting in completely different syntheses for the four pairs of molybdenum and

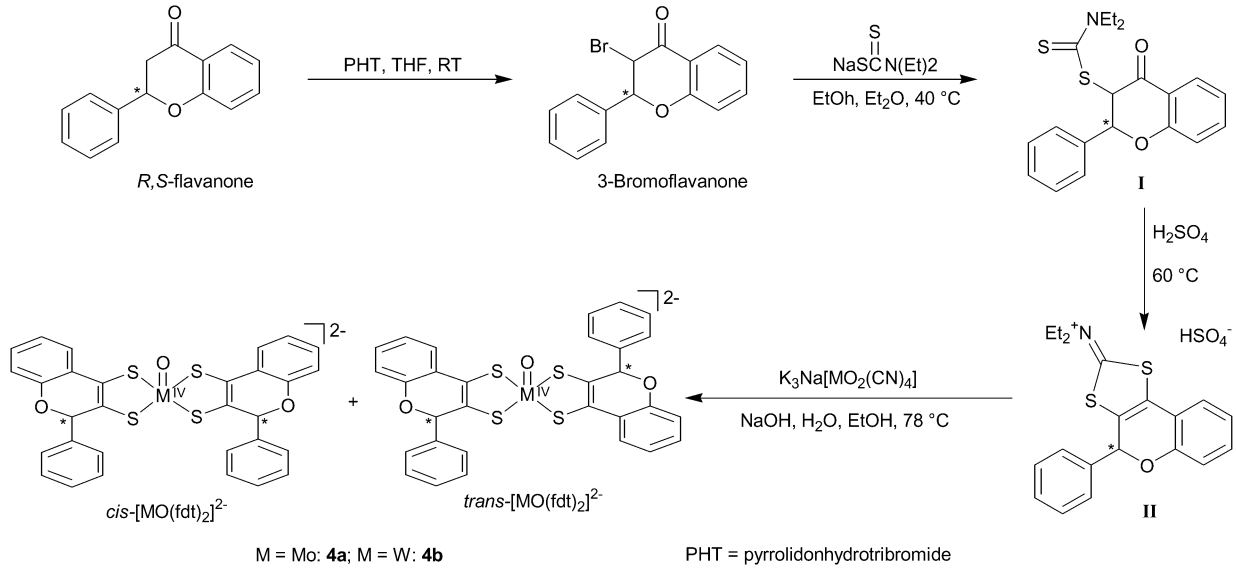

Fig. 4 Synthesis of the compounds $[PPh_4]_2[Mo^{IV}O(fdt)_2]\cdot EtOH$ **4a** and $[PPh_4]_2[W^{IV}O(fdt)_2]\cdot EtOH$ **4b** following a method developed by Joule and Garner.[37]

tungsten compounds that seem not to be exchangeable. The syntheses of the six known molybdenum (**1a**, **2a**, **3a**) and tungsten (**1b**, **2b**, **3b**) complexes were exactly like those described in the respective literature.[22,28–32] The syntheses of the two new complexes (**4a**, **4b**) (see Fig. 4) followed largely a procedure developed by Garner and Joule.[37]

The flavanone was chosen as starting material because it is commercially available and promised the synthesis of a ligand system that not only includes the dithiolene group but also the pyrane feature of the enzymatic molybdopterin and therefore a more accurate modelling of the natural ligand system. The syntheses of the intermediates were carried out according to the literature procedures of similar compounds with the exception of the ring closing step with sulfuric acid. Here the isolation of the product turned out to be rather difficult. Recrystallization from ethanol was not possible because with this solvent the aminodithiolium hydrogensulfate and probably some side products formed a honeylike residue that even *in vacuo* could not be dried to give a solid product. Instead the residue was stirred several times vigorously in acetone in which some side products of the reaction were soluble, leaving the desired product as a microcrystalline precipitate. The synthesis of the molybdenum and tungsten complexes followed again the literature procedures with a rather poor yield for the tungsten compound. Crystals of these complexes could not be obtained possibly due to the fact that these complexes form *cis*- and *trans*-(ligand orientation) and *R*- and *S*- (position of the phenyl group at the pyrane ring) isomers *i.e.* a mixture of diastereomers, which usually results in a poor crystallization behaviour. However, the identity of the new complexes was clearly established by elemental analyses and NMR spectroscopy as well as by IR spectroscopy, showing dominating bands for the PPh_4^+ cation and characteristic though less intense bands for the M=O and M–S valences.

Electrochemistry

The compounds **1a**, **1b**, **2a**, **3a** and **3b** were examined by cyclic voltammetry (CV), the compounds **2b**, **4a** and **4b** by differential pulse voltammetry. The obtained CV spectra of the latter were of inferior quality (for **4a** and **4b** due to the poor solubility of these compounds). Therefore the peak determination was problematic. The differential pulse voltammograms of **4a** and **4b** and for comparison the cyclic voltammogram of **4a** are shown in Figs. 5 and 6. Only the DPV oxidations are shown here but the reductive DPV shows in both cases the same two peaks similar in height.

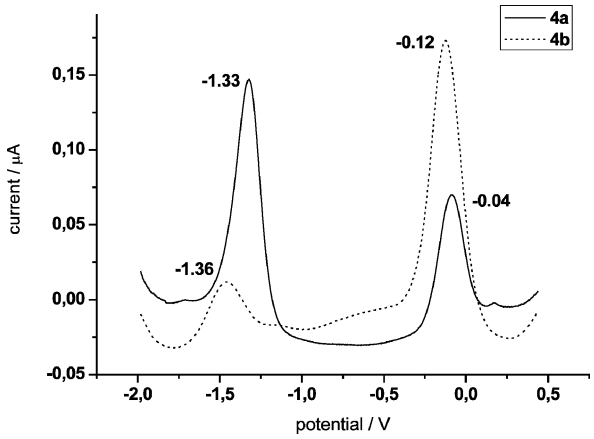

Fig. 5 Differential pulse voltammograms of **4a** (solid) and **4b** (dotted) at 25 °C. Data is referenced against Fc/Fc^+ (= 0 V in this diagram).

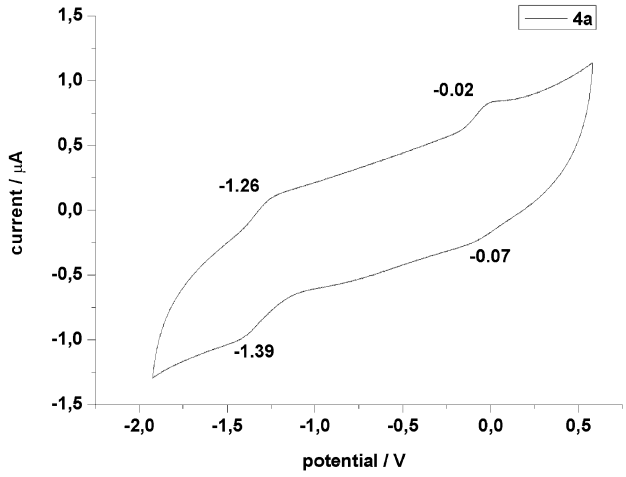

Fig. 6 Cyclic voltammogram of **4a** referenced *vs.* Fc/Fc^+ (= 0 V in this diagram).

Table 1 $E_{1/2}$ ($M^{IV} \leftrightarrow M^{V}$) *vs.* Fc/Fc^{+} and *vs.* SCE of this work, the differences between corresponding molybdenum and tungsten compounds and comparison with literature data

Complex	$E_{1/2}$ *vs.* Fc/Fc^{+}/V[a]	$E_{1/2}$ *vs.* SCE/V[b]	$\Delta E_{1/2}$/V	$E_{1/2}$ *vs.* SCE/V Literature	$\Delta E_{1/2}$/V Literature
$[MoO(mnt)_2]^{2-}$ **1a**	+ 0.09 (*0.005*)	+0.46		+0.48[c]/+0.395[d]	
$[WO(mnt)_2]^{2-}$ **1b**	−0.09 (*0.015*)	+0.28	0.18	+0.2238[e]	0.26/0.17/0.17[f]
$[MoO(bdt)_2]^{2-}$ **2a**	−0.78 (*0.006*)	−0.41		−0.39[c]/−0.35[g]/−0.34[h]	
$[WO(bdt)_2]^{2-}$ **2b**	−1.30 (*0.016*)	−0.93	0.52	−0.63[h]	0.24/0.28/0.29
$[MoO(S_2C_2Me_2)_2]^{2-}$ **3a**	−0.74 (*0.037*)	−0.37		−0.62[i]	
$[WO(S_2C_2Me_2)_2]^{2-}$ **3b**	−1.33 (*0.005*)	−0.96	0.59	−0.91[j]	0.29
$[MoO(fdt)_2]^{2-}$ **4a**	−1.33 (*0.034*)	−0.96			
$[WO(fdt)_2]^{2-}$ **4b**	−1.36 (*0.042*)	−0.99	0.03		

[a] Average over all data at 25 °C for each compound (*standard deviation in parentheses*). [b] Calculated by adding 0.37 V[43] to obtained data *vs.* Fc/Fc^{+}. [c] Ref. 38. [d] Calculated from data *vs.* Ag/AgCl, ref. 39. [e] Calculated from data *vs.* NHE, ref. 40. [f] Value of difference from ref. 35. [g] Ref. 29. [h] Ref. 30; [i] Ref. 31. [j] Ref. 32.

The DPVs of **4a** and **4b** are very similar with the respective peaks of the oxidation process $M^{IV} \rightarrow M^{V}$ as well as for the $M^{V} \rightarrow M^{VI}$ oxidation close together. As expected both redox potentials for the molybdenum compounds are (at 25 °C) more positive than the potentials for the tungsten compound but the difference is extraordinarily small.

The temperature dependent measurements were carried out as follows. Of each compound about 100 data points have been measured at different temperatures from 5 °C to 65 °C. Measurements at temperatures up to 75 °C (about 7 ° below the boiling point of acetonitrile) resulted in a substantial change of the potentials due to the loss of solvent, therefore only a temperature range of 60 °C could be examined with reliable results. This temperature region was measured for every sample in circles by changing the temperature at each step by 10 °C and by 5 °C at the turning points. For instance starting with 25 °C the routine was as follows: 25 °C, 35 °C, 45 °C, 55 °C, 65 °C, 60 °C, 50 °C, 40 °C, 30 °C, 20 °C, 10 °C, 5 °C, 15 °C, 25 °C. The starting points of the routine were also varied for every new sample. This was carried out to achieve neutralization of any phenomenon that might occur due to deterioration or other time dependent change of the set-up. Every sample was finally measured again at 25 °C after ferrocene had been added to the solution for referenciation. Then the ferrocene/ferrocenium couple was measured at 25 °C. (A typical course of the measurements of the $M^{IV} \leftrightarrow M^{V}$ redox potentials with time and with temperature can be found in the ESI.†) The compound **3a** was deteriorating during the measurements possibly due to an electrode mediated disproportionation which could be observed by the fading of the CV signal. Of this compound no full circle could be measured. Of each sample of **3a** only three to eight CVs could be obtained. Therefore of this compound only 80 instead of about 100 data-points were collected and the quality of the data is substantially lower than that for the other compounds.

The compounds average redox potentials at 25 °C determined in this work as well as a comparison with literature data is shown in table 1. Partly there are some discrepancies between literature and the new data (differences up to 300 mV for $[WO(bdt)_2]^{2-}$ **2b**). This may occur due to the fact that there is no truly reliable reference electrode for non-aqueous solutions.[41] In accordance with an IUPAC recommendation[42] potentials should be referenced against the ferrocene/ferrocenium couple for better comparability of different investigations, which was performed here and the data obtained by this method are listed in the first column of table 1. To be able to compare the data of this work and the literature data, the potentials were recalculated against SCE (standard calomel electrode) assuming that the potential of Fc/Fc^{+} against SCE in acetonitrile solution is 0.37 V.[43] The literature data that was referenced *vs.* NHE or *vs.* Ag/AgCl was recalculated to obtain the potentials *vs.* SCE by subtracting 0.2412 V or 0.045 V, respectively, as these are the differences to the potential of the SCE.[44] The difference between the literature data and the new data for **2b** may also be based on the fact that different solvents were used for the measurements (acetonitrile *vs.* dimethylformamide); other details of the experiment are not mentioned and further comparison of the experimental set-up not possible.

As expected for all ligands at 25 °C the redox potentials for the molybdenum compounds are more positive than those for the tungsten compounds, but the differences vary. Garner *et. al.* stated that the difference of redox potentials between corresponding molybdenum and tungsten dithiolene complexes is usually in the range of 225 mV, but the compounds they are referring to are not very diverse with respect to size and mesomeric potential.[37,45,46] On the other hand it was said by Holm *et. al.* that the difference of the redox potentials for the molybdenum and the tungsten complexes depend upon the strength of the non-innocent character of the respective ligands. That means: the more pronounced the non-innocence the smaller the difference of the redox potentials.[35,36] Within the pairs of molybdenum and tungsten compounds examined here the mnt^{2-} ligand (complexes **1a** and **1b**) with the two strongly electron withdrawing CN^{-} groups, that facilitate the breaking of the dithiolene C–C–π-bond, was expected to be the most non-innocent ligand of all. But the pair with the smallest difference between the redox potentials for the molybdenum and the tungsten complex, extraordinary 30 mV, is that with the fdt^{2-} ligand (complexes **4a** and **4b**) which is not only the most voluminous ligand with the highest mesomeric potential but also the ligand system that resembles the natural molybdopterin ligand closest by incorporating the pyrane subunit. Therefore it is likely that this feature plays an important role within the natural molybdopterin with respect to the tuning of the redox properties of the enzymes' active sites.

The differences of redox potentials for **2a**/**2b** and **3a**/**3b** are similar with a slightly smaller difference for the former (the bdt^{2-}-pair) as was expected. The differences for **2a**/**2b** and **3a**/**3b** observed in this study are substantially larger than those reported in the literature. This may be based on the fact that the reported potentials were not measured consecutively under the exact same conditions as was performed here and also on the above mentioned problem with the reference electrodes.

The results of all temperature dependent measurements of the eight compounds are shown in Fig. 7. The redox potentials $E_{1/2}$ were plotted against the temperature, linear fits employed and these fits extrapolated as far as necessary.

The gradients of the eight graphs and the virtual cross-points of the particular two graphs of each pair are assembled in Table 2. Based on eqn. (2)[47] (with $\delta E/\delta T$ = gradient; ΔS = entropy

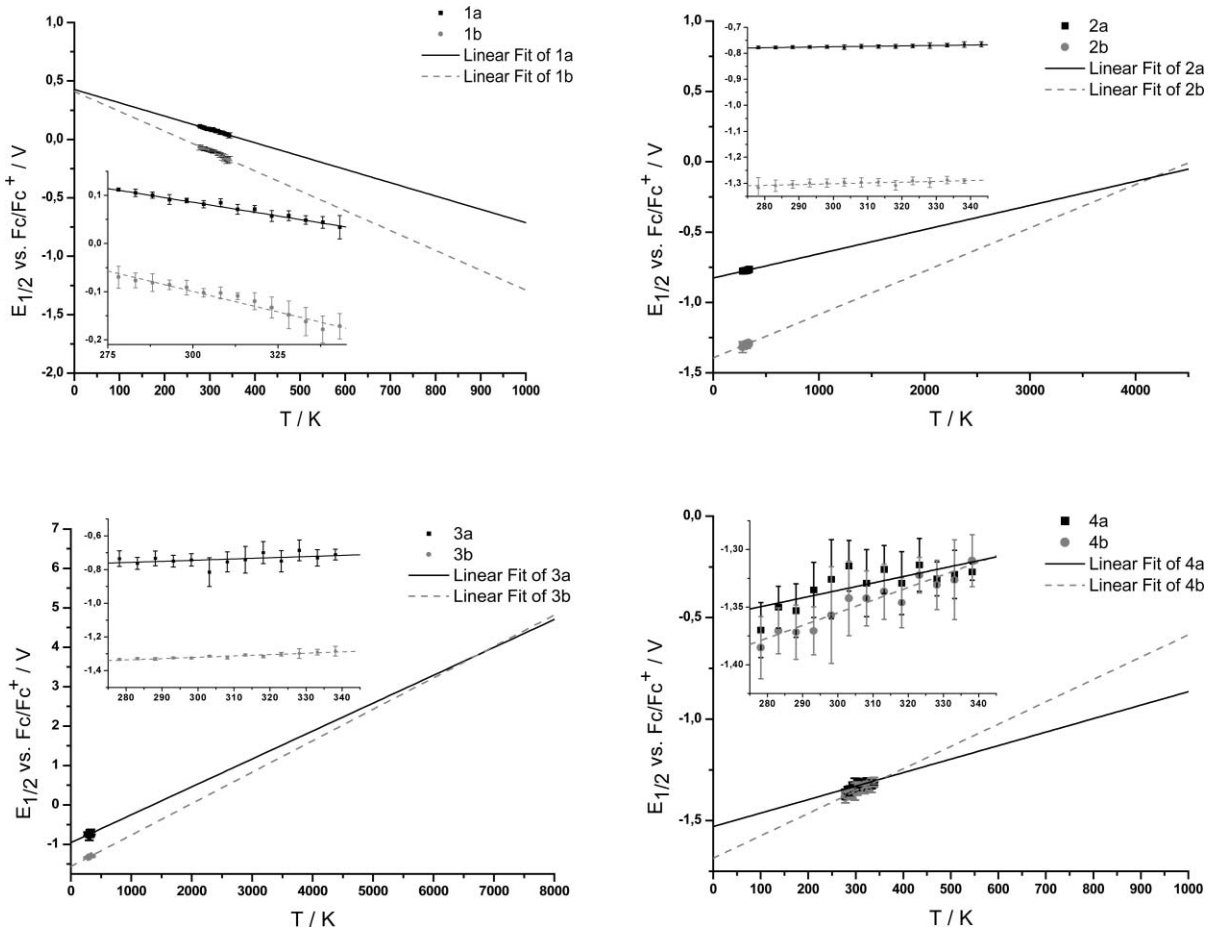

Fig. 7 Plots of the redox potentials $E_{1/2}$ ($M^{IV} \leftrightarrow MV$; *vs.* Fc/Fc^{+}) against temperature and their linear fits. Note that the scales of the diagrams vary.

change for the reduction process; z = number of transferred electrons = 1; F = Faraday constant = 9.64853×10^{-4} C mol^{-1}) the ΔS values for the reduction process were calculated for each compound and inserted into Table 2 as well.

$$\delta E/\delta T = \Delta S/zF \quad (2)$$

The most striking characteristic is the behaviour of compounds **1a** and **1b** that show a reverse response to rising temperature compared with the six other compounds. Obviously for **1a** and **1b** the gradient $\delta E/\delta T$ and thus the entropy change for the reduction is negative. Therefore the term $-T\Delta S$ is positive as well as its contribution to ΔG. This means that from the entropic point of view the oxidative form M^{V} is thermodynamically favoured over the reduced form M^{IV}. Thus with rising temperature, as the influence of ΔS increases, the redox potentials of **1a** and **1b** decrease. Oxidation becomes easier while the gain of Gibbs free energy for the reduction process declines. For the other compounds (**2a**/**2b**, **3a**/**3b** and **4a**/**4b**) ΔS for the reduction is positive and therefore its contribution to ΔG negative. Thus the entropic part of the Gibbs equation favours the reduction over the oxidation.

Comparison of the average redox potentials at 25 °C leads to the following conclusion. The redox potentials of **1a** and **1b** are the most positive ones within this series and hence the reduction process is easier than for all the other compounds. While ΔS for the reduction is negative (favouring the oxidation), obviously ΔH has to be substantially more negative (favouring the reduction) than for the other compounds, and hence the

Table 2 Gradients, cross-points of the linear fits for the temperature dependence of the redox potentials $E_{1/2}$ ($M^{IV} \leftrightarrow M^{V}$) and from the gradients calculated values of the entropy change ΔS for the respective reduction processes

Complex	Gradient/mV K^{-1} [a]	Cross-point temperature/K [b]	ΔS/J mol^{-1} K^{-1} [c]
$[MoO(mnt)_2]^{2-}$ **1a**	−1.14 (*0.043*)		−110.0 (*4.15*)
$[WO(mnt)_2]^{2-}$ **1b**	−1.70 (*0.102*)	Non existent (−31(*30*))	−164.0 (*9.84*)
$[MoO(bdt)_2]^{2-}$ **2a**	0.17 (*0.033*)		16.4 (*3.18*)
$[WO(bdt)_2]^{2-}$ **2b**	0.31 (*0.094*)	4179 (*1000*)	29.9 (*9.07*)
$[MoO(S_2C_2Me_2)_2]^{2-}$ **3a**	0.71 (*0.344*)		68.5 (*33.19*)
$[WO(S_2C_2Me_2)_2]^{2-}$ **3b**	0.80 (*0.072*)	6722 (*1500*)	77.2 (*6.95*)
$[MoO(fdt)_2]^{2-}$ **4a**	0.65 (*0.131*)		62.7 (*12.64*)
$[WO(fdt)_2]^{2-}$ **4b**	1.10 (*0.149*)	343 (*26*)	106.1 (*14.38*)

[a] Standard deviation in parentheses as obtained from the linear fit. [b] Errors in parentheses estimated by the calculation of extreme values. [c] Errors in parentheses calculated from the standard deviation of the gradients.

resulting ΔG is more negative as well. The difference of the ΔH values for instance between **1a** and **3a** (the molybdenum compound with a redox potential most closely to that of **1a**) is about 133 kJ mol^{-1} as can be calculated from the Gibbs equation with the knowledge of the ΔS values and the relation between ΔG and the redox potential. The reason for this particular behaviour has to be the electron withdrawing effect of the CN groups at the dithiolene, which reduces electron density at the dithiolene group and eventually at the metal centre, thus facilitating reduction. In contrast to the other complexes for compounds **1a** and **1b** the reduced is the more stable form (redox potentials referenced *vs.* NHE are positive) and an unrestricted distribution of electrons in a system solely driven by the enthalpy would lead to a complete reversion of the oxidized to the reduced form. This would mean a minimum of disorder to which ΔS for the reduction process is likely to be opposed and therefore negative, as can be observed here. For the other compounds the non-innocence is manifested in an electron distribution to the metal (see Fig. 3) and therefore in a facilitated oxidation. This matches a redox potential referenced *vs.* NHE that is negative. Such a system solely driven by the enthalpy would lead to a complete reversion of the reduced to the oxidized form, resulting in a minimum of disorder to which the entropy is opposed. Therefore ΔS for the reduction process, which increases the disorder, is positive.

The virtual cross-points of the graphs for **2a** and **2b** and **3a** and **3b** are in temperature regions that have no significance for any form of life; that for **1a** and **1b** (at calculated −31 K) does not even exist. But the cross-point for the graphs of **4a** and **4b**, the models that resemble the natural enzymes active sites closest, is at 343 K (70 °C) and hence in a region in which usually tungsten is used within the active sites of the molybdopterin containing enzymes. For these model compounds the usual characteristic that the redox potential for the molybdenum compound is more positive than that for the tungsten compound is reversed at temperatures above 70 °C. This could be an indication for a redox property related change of metal within molybdopterin containing enzymes. It should be noted though that the redox potentials for **4a** and **4b** are only separated by a minimum of 30 mV even at 25 °C, and thus the redox potential may not be the main reason for the incorporation of either of the two metals.

In all cases the gradient of the tungsten compound is of greater value than that for the corresponding molybdenum compound, meaning that the tungsten compounds' response to temperature change is always more drastic than that of the molybdenum compound. The stronger dependence on the temperature of the redox potentials of the tungsten compounds seems to be a disadvantage with respect to the function of the enzymes unless of course the response of the substrates redox potentials is about equal to that of the respective tungsten enzyme.

Conclusion

Only the compounds $[MoO(mnt)_2]^{2-}$ **1a** and $[WO(mnt)_2]^{2-}$ **1b** show gradients $\delta E/\delta T$ that are negative. For these compounds the redox potentials and the gain of Gibbs free energy decreases with rising temperature. They are also the only complexes within this study that show positive redox potentials *vs.* NHE. Therefore ΔS as well as ΔH for the reduction process are negative. This in comparison with the other compounds reverse behaviour is based on the fact that the mnt^{2-}-ligand contains two strongly electron withdrawing CN groups which from the enthalpic point of view support the reduced forms of the complexes. This indicates that **1a** and **1b** are not as good for modelling the molybdopterin containing enzymes, in which no such groups occur, than the other dithiolene complexes.

The redox potentials of **4a** and **4b** (the compounds that include the pyrane subunit of the molybdopterin ligand) are exceptionally close together with a difference at 25 °C of only 30 mV. The cross-point of their linear fits is at 343 K (70 °C) and therefore in a temperature region in which organisms usually utilize tungsten instead of molybdenum. This may hint at a change of metal within molybdopterin containing enzymes caused by a reversal of the usual order ($E_{1/2}$(Mo) > $E_{1/2}$(W)) for the redox potentials of the metal sites at a certain temperature. But because even at 25 °C the redox potentials of **4a** and **4b** are exceptionally close together it can not yet be concluded that this reversal would be the main reason for the metal exchange within theses enzymes.

The cross-points of the other three pairs are in temperature regions that are of no importance for any kind of life or even do not exist (<0 K for **1a** and **1b**) indicating that the pyrane subunit of the natural molybdopterin ligand plays an important role with respect to the tuning of the enzymes redox potentials. Whether this is generally valid will be explored further by the synthesis and electrochemical examination of more pyranedithiolene ligands and their respective molybdenum and tungsten complexes.

The gradients of the linear fits of the tungsten compounds redox potentials are in every case of greater value (positive or negative) than those of the molybdenum compounds. Their redox potentials are more susceptible to temperature change than those of the molybdenum complexes. Should this also be the case within the molybdopterin enzymes, this characteristic would probably be unfavourable unless the substrates potentials behave in similar ways. This will be the subject of further investigations.

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