N,*N*-dimethylhydroxamidovanadium(V). Interactions with sulfhydryl-containing ligands: V(V) equilibria and the structure of a V(IV) dithiothreitolato complex

Sudeep Bhattacharyya, Anette Martinsson, Raymond J. Batchelor, F.W.B. Einstein, and Alan S. Tracey

Abstract: The major aqueous equilibrium complexation reactions of vanadate in the presence of *N*,*N*-dimethylhydroxylamine (DMHA) and with dithiothreitol (DTT), β-mercaptoethanol, glycine, or cysteine in solution have been studied using ^{51}V NMR spectroscopy. Previously unreported DMHA complexes of 2:1 and 2:3 V:DMHA stoichiometry were observed and characterized. Concentration studies showed that, for the three sulphur-containing ligands, the major product of sulphur coordination has a 1:2:1 stoichiometry of vanadate to dimethylhydroxylamine to heteroligand. These products do not carry a charge in neutral to moderately basic solution. A second product type of 1:1:1, V to DMHA to heteroligand, stoichiometry is also formed. These products carry a single negative charge. A reductive reaction between vanadate and excess DTT to form a V(IV) complex was also observed and a solid product was isolated. This product could also be obtained by direct reaction of vanadyl sulphate with DTT. It was characterized by X-ray diffraction studies. Crystal structure of $[\{VO(SCH_2CHOHCHOCH_2S)\}_2]$ $[AsPh_4]_2$: monoclinic, space group $P2_1/n$, $Z = 2$, $a = 10.1607(18)$ Å, $b = 17.8255(42)$ Å, $c = 15.1520(33)$ Å, $\beta = 104.\ 000(15)°$, $V = 2662.8$ Å^3, $R_F = 0.038$ for 2327 data ($I_o \geq 2.5\sigma(I_o)$) and 325 variables.

Key words: vanadate, vanadyl, dithiothreitol, mercaptoethanol, cysteine, glycine, equilibrium constants, crystal structure, X-ray, vanadium NMR.

Résumé : Faisant appel à la RMN du ^{51}V, on a étudié les principales réactions de complexation en équilibre aqueux du vanadate, en présence de *N*,*N*-diméthylhydroxylamine (DHMA), avec le dithiothréitiol (DTT), le β-mercaptoéthanol, la glycine et la cystéine. On a observé et caractérisé des complexes du DMHA de stoechiométries V:DMHA 2:1 et 2:3. Des études de concentrations montrent que, pour les trois ligands contenant du soufre, le produit principal de la coordination du soufre comporte une stoechiométrie 1:2:1 du vanadate par rapport à la diméthylhydroxylamine et à l'hétéroligand. En solution neutre ou faiblement basique, les produits ne portent pas de charge. Il se forme aussi un deuxième type de produit de stoechiométrie 1:1:1 du V au DMHA à l'hétéroligand. Ces produits portent une charge négative simple. On a observé une réaction réductrice entre le vanadate et l'excès de DTT; elle conduit à la formation du'un complexe V(IV) et on a isolé un produit solide. On peut aussi obtenir ce produit par réaction directe du sulfate de vanadyle avec le DTT. On l'a caractérisé par des études de diffraction des rayons X. Les cristaux de $[\{VO(SCH_2CHOHCHOCH_2S)\}_2][AsPh_4]_2$ sont monocliniques, groupe d'espace $P2_1/n$, $Z = 2$, $a = 10{,}1607(18)$ Å, $b = 17{,}8255(42)$ Å et $c = 15{,}1520(33)$ Å, $\beta = 104\ 000(15)°$, $V = 2662{,}8$ Å^3, $R_F = 0{,}038$ pour 2327 données ($I_0 \geq 2{,}5\sigma(I_o)$) et 325 variables.

Mots clés : vanadate, vanadyle, dithiothréiotol, mercaptoéthanol, cystéine, glycine, constantes d'équilibre, structure cristalline, rayons X, RMN du vanadium.

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Dedicated to Professor Brian James on the occasion of his 65th birthday.

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Introduction

It is frequently asserted that vanadium(V) oxides (vanadate) are rapidly reduced by thiolates but this is true only for specific conditions. Indeed, although stable V(IV) thiolates are frequently reported, it is not uncommon to have mixed vanadium (IV/V) states and even vanadium(V) thiolates can be quite stable in solution (1, 2). As a part of our ongoing studies on the reactivities of V(V) and thiolates, we have previously carried out a detailed study of the aqueous equilibria established between vanadate and dithiothreitol (DTT) and have proposed structures for the major product formed in that system (2). A crystal structure of the complex of β-mercaptoethanol with vanadate has also been reported and this structure correlated with aspects of the associated aqueous chemistry (1).

Our interest in these thiolate complexes has evolved from our studies of the insulin-mimetic effect of vanadium compounds and of protein tyrosine phosphatases (PTPases) that may be relevant to that effect. The PTPases are group of signal transduction enzymes that regulate a number of key cellular responses that are initiated by certain tyrosine phosphorylated proteins (3, 4). The event of dephosphorylation of the phosphotyrosine group of the substrate proteins by the PTPase regulates the cellular response. The dephosphorylation takes place via a concerted and cooperative mechanism effected by the catalytic active site residues of the enzyme. The chemistry of the process is complex, and involves the critical active site residues, aspartate and cysteine via a thiophosphate intermediate. Vanadate, a transition-metal analog of phosphate, is an excellent inhibitor of this hydrolytic process. Recently, it has been shown that *N*,*N*-dimethylhydroxylamine (DMHA) derivatives of vanadate (here referred to collectively as DMHAV) have a similar effect. Specifically, bis(*N*,*N*-dimethylhydroxamido)hydroxooxovanadate ($(dmha)_2V(O)OH$) is a very good inhibitor of PTPase activity (5). Furthermore, this compound is functional as an insulin-mimetic in some cell cultures where vanadate is inactive (6).

An understanding of the chemistry involved in the inhibition of PTPase function by the small molecules of DMHAV is important since it provides a basis for the design of other metallorganic inhibitors. To an extent, the identity of the actual inhibiting species is still speculative. The details of the specific interactions of DMHAV with the surrounding active site residues are not yet known although the interactions very likely involve direct reaction at the sulphur of the active site cysteine (7). The influence of externally added thiolate redox buffers like dithiothreitol (DTT) and β-mercaptoethanol is also not fully understood. Both of these buffers are known to form complexes with oxovanadium species (1, 8) and these, and various other sulphur-containing ligands are highly reactive with DMHAV (9). An understanding of the chemistry promoted by sulfur-containing reagents is critically important for the development of our understanding of the chemistry of the inhibition itself. Some time ago we reported a preliminary study of the reactions of DMHA complexes of vanadate with DTT (9). We are now able to provide a much more detailed description of the aqueous chemistry and also are able to draw some comparisons with the closely related ligand β-mercaptoethanol and with the amino acids glycine and cysteine. We also report an X-ray crystal structure of a V(IV) complex with DTT which is compared to and contrasted with the structure we propose for its V(V) analog.

Experimental section

Materials

All chemicals used in this work were of reagent or better quality. Divanadium(V) pentoxide, *N*,*N*-dimethylhydroxylamine hydrochloride, dithiothreitol, tetraphenylarsonium chloride, glycine, cysteine and β-mercaptoethanol were obtained from Sigma–Aldrich and used without further purification. Potassium chloride (99.5% analytical reagent) was from BDH Inc.

Solutions

Preparative methods for stock solutions of sodium vanadate, DMHA, KCl, and DTT are described elsewhere (9). The ionic strength of all solutions were maintained at 1.0 M with KCl. In each case, the solutions at different pH were prepared following a two-step addition. Initially, appropriate amounts, for the desired final concentrations, of the stock KCl, DMHA, and DTT solutions were combined. The pH of the solution was then adjusted, using appropriate amounts of HCl or KOH, to a value so that after, the addition of the stock vanadate solution, the pH would be close to, but below the desired value. In the second step the vanadate was added, the pH adjusted upward with KOH, and finally the final desired volume was made up with water previously brought to the particular pH. This procedure avoided the formation of decavanadate which tends to form in solutions below the neutral pH.

Synthesis of $As_2C_{56}H_{54}O_6S_4V_2$

At room temperature, an aqueous solution (2 mL) of DTT (0.15 g, 1 mmol), adjusted to pH 8.5, was added dropwise, with stirring, to a 2.5 mL aqueous solution of $VOSO_4.xH_2O$ (0.2 g, 1 mmol). The resultant solution gradually turned green. It was stirred for an additional 5 min and the desired compound was then precipitated by adding a warm saturated solution of tetraphenylarsonium chloride (0.42 g, 1 mmol), in 5 mL of water. The product, obtained in green microcrystalline form, was recrystallized from warm acetonitrile. Yield: (0.4 g) 67%. FT-IR (KBr) (cm^{-1}): 961, V=O stretching. Anal. calcd. for $As_2C_{56}H_{54}O_6S_4V_2$: C 55.9, H 4.5; found: C 55.78, H 4.52.

^{51}V NMR Spectroscopy

^{51}V NMR spectra were obtained either from a Bruker AMX400 NMR spectrometer operating at 105.2 MHz at ambient temperature or from a Bruker AMX600 operating at 157.7 MHz. Vanadium chemical shifts are reported relative to the chemical shift of the external reference $VOCl_3$ at 0 ppm. The following NMR parameters were used: pulse width 60°; spectral width 62.5 kHz; acquisition time 0.065 s. The data was process using the spectrometer manufacturers software WINNMR. A line-broadening factor of 40 Hz was applied to all spectra and after Fourier transformation, the

Table 1. Crystallographic data for the structure determination of $[\{VO(SCH_2CHOHCHOCH_2S)\}_2]$ $[AsPh_4]_2$.

Formula	$As_2V_2S_4O_6C_{56}H_{54}$	Crystal system	Monoclinic
Fw	1202.99	Space group	$P2_1/n$
a (Å)[a]	10.1607(18)	ρ_c (g cm^{-3})	1. 500
b (Å)	17.8255(42)	λ (Mo Kα_1) (Å)	0.70930
c (Å)	15.1520(33)	μ (Mo Kα) (cm^{-1})	17.7
b (°)	104. 000(15)	Min-max 2θ (°)	4–48
V (Å^3)	2662.8	Transmission[b]	0.634–0.818
Z	2	R_F[c]	0.038
Temperature (K)	295	R_{wF}[d]	0.040
Observed reflns[e]	2327	Parameters	325

[a]Cell dimensions were determined from 57 reflections ($40° < 2\theta < 46°$).
[b]The data were corrected for the effects of absorption by the Gaussian integration method.
[c]$R_F = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$ for observed data.
[d]$R_{wF} = [\Sigma(w(|F_o| - |F_c|)^2)/\Sigma(wF_o^2)]^{1/2}$ for observed data.
[e]$I_o \geq 2.5\sigma(I_o)$.

Table 2. Fractional atomic coordinates and equivalent isotropic or isotropic displacement parameters (Å^2) for the non-hydrogen atom sites $[\{VO(SCH_2CHOHCHOCH_2S)\}_2]$ $[AsPh_4]_2$.

Atom	x	y	z	U_{eq}[a]
As	0.28671(6)	0.07988(3)	0.11069(4)	0.0342(3)
V	0.56794(10)	0.53509(6)	–0.07304(6)	0.0374(6)
S(1)	0.80324(16)	0.52528(11)	–0.05604(12)	0.0557(11)
S(4)	0.41296(18)	0.33340(10)	0.06651(12)	0.0563(11)
O(1)	0.4998(4)	0.50966(23)	–0.1743(3)	0.051(3)
O(2)	0.8427(5)	0.4026(3)	0.0919(3)	0.057(3)
O(3)	0.5722(4)	0.44609(20)	0.0048(3)	0.0404(22)
C(1)	0.8221(7)	0.4248(4)	–0.0675(4)	0.060(5)
C(2)	0.7774(6)	0.3777(4)	0.0027(4)	0.052(4)
C(3)	0.6258(6)	0.3739(3)	–0.0068(4)	0.047(4)
C(4)	0.5853(7)	0.3192(3)	0.0583(4)	0.057(4)
C(11)	0.1451(6)	0.1396(3)	0.1361(4)	0.040(4)
C(21)	0.2471(5)	–0.0244(3)	0.1173(4)	0.034(3)
C(31)	0.4517(6)	0.1022(3)	0.1969(4)	0.036(3)
C(41)	0.2981(6)	0.1034(3)	–0.0099(4)	0.036(3)
C(12)	0.0591(7)	0.1122(4)	0.1845(4)	0.060(5)
C(13)	–0.0352(8)	0.1573(6)	0.2057(5)	0.090(7)
C(14)	–0.0445(9)	0.2312(6)	0.1792(6)	0.094(7)
C(15)	0.0417(8)	0.2590(4)	0.1313(6)	0.081(6)
C(16)	0.1366(7)	0.2141(4)	0.1088(5)	0.056(4)
C(22)	0.2434(6)	–0.0552(3)	0.2008(4)	0.047(4)
C(23)	0.2121(6)	–0.1295(4)	0.2053(5)	0.055(4)
C(24)	0.1855(7)	–0.1732(4)	0.1289(5)	0.059(5)
C(25)	0.1891(7)	–0.1431(4)	0.0468(5)	0.054(4)
C(26)	0.2199(6)	–0.0690(3)	0.0404(4)	0.043(4)
C(32)	0.5484(7)	0.0498(4)	0.2233(5)	0.071(5)
C(33)	0.6661(7)	0.0656(4)	0.2870(5)	0.079(5)
C(34)	0.6855(7)	0.1333(5)	0.3271(5)	0.066(5)
C(35)	0.5891(8)	0.1859(4)	0.3002(5)	0.079(5)
C(36)	0.4709(7)	0.1720(4)	0.2363(5)	0.063(5)
C(42)	0.1849(6)	0.0966(3)	–0.0796(4)	0.047(4)
C(43)	0.1908(7)	0.1088(4)	–0.1676(4)	0.055(5)
C(44)	0.3124(8)	0.1279(4)	–0.1850(4)	0.057(5)
C(45)	0.4250(7)	0.1369(4)	–0.1161(5)	0.056(4)
C(46)	0.4201(7)	0.1238(3)	–0.0273(4)	0.046(4)

[a]U_{eq} is the mean of the principal axes of the displacement ellipsoid.

spectra were baseline corrected before integration. Where necessary, signal deconvolution was also carried out using the WINNMR program.

Data analysis

The procedures for analysis of the NMR data are outlined in the text. All errors in parameters are reported at the 95% (3σ) confidence level.

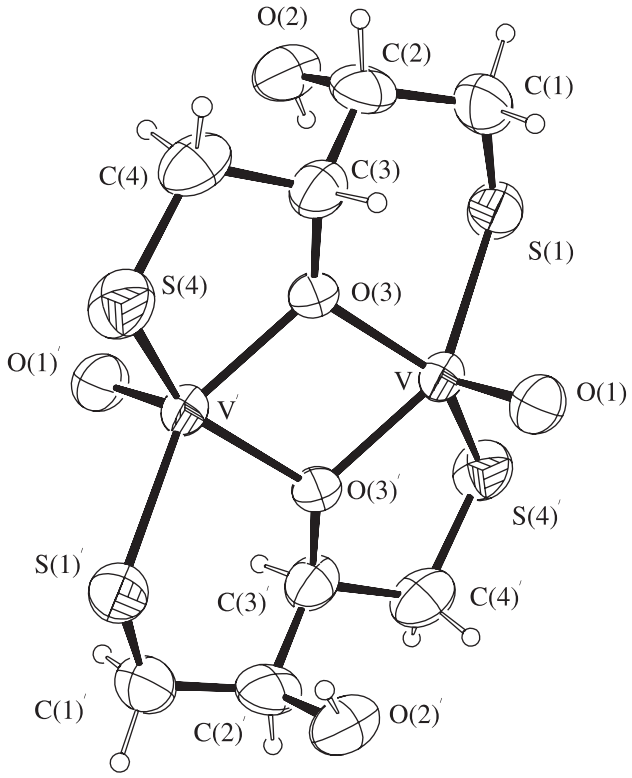
X-ray crystallography

A cleaved segment of a light green, plate-shaped crystal was mounted on a glass fiber using epoxy glue as an adhesive. Data were recorded with an Enraf Nonius CAD4F diffractometer using graphite-monochromatized Mo K_α radiation. Data reduction included corrections for Lorentz and polarization effects.

All non-hydrogen atoms' coordinates and anisotropic displacement parameters as well as the hydroxyl hydrogen atoms' coordinates and isotropic displacement parameter were refined. All other hydrogen atoms were placed in calculated positions and their coordinates were linked with those of their respective carbon atoms during refinement. Isotropic displacement parameters for these latter hydrogen atoms were initially assigned proportionately to the equivalent isotropic displacement parameters of their respective carbon atoms. Subsequently, these hydrogen atom displacement parameters were refined, but were constrained such that those within each phenyl group or those within the vanadium complex had the same shifts.

The data collection was performed using the program DIFRAC (10). The programs used for absorption corrections, data reduction, structure solution, and graphical output were from the NRCVAX Crystal Structure System (11). Full-matrix least-squares refinement was carried out using CRYSTALS (12). Complex scattering factors for neutral atoms (13) were used in the calculation of structure factors. Crystal data and experimental details are given in Table 1. The atomic coordinates, equivalent isotropic thermal parameters, and site occupancies for the non-hydrogen atoms are listed in Table 2.

Fig. 1. Molecular structure of the complex anion formed from dithiothreitol and vanadyl sulphate. 50% probability ellipsoids are shown for all non-hydrogen atoms. Hydrogen atoms are indicated by spheres of arbitrary radius.



Results and discussion

The complexation of the vanadium(V) oxoanion, vanadate, by dithiothreitol (DTT) has been studied for the pH range 7.1–9.7 and reduction of V(V) to V(IV) reported to occur in about a 90 min timescale (2). In the solutions utilized for this study at pH levels above neutral pH, partial reduction of vanadium(V) to V(IV) was observable after about 1–1.5 h. Consequently, the formation of the various V(V) solution products was readily studied by ^{51}V NMR spectroscopy without needing to take into account the redox reactions. However, at pH values below neutrality, and particularly at the higher concentrations of DTT, the reduction rate increases substantially. For example, at pH 6.2, in the reaction of 40 mmol DTT with 3 mmol vanadate, discoloration of the solution as V(IV) is formed becomes clearly evident in about 20 min. In the presence of sufficient *N*,*N*-dimethylhydroxylamine (DMHA) to effectively remove any free vanadate from solution, the reduction of V(V) to V(IV) is greatly slowed.

Efforts to isolate a crystalline V(V)–DTT–DMHA complex was not successful. However, as a result of V(V) reduction to V(IV) in the reaction medium, a V(IV) complex was obtained in crystalline form. The product contained DTT but no DMHA. A successful procedure for preparation of this V(IV)–DTT product from vanadyl sulphate was developed and is reported in the *Experimental section* and summarized below.

Crystal structure

The formation of the V(IV) dithiothreitol complex ($As_2C_{56}H_{54}O_6S_4V_2$) was carried out in water by adding a stoichiometric amount of dithiothreitol to vanadyl sulphate. The product was precipitated from solution as the tetraphenylarsonium salt. The microcrystalline compound appeared to be chemically stable. It was found to have medium solubility in common organic solvents such as chloroform and acetone and, in solution, did not show signs of decomposition even after several days at ambient temperature. An acetonitrile solution of the compound was allowed to slowly evaporate to give green crystals suitable for X-ray crystallographic analysis.

The molecular structure of the $[\{VO(SCH_2CHOHCHOCH_2S)\}_2]^{2-}$ anion is shown in Fig. 1. The anion has crystallographic inversion symmetry and the associated tetraphenylarsonium cation is in a general position. There are no inter-ionic contacts significantly shorter than the appropriate sums of accepted van der Waals radii and there is no water in the cell. Each vanadium atom is coordinated to one thiolate and one bridging alkoxy group from two oppositely directed dithiothreitol molecules. One hydroxyl group of each DTT is not coordinated and there are no close intermolecular contacts of the oxygen of this hydroxyl group to any other group. Consequently, the hydroxyl group does not undergo hydrogen bonding interactions.

The bond distances and bond angles obtained for the anion are listed in Table 3. Each vanadium(IV) is in an irregular five-coordinate environment which is close to a square-pyramidal structure with the V=O (O(1)) in an axial position. The six-membered chelate ring (V-S(1)-C(1)-C(2)-C(3)-O(3)) has a chair conformation with the hydroxyl group (O(2)) in an axial position. The five-membered chelate ring (V′-S(4)-C(4)-C(3)-O(3)) has an envelope conformation with C(4) constituting the flap. The arrangement around O(3) is close to planar (cf. the sum of the three bond angles at O(3) is 356.8°).

Solution studies

In aqueous solution, both dithiothreitol (2) and *N*,*N*-dimethylhydroxylamine (14–16) react spontaneously with vanadate. If DTT is designated as T, then complexes of V_2T stoichiometry are formed with DTT. The chemistry with DMHA is quite complex but under moderate total vanadate concentrations, the predominent product stoichiometries with DMHA (L) are VL and VL_2. Additional mixed products of varying stoichiometry are formed when the two ligands are together in solution. In anticipation of the results summarized below, Fig. 2 identifies the various signals and Table 4 gives their ^{51}V chemical shifts.

These studies of vanadate complexation reactions with *N*,*N*-dimethylhydroxylamine and associated heteroligands have utilized overall vanadium concentrations considerably higher than we have previously used in studies of this type. One consequence of this was the observation of NMR signals that corresponded to multinuclear vanadium–DMHA complexes. The concentrations of these compounds were sufficiently high that it was necessary to identify them so that proper characterization of the mixed ligand complexes of predominant interest could be carried out. Initial studies indicated that the compounds were binuclear complexes of

Table 3. Selected intramolecular distances (Å) and angles (°) for $\{VO(SCH_2CHOHCHOCH_2S)\}_2]^{2-}$.

Distances (Å)			
V—S(1)	2.3484(20)	S(4)—C(4)	1.803(7)
V—S(4)′	2.3524(21)	O(2)—C(2)	1.424(7)
V—O(1)	1.589(4)	O(3)—V′	1.980(4)
V—O(3)	1.971(4)	O(3)—C(3)	1.424(7)
V—O(3)′	1.980(4)	C(1)—C(2)	1.510(10)
S(1)—C(1)	1.814(7)	C(2)—C(3)	1.514(9)
S(4)—V′	2.3524(21)	C(3)—C(4)	1.513(10)
Angles (°)			
S(1)-V-S(4)′	89.89(7)	V-O(3)-V′	105.16(18)
S(1)-V-O(1)	106.19(17)	V-O(3)-C(3)	127.4(4)
S(1)-V-O(3)	90.00(13)	V′-O(3)-C(3)	124.2(4)
S(1)-V-O(3)′	142.84(12)	S(1)-C(1)-C(2)	115.2(5)
S(4)′-V-O(1)	109.70(16)	O(2)-C(2)-C(1)	110.1(5)
S(4)′-V-O(3)	141.93(12)	O(2)-C(2)-C(3)	109.5(6)
S(4)′-V-O(3)′	82.63(12)	C(1)-C(2)-C(3)	115.5(5)
O(1)-V-O(3)	106.84(19)	O(3)-C(3)-C(2)	111.0(5)
O(1)-V-O(3)′	110.61(19)	O(3)-C(3)-C(4)	109.2(5)
O(3)-V-O(3)′	74.84(16)	C(2)-C(3)-C(4)	113.4(5)
V-S(1)-C(1)	101.03(23)	S(4)-C(4)-C(3)	112.1(4)
V′-S(4)-C(4)	94.09(21)		

Note: ′ represents the symmetry operation $1 - x, 1 - y, -z$.

V_2L and V_2L_3 stoichiometries. The stoichiometry of these complexes was most easily established through the use of the known mononuclear complex, VL (15), as a reference compound. Equations [1] –[3] then describe the formation of these products. The

$$[1] \quad V + L \overset{K_{110}}{\rightleftharpoons} VL \qquad [V][L]K_{110} = [VL]$$

$$[2] \quad 2VL \overset{K_{110,210}}{\rightleftharpoons} V_2L + L \qquad [VL]^2 K_{110,210} = [V_2L][L]$$

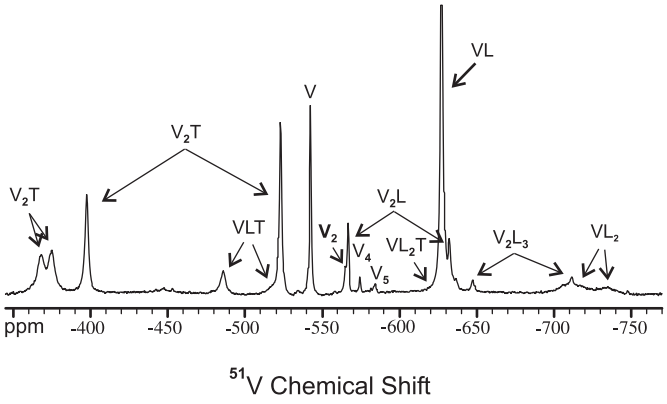
$$[3] \quad 2VL + L \overset{K_{110,230}}{\rightleftharpoons} V_2L_3 \qquad [VL]^2[L]K_{110,230} = [V_2L_3]$$

formation constant determined for VL ($K_{110} = (1.4 \pm 0.1) \times 10^3$ M^{-1}) agrees well with that previously reported (15).

The characterization of V_2L was complicated by the fact that one NMR signal (–632 ppm) from this compound occurred very close to that from VL while the second (–567 ppm) was partially superimposed on that of divanadate. As near as could be judged, both of these signals had the same intensity and exhibited the same concentration dependencies. They were therefore assigned to the individual vanadiums of the same product. On that basis, the formation constant was $K_{110,210} = (0.12 \pm 0.01)$.

Similarly to those of V_2L, two NMR signals (–648 ppm, –712 ppm) had a concentration dependence corresponding to a V_2L_3 stoichiometry. The second of these signals was superimposed on one of the VL_2 signals but appropriate concentrations of reactants could be obtained that allowed it to be established that these signals were of equal intensity. Consequently, they were assigned to the individual signals of V_2L_3. Fig. 3 shows the equilibrium data plotted according to

Fig. 2. ^{51}V NMR spectrum of vanadate in the presence of dithiothreitol (T) and *N*,*N*-dimethylhydroxylamine (L). The signals of various products discussed in the text are identified. Conditions of the experiment: vanadate (10 mM), dithiothreitol (10 mM), *N*,*N*-dimethylhydroxylamine (5 mM), KCl (1.0 M), pH 8.54.



eq. [3]. The value determined for the formation constant was $K_{110,230} = (4.2 \pm 0.2) \times 10^3$ M^{-2}.

From the relative values of $K_{110,210}$ and $K_{110,230}$ (note different units) it is evident that V_2L and V_3L_2 are found in equal proportions in solution when the free DMHA concentration is about 5 mM. Because of the L stoichiometry, the relative proportion of V_2L_3 increases with higher concentration of L free in solution and decreases with a lower concentration. This is evident in Fig. 2 where, as reflected in the NMR spectrum, the V_2L concentration is significantly higher than that of V_3L_2.

Of critical importance is the observation that VL (–627 ppm) and a mixed ligand product VL_2T (–626 ppm), have overlapping NMR signals. Products that have VLT stoichiometry have been assigned to NMR signals at –485 and –517 ppm. Both of these signals occur with low relative intensities, with the –515 ppm signal being much the smaller of the two. The identity of the –517 ppm product was inferred from that of the –485 ppm signal because the relative intensities of the two signals, as well as could be judged, maintained a constant proportionality throughout the various concentration studies. Because of the ligand and proton stoichiometries, the –485 ppm signal is observable at pH 6.73 only under low DMHA and quite high DTT concentrations. This condition also ensures formation of low amounts of VL_2T so that there is selective suppression of this –626 ppm product. The situation is quite different at pH 8.54 where the –485 ppm signal can be observed even when the concentration of DTT is low (Fig. 2).

Under neutral conditions, preliminary experiments identified the concentrations of vanadate, DMHA, and DTT, that provided a useful distribution of free vanadate and its products. Using appropriate concentrations of reactants, as established above, a pH variation study was carried out. At low pH (below 6.00), vanadate is a reasonably effective oxidant of DTT while at high pH (above 9.5) the vanadate complexes are highly dissociated. A pH range for the study of product formation from pH 6.7 to 8.7 was therefore adopted. Detailed solution studies were then carried out at both pH 6.73 and pH 8.54. In each case the concentrations of V,

Table 4. Equilibrium equations, product formation constants, and ^{51}V chemical shifts.[a]

Equilibrium equation	Formation constant	pH	ppm
$2VL \rightleftharpoons V_2L + L$	0.12 ± 0.01	8.54	–567, –632
$2VL + L \rightleftharpoons V_2L_3$	(4.2 ± 0.2) × 10^3 M^{-2}	8.54	–648, –712
$VL + T \rightleftharpoons VLT$[b]	13.4 ± 0.6 M^{-1}	6.73	–485
	12.6 ± 0.4 M^{-1}	8.54	–485
$VL + M \rightleftharpoons VLM$[b]	5.0 ± 0.2 M^{-1}	8.54	–487
$VL + Cys \rightleftharpoons VLCys$[b]	6.7 ± 1.2 M^{-1}	8.50	–496
$VL_2 + T \rightleftharpoons VL_2T$[b]	35.2 ± 0.9 M^{-1}	6.73	–626
	28.0 ± 1.0 M^{-1}	8.54	–626
$VL_2 + M \rightleftharpoons VL_2T$[b]	6.5 ± 0.6 M^{-1}	8.54	–628
$VL_2 + Gly \rightleftharpoons VL_2Gly$[c]	5.5 ± 0.4 M^{-1}	8.55	–700, –724, –734
$VL_2 + Cys \rightleftharpoons VL_2Cys$[c]	10.5 ± 3.5 M^{-1}	8.50	–729, –734, –741
$VL_2 + Cys \rightleftharpoons VL_2Cys$[b]	8.2 ± 0.5 M^{-1}	8.50	–632, –636

[a]Abbreviations: *N*,*N*-dimethylhydroxylamine (L), dithiothreitol (T), β-mercaptoethanol (M), cysteine (Cys), glycine (Gly). All formation constants are for aqueous 1.0 M ionic strength solutions maintained with KCl.
[b]Data is for sulphur-coordinated products.
[c]Data is for *N*,*O*-coordinated products.

Fig. 3. Graphical representation of the formation of V_2L_3 from VL and free *N*,*N*-dimethylhydroxylamine (L) according to the formalism of eq. [3]. Data for pH 8.54.

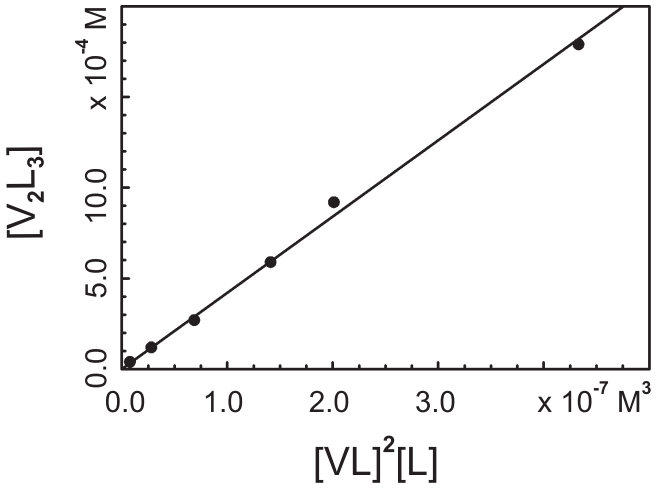
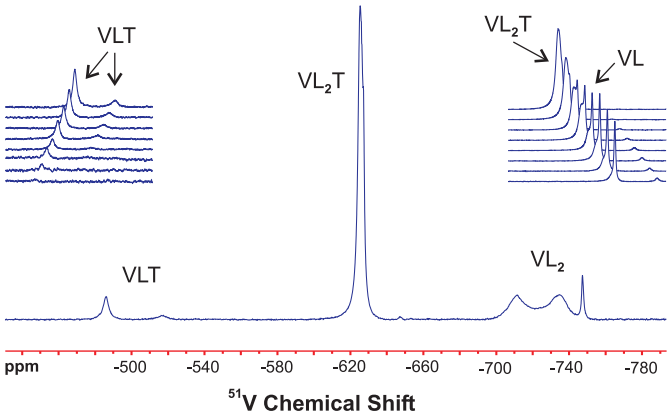


Fig. 4. ^{51}V NMR spectrum of vanadate in the presence of 60 mM dithiothreitol. The insets show the influences of varying amounts of dithiothreitol (T) with fixed total concentrations of vanadate and *N*,*N*-dimethylhydroxylamine (L). Conditions of the experiments: vanadate (5 mM), *N*,*N*-dimethylhydroxylamine (40 mM), dithiothreitol (60 mM) (main trace), pH 8.54, KCl (1.0 M); total dithiothreitol concentrations: 2, 5, 8, 10, 20, 30, 60, 120 mM bottom to top traces of the insets.



DMHA, and DTT were selectively varied in independent experiments. Figure 4 shows the results of one such concentration study.

There, however, is an analytical problem that arises because some product formation constants are large. Under certain reaction conditions, free DMHA ([L]) and free DTT ([T]) concentrations can be very low and thus difficult to accurately specify from the conservation equations. This arises because the errors in the determination of product concentrations are comparable to the free DMHA or free DTT concentrations. However, these problems were circumvented by a judicious choice of reactant concentrations. Studies were initially carried out under the assumption that the mixed ligand product at –626 ppm was of stoichiometry VLT, as previously assigned (5). It was possible to show that that assignment of stoichiometry was incorrect by studying first the formation of the minor product giving a signal at –485 ppm. The product giving rise to this latter signal could unequivocally be shown to have a VLT stoichiometry and also it could be shown that the stoichiometry of this product was different from the product giving rise to the partially obscured signal at –626 ppm.

The formation of two V_2T complexes (eq. [4]) has previously been reported (2) and, as is evident from Fig. 2, it was necessary to take them into account in this study.

$$[4] \qquad 2V + T \overset{K_{201}}{\rightleftharpoons} V_2T \qquad [V]^2[T]K_{201} = [V_2T]$$

The stoichiometry corresponding to the –485 ppm (and by inference the –517 ppm) signal was established by varying the total concentrations of V, L, and T in separate experiments. Figure 4 provides a graphical display of the influence of varying the concentration of DTT while total vanadate and total DMHA were maintained constant. The constant ratio of signal intensities of the –485 and –517 ppm signals is

Fig. 5. Correlation indicating the formation of VLT from VL_2 and dithiothreitol (T) with the release of 1 equiv of *N*,*N*-dimethylhydroxylamine (L) as described by eq. [8]. Data for pH 8.54.

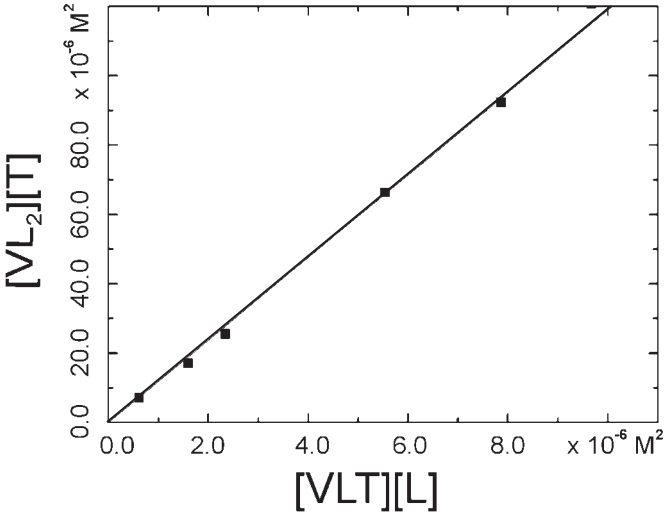
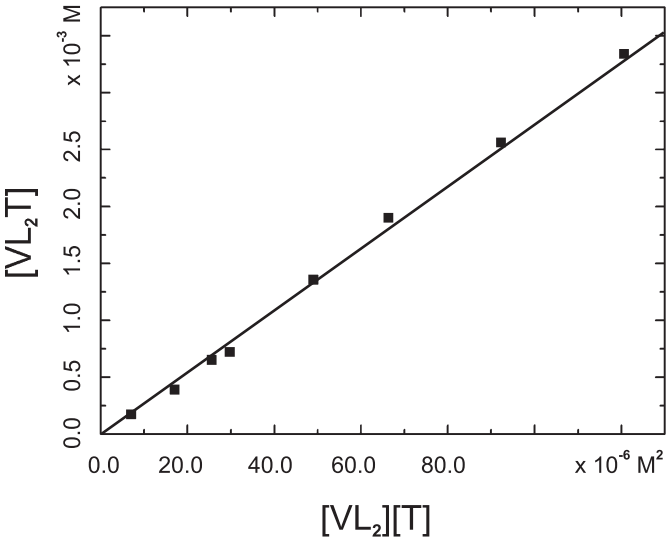


Fig. 6. Linear relationship between the product described as VL_2T and the VL_2 concentration multiplied by the dithiothreitol (T) concentration as required by eq. [9]. Data for pH 8.54.



evident in the inset of Fig. 4. In a similar experiment where DMHA was varied with constant total DTT, the two signals also maintained a constant proportionality with each. This indicates that these products have the same V to DMHA to DTT stoichiometry and therefore probably correspond to isomers, possibly arising from rotational isomerization of the hydroxamido group (15). From the concentration study, it was also evident that the signal corresponding to the product designated VL_2T at –626 ppm gained in magnitude relative to the VL and VLT signals as the L (DMHA) concentration was increased. These concentration studies are consistent with the equilibria expressed in eqs. [5] and [6] for the –485, –517 (eq. [5]), and –626 (eq. [6]) ppm products, respectively.

$$[5] \qquad V + L + T \overset{K_{111}}{\rightleftharpoons} VLT \qquad [V][L][T]K_{111} = [VLT]$$

$$[6] \qquad V + 2L + T \overset{K_{121}}{\rightleftharpoons} VL_2T \qquad [V][L]^2[T]K_{121} = [VL_2T]$$

Since, with a 600 MHz NMR spectrometer, it was possible to obtain a good enough resolution of the overlapped –626 and (or) –627 ppm signals to use lineshape fitting to resolve them into the two component parts it was possible to unambiguously establish the identity of the –485 (–517) and the –626 ppm products. The –627 ppm product is known to correspond to VL (15) and that identity has been confirmed in this study. Addition of 1 equiv of DTT to VL provides VLT (–485 ppm) as described by eq. [7]. Figure 5 shows the results of a concentration study carried out at pH 8.54 with the relevant quantities plotted according to eq. [7]. From this graph,

$$[7] \qquad VL + T \overset{K_{110,111}}{\rightleftharpoons} VLT \qquad [VL][T]K_{110,111} = [VLT]$$

$K_{110,111} = 12.8 \pm 0.9\ M^{-1}$. The corresponding value obtained for pH 6.73 was $13.0 \pm 1.1\ M^{-1}$. VL is singly negatively charged and, throughout the pH range of this study, does not form VL^{2-}. As a consequence of this, the finding that the formation constants are pH independent shows that VLT carries a single negative charge and, like VL^-, does not lose an additional proton.

It is possible to obtain the stoichiometry of the VLT product by an alternative method. Its formation can be written as occurring from VL_2, as described by eq. [8] so that VL_2 is the

$$[8] \qquad VL_2 + T \overset{K_{120,111}}{\rightleftharpoons} VLT + L$$

reference compound rather than VL. The observation of good straight lines when plotting the data according to eq. [8] was in accord with the assignment of VLT stoichiometry to the product providing the –485 ppm signal (and also, for the reasons described above, to the –517 ppm product).

The VL_2 complexes also provide a suitable reference for characterizing the product providing the –626 ppm signal, here assigned to VL_2T. Its formation can be written as in eq. [9], where cVL_2 represents the overall concentration of VL_2 complexes, and the

$$[9] \qquad VL_2 + T \overset{K_{120,121}}{\rightleftharpoons} VL_2T \qquad cVL_2[T]K_{120,121} = [VL_2T]$$

experimental results appropriately plotted. Figure 6 shows the good linear correlation obtained at pH 8.54, $K_{120,121} = (28.5 \pm 1.0)\ M^{-1}$. A similar correlation was obtained for the pH 6.73 study, $K_{120,121} = (35.6 \pm 0.9)\ M^{-1}$. The VL_2 complexes, which do not carry a charge in the slightly acidic to slight basic pH range, have a pK_a in the order of 9.0 (see below). The decrease in formation of VL_2T from VL_2 on going from pH 6.73 to pH 8.54 is fully accounted for by the averaged pK_a of VL_2 and therefore VL_2T does not have a pK_a within the range of pH of this study and is non-ionic.

The reactions of β-mercaptoethanol (M) with dimethylhydroxamidovanadates are quite similar to those of

Fig. 7. ^{51}V NMR spectra of vanadate in the presence of varying amounts of β-mercaptoethanol (M) with fixed total concentrations of vanadate and *N*,*N*-dimethylhydroxylamine (L). The chemical shift scale refers to the lower trace only, the remaining spectra are offset from that trace. Conditions of the experiments: vanadate (5 mM), *N*,*N*-dimethylhydroxylamine (40 mM), β-mercaptoethanol (as indicated), pH 8.54, KCl (1.0 M).

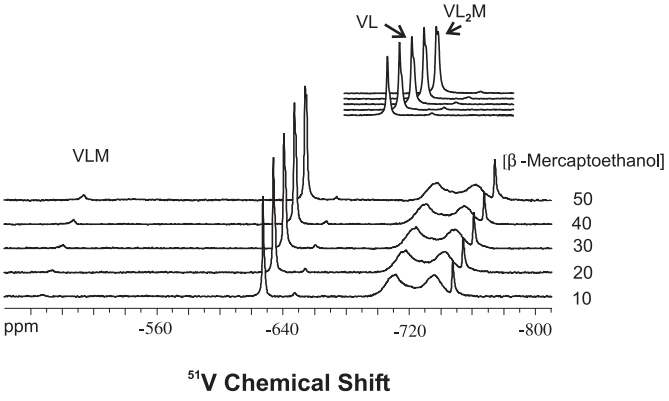
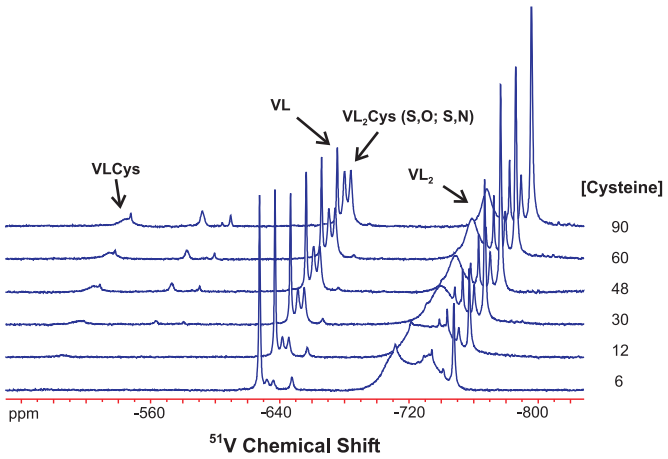


Fig. 8. The influence of varying amounts of cysteine on the ^{51}V NMR spectra of vanadate in the presence of a fixed total concentration of *N*,*N*-dimethylhydroxylamine (L). The chemical shift scale refers to the lower trace only, the remaining spectra are offset from that trace. Conditions of the experiments: vanadate (5.0 mM), *N*,*N*-dimethylhydroxylamine (40 mM), cysteine (as indicated), KCl (1.0 M), pH 8.50.



DTT. Figure 7 displays NMR spectra from a pH 8.54 β-mercaptoethanol concentration study. The VLM product gives a signal at –487 ppm, a 2 ppm shift to higher field of the corresponding VLT derivative at –485 ppm. This behaviour is mirrored in the VL_2M product where there is a small change of chemical shift to –628 ppm from –626 of the DTT complex. The formation of VLM can be described by an equation that is equivalent to eq. [7] and the data plotted appropriately. An excellent linear relationship was obtained. The formation constant for formation of VLM from VL and β-mercaptoethanol was 5.0 ± 0.2 M^{-1}. This value is close to one-half of that observed for formation of VLT and this is consistent with the fact that, structurally, DTT is equivalent to a dimer of β-mercaptoethanol and statistically, this will double the forward rate of complexation. There seems to be little stabilizing influence of the additional potentially ligating groups of the DTT ligand. In contrast to this, formation of VL_2M is less favoured by about a factor of four ($K_{120,121}$ = 6.5 ± 0.6 M^{-1}) compared to the formation of VL_2T ($K_{120,121}$ = 28.0 ± 1.0 M^{-1}).

The reaction of DMHA–vanadate with cysteine is considerably more complex than with DTT or β-mercaptoethanol. Figure 8 shows a cysteine concentration study carried out at pH 8.54. A previous study (9) has shown that bidentate complexation occurs through both *N*,*S* and *O*,*S* linkages and also through the *O*,*N*. However, there are problems arising because of a reaction involving the two VL_2 compounds with NMR signals at –710 and –732 ppm. With increase in cysteine content these signals collapsed into a single signal (–720 ppm, Fig. 8) corresponding to a complex of unknown identity. This behaviour was not observed with either dithiothreitol or β-mercaptoethanol. The reaction offered some complications in characterizing the cysteine system so an initial study of the simpler amino acid, glycine (G) was carried out. The coalescence of the –710 and –732 ppm signals was also observed with glycine, and, as for cysteine, new signals appeared in the –740 ppm region of the spectrum. Signals of the latter type have also been observed when simple peptides have been included in the medium (17) but the coalescence behaviour was not observed. The collapse of the –710 and –732 ppm signals into a single signal suggested the formation of a new product. However, a number of concentration studies, where the concentrations of V, L, or glycine were varied, showed that the single product signal corresponded to a VL_2 stoichiometry. If the formation of VL_2 is written from VL as described in eq. [10], and that of the glycine (N,O) complex as in eq. [11],

$$[10]\quad VL + L \overset{K_{110,120}}{\rightleftharpoons} VL_2 \qquad [VL][L]K_{110,120} = [VL_2]$$

$$[11]\quad VL_2 + G \overset{K_{120,121}}{\rightleftharpoons} VL_2G \qquad [VL_2][G]K_{120,121} = [VL_2G]$$

then summation and rearrangement of the two equations gives eq. [12] where cVL_2 refers to

$$[12]\quad cVL / [VL][L] = K_{110,120} + K_{110,120}K_{120,121}[G]$$

the total concentration of VL_2 + VL_2G products. Figure 9 shows the results plotted according to eq. [12]. A good linear correlation is in accord with this description of the equilibria. The intercept of the graph gives a value of 140 ± 3 M^{-1} for $K_{110,120}$ while the value for the slope (770 ± 50 M^{-2}) divided by the value for the intercept gives $K_{120,121}$ = 5.5 ± 0.4 M^{-1} for pH 8.54. This study is fully consistent with a VL_2 stoichiometry for the –720 ppm signal and suggests that the amino acid is catalyzing an exchange process between the VL_2 isomers. This might, for instance, be caused by the formation of a transient outer sphere complex that promotes rotational isomerization of the DMHA ligands. This isomerization already occurs in the timescale of a few milliseconds at room temperature (15).

Fig. 9. Relationship between the quantities, as described in eq. [12], for the formation of VL_2 and VL_2G from VL, L (*N*,*N*-dimethylhydroxylamine) and glycine (G). cVL_2 represents the sum of concentrations of VL_2 and VL_2G products.

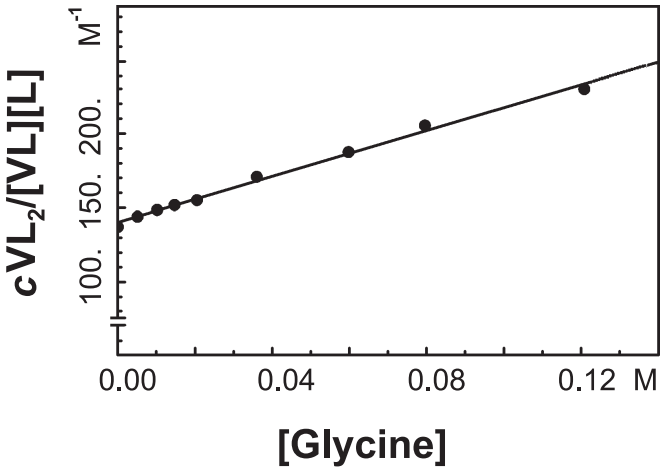
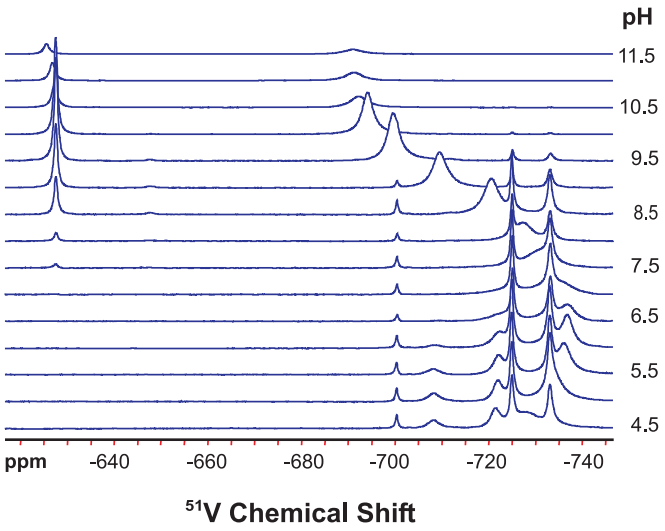
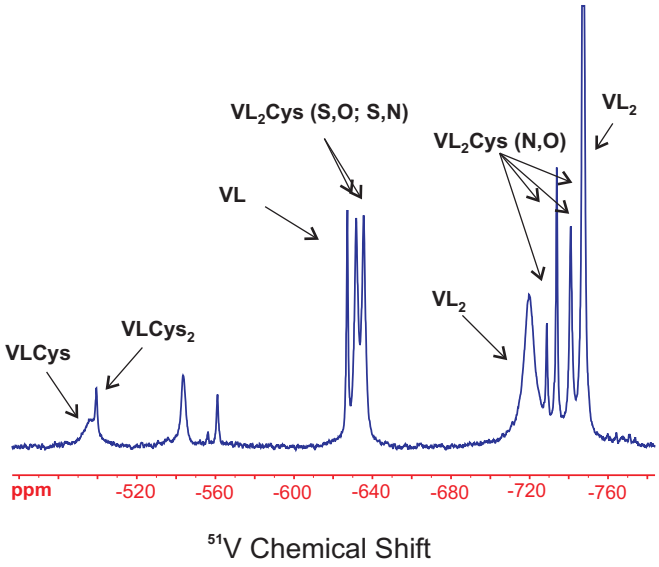


Fig. 10. Influence of pH on the NMR spectra of vanadate in the presence of *N*,*N*-dimethylhydroxylamine (DMHA) and glycine. Conditions of the experiments: vanadate (5 mM), DMHA (40 mM), glycine (120 mM) KCl (1.0 M), variable pH as indicated.



A pH variation study (pH 4.5–11.5) in the presence of 120 mM glycine revealed a systematic change in position of the –720 ppm signal (Fig. 10). Removal of glycine from the solution at pH 10 had no influence on peak position. Furthermore, a more detailed glycine concentration study at pH 10 revealed no reaction between glycine and any DMHA complex in solution. This is expected if the mixed ligand (glycine–DMHA) vanadate complexes do not have a pK_a in the high pH range. An additional pH study in the absence of glycine revealed that an increase in pH leads to generation of a single DMHA product signal above pH 9. Given then, that the –720 ppm signal is a time-averaged signal, the pH titration curve represents an averaged pK_a for the VL_2 products. Because of this, the pK_a from the titration curve (pK_a = 9.0) is not particularly meaningful. The VL_2 complexes can also be protonated at low pH to form cationic species (15) and this accounts for the downfield shifts observed in the spectral in the low pH region of Fig. 10. An interesting point to be made from Fig. 10 is that, near the high pH limit of the study, the signal of VL^- starts to change position. This indicates that VL has a second pK_a under strongly basic conditions.

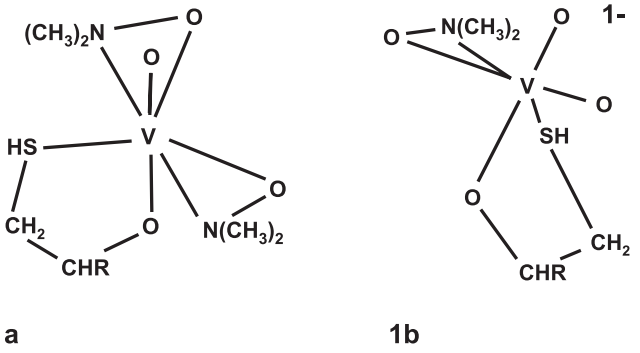
Fig. 11. ^{51}V NMR spectrum of vanadate (5 mM) in the the presence of *N*,*N*-dimethylhydroxylamine (L, 40 mM) and cysteine (Cys, 120mM) obtained at pH 8.50 with 1.0 M KCl.



With the identification of the –720 ppm product signal as VL_2, characterization of the cysteine–DMHA complexes was reasonably straightforward. Cysteine (Cys) forms product complexes both with vanadate and with DMHA–vanadate. Vanadate, in the presence of only cysteine gives product complexes with ^{51}V NMR signals at –243, –309, –393, and –405 ppm. Under the conditions utilized in this study, oxidative instability precluded detailed characterization of these products. However, a cysteine concentration study suggested that the –243 and –309 ppm products contained at least two cysteine moieties per vanadium while the products giving signals at –393 and –405 contained less cysteine, most likely only one cysteine per vanadium.

In the presence of DMHA, both VLCys and VL_2Cys products were observed. Figure 11 shows a spectrum from a cysteine concentration study. The broad signal at –496 ppm derives from VLCys whiles those at –631 and –635 ppm arise from VL_2Cys products of *S*,*O* and *S*,*N* coordination. As for glycine, the broad VL_2 signals at –710 and –732 ppm collapsed into one signal (–720 ppm) with addition of cysteine. The sharp signal found at –747 ppm also arises from a VL_2 complex but the concentration studies revealed that an additional product signal is superimposed on the –747 ppm signal. The broad and sharp signals from VL_2 have been assigned to 6 ($(dmha)_2V(O)OH$) and 7 ($(dmha)_2V(O)(OH)H_2O$) coordinated products, respectively, (15, 18). Cysteine *N*,*O*-coordinated products give NMR signals at –729, –734, and –741 and, as mentioned, an additional product signal is found at –747 ppm. This latter

Scheme 1.



1a 1b

product was not observed at pH 7.3 (9) and apparently corresponds to a product of reaction with $(dmha)_2V(O)(OH)H_2O$. At pH 8.5, this coordination, relative to that of $(dmha)_2V(O)OH$, is much more highly favoured than it is at pH 7.3. The signals at –544, –556, and –561 apparently correspond to VLCys products. These latter signals are not observed in the absence of DMHA, so it seems likely that DMHA is complexed in an end-on fashion in these complexes. If so, the chemical shifts of these compounds suggest they are not coordinated through sulphur. The remaining NMR signal occurs at –500 ppm and is assigned to $VLCys_2$. Since the influence on chemical shift of this compound is small relative to the chemical shift of sulphur-coordinated VLCys (–496 ppm), it is likely that the second cysteine of $VLCys_2$ is not coordinated through a sulphur.

The formation constant for VLCys from VL and cysteine was 6.7 ± 1.2 M^{-1} while that of VL_2Cys was 8.2 ± 0.5 M^{-1} overall for the *S*,*O* and *S*,*N*-coordinated products from VL_2 and cysteine. As can be judged from Fig. 11, there is little selectivity of reaction of *N*,*S*- vs. *O*,*S*-coordination in these complexes. The magnitude of these formation constants is similar to those with the ligands DTT and β-mercaptoethanol. The overall constant for formation of the *N*,*O*-coordinated cysteine products was 11 ± 2 M^{-1}.

Discussion

^{51}V chemical shifts

The influence of ligating atoms on vanadium(V) chemical shifts has been of interest for a number of years (19–21) and various relationships have been drawn. Sulphur is known to have a large influence on chemical shifts compared to oxygen whereas the influence of nitrogen seems quite small. For instance, the complex formed between vanadate and β-mercaptoethanol (–362 ppm) is 160 ppm to low field of the structurally similar ethylene glycol complex (–522 ppm). The chemical shifts of VLT (–485 ppm) and VLM (–487 ppm) are 140 ppm to low field of the parent complex VL (–626 ppm) while those of VL_2T and VL_2M are about 95 ppm to low field of the precursor VL_2 complexes. Similarly, sulphur in cysteine complexes with VL_2 causes a shift of about +105 ppm compared to the *N*,*O* complexes of cysteine and serine (9). It seems evident then, that inclusion of a single sulphur in the coordination shell of vanadate complexes will cause a shift of about +100 to +150 ppm in the signal position compared to oxygen and changes in signal position of this magnitude should be looked for when studying thiolate complexes.

N,*N*-dimethylhydroxylamine has a large influence on vanadium chemical shifts that is comparable to the influence of hydrogen peroxide. Ligation of *N*,*N*-dimethylhydroxylamine causes a high field shift in resonance positions of about 100 ppm per ligand. This large change was used as a basis for assigning the individual vanadium signals of V_2L and V_2L_3. It is somewhat surprising that no product signals corresponding to the dimers V_2L_2 and V_2L_4, were observed in this study. It may be that the asymmetry in the V_2L and V_2L_3 complexes adds an additional element of stability to the formation of these dimer-like complexes. At higher overall vanadate concentrations, V_2L_2 and V_2L_4 dimers will probably be observable; certainly V_2L_4 ($\{(dmha)_2V(O)\}_2O$) is the crystalline form of the complex (15).

Coordination

The dimeric coordination of V_2L_4, $\{(dmha)_2VO\}_2O$, as observed in the crystal structure, and also assigned to the structure in aqueous solution (15) together with the observation of large differences in chemical shifts between the individual vanadiums (Fig. 2, Table 4), as expected from selective sulphur coordination, provides a firm foundation for assigning the coordination structures of V_2L and V_2L_3. The coordination is assigned here to be similar to that of V_2L_4 except that the compounds are not symmetrical, being $\{dmhaV(O)_2\}O\{V(O)_2OH\}^{2-}$ and $\{(dmha)_2VO\}O\{dmhaV(O)_2\}^{1-}$. No information about the acidity constants of these compounds was obtained in this study.

It seems likely that coordination of a thiolate sulphur does not induce a significant change in coordination geometry compared to oxygen. Certainly this is the case for the β-mercaptoethanol complex. The structure of this vanadate complex is not much different from the glycolate complexes with adenosine (22), with a glycoside (23), or with the proposed ethylene glycol complex (24). All are dimeric structures with the cyclic $[VO]_2$ core that is characteristic of many vanadium complexes.

X-ray structures of a number of VL_2 complexes with oxygen- and (or) nitrogen-coordinated bidentate heteroligands have been reported (14, 16) and their structures are very similar. It seems likely that the DTT and β-mercaptoethanol derivatives are, structurally, not very different from those complexes, all of which are pentagonal bipyramidal.

It is interesting to speculate on the position of the thiolate sulphur in the complex. Does it occupy an equatorial or apical position? A recent crystal structure of a tridentate $O(CH_2CH_2S)_2^{2-}$ complex of vanadium(V) shows both sulphurs in equatorial positions (25). In the β-mercaptoethanol–V(V) complex, the sulphur is also in an equatorial position but, in this case, the formation of the $[VO]_2$ core of the complex forces the equatorial location for the sulphur. It is suggested here that the sulphur will be found in the equatorial position in these complexes as indicated in Scheme 1a. Since VL_2T, like the parent VL_2 does not carry a charge under close to neutral conditions, then the ligand must retain one proton, either on the S or the O of the

ligating group. It seems likely that it is retained on the sulphur since the *S*-substituted cysteine (-SC(O)OBz) forms a product complex (–627 ppm) with VL_2 (9). In a related case where a bidentate ethylene glycol complex is formed under forcing conditions, a proton is retained on one of the oxygens of the ligand. The resultant bond to vanadium is, however, very long (2.321(4) Å) (26) compared to a more typical distance of about 1.9 Å for a V—O bond.

The assignment of a coordination geometry to VLT is complicated by the fact that there are no crystal structures of closely related compounds. However, if the bonding to the hydroxamido ligand is formally considered to be unidentate then the vanadiums in the crystalline dimer $\{(dmha)_2V(O)\}_2O$, can be considered to have a tetrahedral coordination (15). Since vanadate, itself, also has tetrahedral coordination, it seems unlikely that VL will have other than tetrahedral coordination. Bidentate complexation of the thiolate can reasonably be expected to expand the coordination sphere (Scheme 1b). On this basis, and if the DMHA ligand is formally considered unidentate, both VL_2T and VLT may be considered to be trigonal bipyramidal. Since there is no indication from these studies that more than one sulfhydryl or more than one hyroxyl group are involved in the complexation of DTT with VL or with VL_2, the β-mercaptoethanol complexes are unlikely to show significant structural differences from the corresponding DTT complexes.

These proposed coordination modes of Scheme 1 are very different from the coordination found in the V(IV) complex with DTT (V_2T_2, Fig. 1) and indeed from the structure previously proposed for the V(V) complex of DTT (2) where the stoichiometry is V_2T. The structure that was suggested for V_2T is quite similar to that observed here for the V(IV) complex. However, there is a significant difference in that the V(V) complex has only one DTT ligand that binds each vanadium differently while the V(IV) complex has two ligands and identical vanadiums. The proposed structures of Scheme 1 are similar to those observed for some amino acid and dipeptide complexes (16, 17) except that the latter complexes do not have sulphur ligation.

Although these β-mercaptoethanol and dithiothreitol complexes are of interest in their own right, they serve as useful models for the study of other thiolate complexes. In particular, they have aided in unraveling the complex equilibrium pattern of the cysteine ligand and have also provided critical information for the study of DMHAV complexation in the active site of protein tyrosine phosphatases (7). The structures of the VLCys and VL_2Cys complexes are probably not much different from those proposed above for the DTT and β-mercaptoethanol complexes except that, in the one case, a nitrogen takes the place of the oxygen. The fact that the *S,O* and *S,N* VL_2Cys complexes are formed in almost equal amounts suggests there is little to choose between nitrogen and oxygen in terms of binding affinity to vanadium in these types of complexes. Since only a single broad signal is observed for the VLCys products, it is not possible to know whether this observation also applies to this type of complex. Although, in VL_2Cys, *N,O*-coordination is as well favoured as is *S,O*- (*S,N*-) coordination at pH 8.5, this will be a pH-dependent phenomenon that is determined by the pK_a values of the various reactants and products.

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