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Mimicking the oxidized glutathione-$V^{IV}O^{2+}$ species †

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Sequential addition of bipy, or phen followed by H_3mpg (a sulfhydryl-containing pseudopeptide) and $AcONa\cdot 2H_2O$ to $V^{IV}OSO_4\cdot 5H_2O$ in the presence of oxygen yields $[(V^{IV}O)_2(\mu\text{-}OH)(\mu\text{-}OAc)(\mu\text{-}H_2mpgS\text{–}S)_2L_2]\cdot x MeOH\cdot y H_2O$ (L = bipy, $1\cdot 1.68MeOH\cdot 0.75H_2O$ or phen, $2\cdot 1.40MeOH\cdot 0.81H_2O$), containing the oxidized-*S*,*S* dimer of H_3mpg $[H_2mpgS\text{–}S^{2-}]$; $[(V^{IV}O)_2(\mu\text{-}OH)(\mu\text{-}OAc)(OAc)_2(bipy)_2]$ 3 was also prepared in a similar way to $1\cdot 1.68MeOH\cdot 0.75H_2O$ using reduced glutathione instead of H_3mpg.

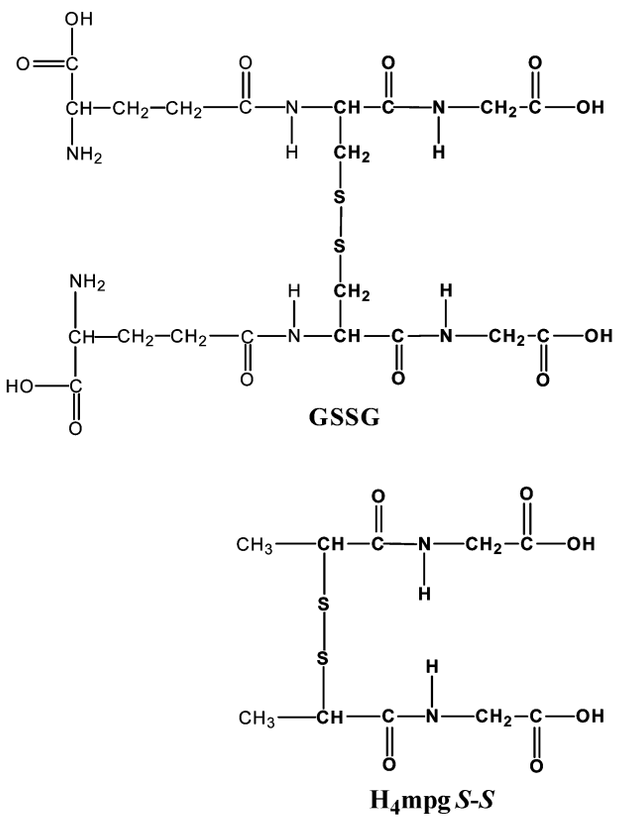
Glutathione is a major intracellular reducing agent present in almost all biological tissues (with typical intracellular concentrations of 1–2 mmol dm^{-3}),[1] which plays important roles in biosynthesis, metabolism, transport and the protection against adventitious free radicals.[2] Remarkably, *in vitro* studies have shown that depression of intracellular glutathione levels decreases cell survival,[3] alters T-cell functions[4] and increases HIV replication,[5] while clinical studies directly link glutathione deficiency to impaired survival in HIV infections.[6] Glutathione, in its reduced (GSH) or oxidized form (GSSG) (Scheme 1), also plays a crucial role in the biochemistry of a series of metal ions.[7,8] For example, inside the red blood cells GSH reduces vanadium(v) to $V^{IV}O^{2+}$ with the simultaneous formation of GSSG and in addition, GSH or GSSG might act as ligands for the generated oxovanadium(IV) cation.[8]

The characterization of metal-GSH/-GSSG species is quite difficult because of their instability. As a consequence, to date, only one crystal structure of a metal-GSSG species has been reported[9] and no metal-GSH complex has been crystallographically characterized. Alternatively, model compounds of metal ions with sulfhydryl-containing peptides or pseudopeptides might provide valuable information concerning the possible coordination modes of GSH/GSSG to a metal ion.[10] Similarity between the constitution of the disulfide containing pseudopeptide H_4mpg*S*–*S*, which is the oxidized-*S*,*S* dimer form of *N*-(2-mercaptopropionyl)glycine (H_3mpg), and the right hand portion of GSSG is clearly evident from Scheme 1. Herein, we present the isolation, structural and physical studies of the two dimeric $V^{IV}O^{2+}$ compounds $1\cdot 1.68MeOH\cdot 0.75H_2O$ and $2\cdot 1.40MeOH\cdot 0.81H_2O$ containing the oxidized-*S*,*S* dimer of H_3mpg. In addition, the synthesis, crystal structure and physicochemical characterization of the dimeric species **3** are also reported. ‡

Compounds $1\cdot 1.68MeOH\cdot 0.75H_2O$ and $2\cdot 1.40MeOH\cdot 0.81H_2O$ were prepared in 71 and 66% yields respectively by treating $V^{IV}OSO_4\cdot 5H_2O$ (0.8 mmol), in methyl alcohol (7 ml), with bipy (0.8 mmol) or phen (0.8 mmol), H_3mpg (0.8 mmol) and $AcONa\cdot 2H_2O$ (1.6 mmol) [eqn. (1)].

Compound **3** was prepared by an identical procedure in 40% yield except that GSH was used instead of H_3mpg. Compound **3** was isolated in our effort to isolate a $V^{IV}O^{2+}$/GSSG species.

† Abbreviations used: bipy, 2,2′-bipyridine; phen, 1,10-phenanthroline; H_3mpg, *N*-(2-mercaptopropionyl)glycine; H_2mpg*S*–S^{2-}, the oxidized-*S*,*S* dimer of H_3mpg (see Scheme 1).



Scheme 1 Drawing of oxidized glutathione (GSSG) and *N*-2-mercapto-propionyl-glycine disulfide H_4mpg*S*–*S*. Note the close similarity between the Gly–Cys–S–S–Cys–Gly part (highlighted by using bold characters) of GSSG and H_4mpg*S*–*S*.

In all preparations, the reaction mixtures were stirred for ~1 h. Then, they were filtered (in order to remove Na_2SO_4) and the filtrate was allowed to stand for ~3–4 days after which $1\cdot 1.68MeOH\cdot 0.75H_2O$, $2\cdot 1.40MeOH\cdot 0.81H_2O$ and **3** precipitated as crystalline materials. The UV-Vis solid state reflectance spectrum of $1\cdot 1.68MeOH\cdot 0.75H_2O$ contained three peaks in the visible region, at 800, 556 and 413 nm as well as two peaks in the ultraviolet region, at 308 and 254 nm. The ultraviolet spectrum of $1\cdot 1.68MeOH\cdot 0.75H_2O$ in methyl alcohol§ exhibited a shoulder at 313(sh) nm ($\varepsilon = 2400\ M^{-1}\ cm^{-1}$) as well as two peaks at 283 nm ($\varepsilon = 6900\ M^{-1}\ cm^{-1}$) and 234 nm ($\varepsilon = 7900\ M^{-1}\ cm^{-1}$). The infrared spectrum of $1\cdot 1.68MeOH\cdot 0.75H_2O$ contained characteristic bands at 3356 cm^{-1} [ν(N–H)], 1664 cm^{-1} [ν(CO)], 1557 cm^{-1} [$\nu_{as}(CO_2^-)$], 1386 cm^{-1} [$\nu_s(CO_2^-)$], 964 cm^{-1} [ν(V=O)] and 610 cm^{-1} [ν(S–S)]. The room temperature magnetic moment of compound $1\cdot 1.68MeOH\cdot 0.75H_2O$ was measured to be 2.40 μ_B. Variable temperature magnetic measurements for $1\cdot 1.68MeOH\cdot 0.75H_2O$ are underway. IR, UV-Vis and magnetic data for $2\cdot 1.40MeOH\cdot 0.81H_2O$ and **3** are included in the Notes and references.¶

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$$2V^{IV}OSO_4\cdot 5H_2O + 2L + 2H_3mpg + 4CH_3CO_2Na\cdot 2H_2O + 1/2O_2 \xrightarrow{MeOH}$$
$$[(V^{IV}O)_2(\mu\text{-}OH)(\mu\text{-}CH_3CO_2)(\mu\text{-}H_2mpgS\text{-}S)_2(L)_2] + 2Na_2SO_4 + 3CH_3CO_2H + 18H_2O \quad (1)$$
1 or **2** {L = bipy, phen}

An X-ray structural determination of **1**·1.68MeOH·0.75H_2O‖ revealed that **1**·1.68MeOH·0.75H_2O is a non-centrosymmetric dimer (Fig. 1A) containing two independent vanadium atoms (**2**·1.40MeOH·0.81H_2O has a similar gross structure, without significant differences in the coordination spheres and in the disulfide ligand $H_2mpgS\text{–}S^{2-}$, Fig. 1B). Both vanadium(IV) centers reside in a distorted octahedral environment defined by two carboxylate oxygen atoms, two bipy nitrogens and a μ-OH^-, in addition to the O^{2-} group. The V(1) and V(2) are displaced above the mean equatorial NO_3 plane, $N_{bipy}(O_{carboxylate})_2(\mu\text{-}OH)$, by 0.32 and 0.29 Å respectively, towards the oxo ligand. The V(1)–V(2) separation is 3.60 Å and the V(1)–O(3)–V(2) angle is 134.9(2)°.

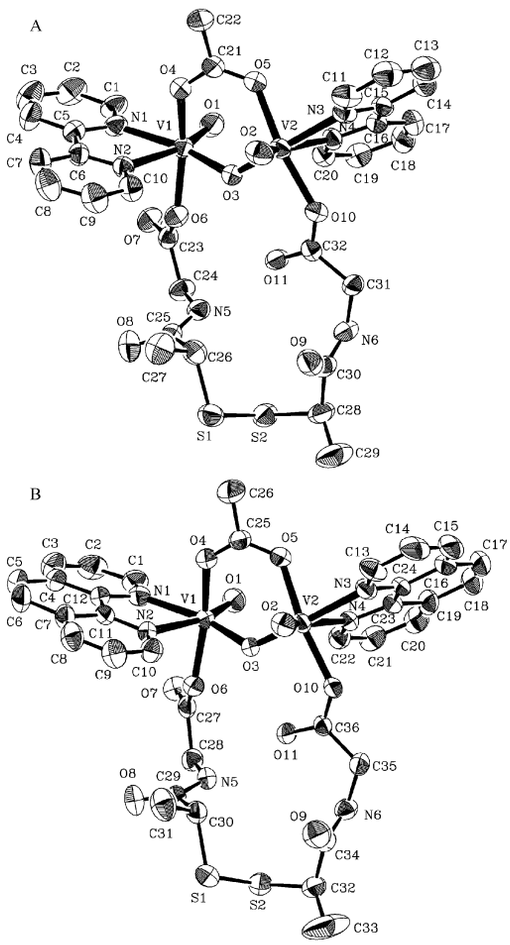

Fig. 1 ORTEP[14] plots of **1**·1.68MeOH·0.75H_2O (A) and **2**·1.40MeOH·0.81H_2O (B). Displacement ellipsoids are plotted at the 30% probability level. Selected interatomic distances (Å) for **1**·1.68MeOH·0.75H_2O, **2**·1.40MeOH·0.81H_2O (square brackets): V(1)–O(1) 1.593(4) [1.597(5)], V(1)–O(3) 1.966(4) [1.958(4)], V(1)–O(6) 1.994(4) [1.996(5)], V(1)–O(4) 2.021(5) [2.023(5)], V(1)–N(1) 2.141(5) [2.153(6)], V(1)–N(2) 2.278(5) [2.311(6)], V(2)–O(2) 1.584(4) [1.599(5)], V(2)–O(3) 1.936(4) [1.939(4)], V(2)–O(10) 2.029(4) [2.046(4)], V(2)–O(5) 2.029(4) [2.018(5)], V(2)–N(3) 2.160(5) [2.151(6)], V(2)–N(4) 2.306(5) [2.327(5)].

The configuration of the $[OV^{IV}(\mu\text{-}OH)(\mu\text{-}OAc)V^{IV}O]^{2+}$ core can be defined as anti-orthogonal[11] by taking into account the orientation of the two oxo groups which are orthogonal to the plane defined by V(1), V(2), O(3), O(4) and O(5) and *trans* to each other (Fig. 2). The notable structural feature of **1**·1.68MeOH·0.75H_2O is the presence of the ligated disulfide

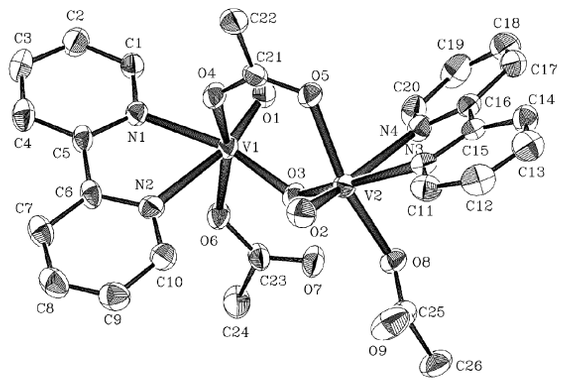

Fig. 2 ORTEP plot of **3**. Displacement ellipsoids are plotted at the 30% probability level. Selected interatomic distances (Å) for **3**: V(1)–O(1) 1.593(4), V(1)–O(3) 1.944(4), V(1)–O(6) 2.027(3), V(1)–O(4) 2.041(4), V(1)–N(1) 2.160(4), V(1)–N(2) 2.276(5), V(2)–O(2) 1.577(4), V(2)–O(3) 1.964(4), V(2)–O(8) 1.991(4), V(2)–O(5) 2.050(4), V(2)–N(3) 2.170(5), V(2)–N(4) 2.324(5).

$H_2mpgS\text{–}S^{2-}$, which forms a fifteen(!)-membered ring with the V(1)–O(3)–V(2) unit and is ligated to each vanadium atom through one carboxylate oxygen atom. The dihedral angle around the disulfide bond, C(26)–S(1)–S(2)–C(28), is 92.8(6)°, which is close to the ideal value of 90° expected for the disulfides.[12]

Concerning the two peptide functionalities of the disulfide ligand, they are planar within the limits of precision. Moreover, the presence of two moderate intermolecular hydrogen bonds between two neighboring dimers results in the formation of tetramers [H(N6) ··· O(7′) (2 − *x*, 1 − *y*, −*z*) = 2.030 Å, N(6) ··· O(7′) = 2.881 Å, N(6)–H ··· O(7′) = 170.4°]. **1**·1.68MeOH·0.75H_2O and **2**·1.40MeOH·0.81H_2O represent the first examples of metal complexes containing the disulfide ligand $H_4mpgS\text{–}S$. The doubly bridging O(3) oxygen atom shows a bond valence sum (BVS) value of 1.21,[13] thus indicating monoprotonation for this oxygen atom. Furthermore, estimation of the oxidation state of the two vanadium centers by using BVS calculations[13] gives values of 4.14 and 4.13 for V(1) and V(2) respectively, which are in agreement with those based on stoichiometry and the crystal structure. The overall structure of **3** is similar to that of **1**·1.68MeOH·0.75H_2O, without significant differences in the coordination spheres, except that **3** contains two unidentate acetate ligands instead of the bridged(μ-) disulfide ligand $H_2mpgS\text{–}S^{2-}$ present in **1**·1.68MeOH·0.75H_2O (Fig. 2).

In conclusion, two dimeric oxovanadium(IV) compounds **1**·1.68MeOH·0.75H_2O and **2**·1.40MeOH·0.81H_2O were isolated by treating $V^{IV}O^{2+}$ species with bipy or phen, sodium acetate and H_3mpg. The disulfide ligand $H_2mpgS\text{–}S^{2-}$, which is present in these compounds, models the Gly–Cys–S–S–Cys–Gly part of the oxidized glutathione. Its complexation to the two vanadium(IV) atoms in **1**·1.68MeOH·0.75H_2O and **2**·1.40MeOH·0.81H_2O through unidentate carboxylate groups is possibly indicative of a strong tendency of GSSG to coordinate to $V^{IV}O^{2+}$ through unidentate carboxylate group of its glycyl residues. The isolation and characterization of the $H_4mpgS\text{–}S$ ligand as well as the $V^{IV}O^{2+}/H_4mpgS\text{–}S$ interaction in solution will be the subject of future studies. Furthermore, we have not been able to isolate any $V^{IV}O^{2+}$/GSSG species using an identical procedure to that used for isolation of **1**·1.68MeOH·0.75H_2O and **2**·1.40MeOH·0.81H_2O and this might be an indication that only unstable intermediate $V^{IV}O^{2+}$/

GSSG species can be formed *in vivo*. However, we are currently trying to synthesize stable $V^{IV}O^{2+}$/GSSG compounds using various bulky organic groups as supporting ligands.

Notes and references

‡ Elemental analysis (%) calc. for **1**·1.68MeOH·0.75H_2O ($C_{33.68}H_{42.22}N_6O_{13.43}S_2V_2$): C, 44.35; H, 4.67; N, 9.22; S, 7.03; V, 11.17. Found: C, 44.56; H, 4.55; N, 9.43; S, 7.20; V, 11.34. Elemental analysis (%) calc. for **2**·1.40MeOH·0.81H_2O ($C_{37.4}H_{42.22}N_6O_{13.21}S_2V_2$): C, 47.13; H, 4.46; N, 8.82; S, 6.73; V, 10.69. Found: C, 47.25; H, 4.05; N, 9.05; S, 6.85; V, 10.80. Elemental analysis (%) calc. for **3** ($C_{26}H_{26}N_4O_9V_2$): C, 48.73; H, 4.09; N, 8.75; V, 15.91. Found: C, 48.25; H, 4.29; N, 8.90; V, 15.80.

§ Because of the low solubility of **1**·1.68MeOH·0.75H_2O and **2**·1.40MeOH·0.81H_2O in any common solvent, we were not able to obtain a satisfactory visible spectra for these compounds in solution.

¶ Selected IR data for **2**·1.40MeOH·0.81H_2O: 3352 [ν(N–H)], 1664 [ν(CO)], 1557 [$\nu_{as}(CO_2^-)$], 1386 [$\nu_s(CO_2^-)$], 964 [ν(V=O)], 619 cm^{-1} [ν(S–S)]. Electronic spectrum in MeOH: λ_{max}/nm (ε_M/M^{-1} cm^{-1}); 292sh (6571), 273 (26751), 227.5 (30000), 202 (20952). UV-Vis solid state reflectance data: λ_{max}/nm; 803, 462, 272, 228. μ_{eff} (25 °C) = 2.30 μ_B.

Selected IR data for **3**: 1607 [ν(C=C, C=N)], 1637, 1578 [$\nu_{as}(CO_2^-)$], 1410, 1315 [$\nu_s(CO_2^-)$], 966, 958 cm^{-1} [ν(V=O)]. Electronic spectrum in MeOH: λ_{max}/nm (ε_M/M^{-1} cm^{-1}); 777 (53), 329sh (10000) 306 (40640), 235 (30430). μ_{eff} (25 °C) = 2.35 μ_B.

‖ Crystal data for **1**·1.68MeOH·0.75H_2O: $C_{33.68}H_{42.22}N_6O_{13.43}S_2V_2$, $P\bar{1}$, triclinic, a = 13.974(4), b = 12.581(3), c = 15.157(4) Å, α = 110.694(8), β = 92.144(9), γ = 116.050(8)°, V = 2180(1) Å^3, Z = 2, D_c = 1.389 Mg m^{-3}, M = 911.99, T = 298 K, $R1/wR2$ [for 5062 reflections with $I > 2\sigma(I)$] = 0.0804/0.2352. CCDC reference number 192413.

Crystal data for **2**·1.40MeOH·0.81H_2O: $C_{37.4}H_{42.22}N_6O_{13.21}S_2V_2$, $P\bar{1}$, triclinic, a = 14.145(6), b = 12.324(5), c = 15.266(6) Å, α = 71.03(1), β = 75.81(1), γ = 67.37(1)°, V = 2301(2) Å^3, Z = 2, D_c = 1.376 Mg m^{-3}, M = 953.15, T = 298 K, $R1/wR2$ [for 4050 reflections with $I > 2\sigma(I)$] = 0.0709/0.1841. CCDC reference number 192414.

Crystal data for **3**: $C_{26}H_{26}N_4O_9V_2$, $Pna2_1$, orthorhombic, a = 20.38(1), b = 8.235(4), c = 16.64(1) Å, V = 2794(1) Å^3, Z = 4, D_c = 1.523 Mg m^{-3}, M = 640.39, T = 298 K, $R1/wR2$ [for 3982 reflections with $I > 2\sigma(I)$] = 0.0446/0.1000, absolute structure parameter 0.28(3). CCDC reference number 200153. See http://www.rsc.org/suppdata/dt/b2/b212419j/ for crystallographic data in CIF or other electronic format.

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