Is Vanadate Reduced by Thiols under Biological Conditions? Changing the Redox Potential of V(V)/V(IV) by Complexation in Aqueous Solution

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Although dogma states that vanadate is readily reduced by glutathione, cysteine, and other thiols, there are several examples documenting that vanadium(V)−sulfur complexes can form and be observed. This conundrum has impacted life scientists for more than two decades. Investigation of this problem requires an understanding of both the complexes that form from vanadium(IV) and (V) and a representative thiol in aqueous solution. The reactions of vanadate and hydrated vanadyl cation with 2-mercaptoethanol have been investigated using multinuclear NMR, electron paramagnetic resonance (EPR), and UV−vis spectroscopy. Vanadate forms a stable complex of 2:2 stoichiometry with 2-mercaptoethanol at neutral and alkaline pH. In contrast, vanadate can oxidize 2-mercaptoethanol; this process is favored at low pH and high solute concentrations. The complex that forms between aqueous vanadium(IV) and 2-mercaptoethanol has a 1:2 stoichiometry and can be observed at high pH and high 2-mercaptoethanol concentration. The solution structures have been deduced based on coordination induced chemical shifts and speciation diagrams prepared. This work demonstrates that both vanadium(IV) and (V)-thiol complexes form and that redox chemistry also takes place. Whether reduction of vanadate takes place is governed by a combination of parameters: pH, solute- and vanadate-concentrations and the presence of other complexing ligands. On the basis of these results it is now possible to understand the distribution of vanadium in oxidation states (IV) and (V) in the presence of glutathione, cysteine, and other thiols and begin to evaluate the forms of the vanadium compounds that exert a particular biological effect including the insulin-enhancing agents, antiamoebic agents, and interactions with vanadium binding proteins.

Introduction

The presence of oxidants and reductants in solution usually leads to redox chemistry.[1] A range of vanadium−sulfur complexes and their chemistries have demonstrated that vanadium−sulfur complexes have interest as models for nitrogenase chemistry,[2] as the vanadium binding proteins,[3,4] as antiamoebic agents,[5−7] as antidiabetic agents,[8] and as catalysts.[9,10] The literature provides many examples that rapid reduction of vanadium(V) compounds takes place in the presence of biological thiols such as glutathione, cysteine, 2-mercaptoethanol, and dithiothreitol (DTT) under physiological conditions.[11−17] Redox reactions of glutathione and cysteine

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(1) Preliminary presentation was presented in: Crans, D. C.; Zhang, B. Y.; Gaidamauskas, E.; Keramidas, A.; Roberts, C. *Abstracts of Papers*, 223rd National Meeting of the American Chemical Society, Orlando, FL, April 7−11, 2002; American Chemical Society: Washington, DC, 2002; INOR-242.

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have also been reported with chromium(VI),[18,19] iron(III),[20,21] and ruthenium(III)[22,23] compounds. Reports on formation and characterization of stable vanadium(V) complexes with thiol-containing ligands[2,5−7,24−26] and their solution chemistry show[26−28] that these complexes can be stable. Slow reduction of vanadium(V) by thiols in biological systems[15,29] implies that vanadium(V) complexes can form, be characterized, and possibly isolated.[24−28,30,31] It was our objective in this study to investigate the seemingly contradictory reports by exhaustively characterizing the reactions of vanadium(V) (oxidant) with 2-mercaptoethanol (reductant), as well as the solution chemistry of both the vanadium(V) and (IV) products. This work provides guidance reconciling this controversy.

Vanadium salts and other vanadium coordination compounds induce insulin-enhancing effects in vitro and in vivo. The human studies with vanadyl sulfate and sodium metavanadate show that simple vanadium salts have reasonable effects even at doses significantly below the "efficacious" dose.[32−34] Studies with bis(maltolato)-oxovanadium(IV),[35−37] bis(acetylacetonato)-oxovanadium(IV),[38] and many other oxovanadium(IV) complexes[39−42] demonstrate that these coordination complexes have higher potency than simple vanadium salts. Although the mechanism of action of vanadium compounds remains elusive, it is generally accepted that inhibition of protein tyrosine phosphatase(s), and subsequent

Table 1. Standard Apparent Reduction Potentials ($E^{0'}$) of Vanadate and Thiols at 298 K and pH $= 7.0$ or pD $= 7.0^a$

redox couple	$E^{0'}$/V vs SHE (pH 7 or pD 7)
$H_2VO_4^- + 4H^+ + e^- \rightleftarrows VO^{2+} + 3H_2O$	-0.341^{49}
$2H_2VO_4^- + 4H^+ + 2e^- \rightleftarrows V_2O_4 + 4H_2O$	0.195^{49}
$1/2$ RSSR $+ H^+ + e^- \rightleftarrows$ RSH	
R = HOCH$_2$CH$_2$ (2-Mercaptoethanol)	$-0.253^{a,51}$; $-0.260^{a,52}$
R = H$_2$NCH(CO$_2$H)CH$_2$CH$_2$CONHCH-(CONHCH$_2$CO$_2$H)CH$_2$ (Glutathione)	$-0.252^{a,52}$; $-0.262^{a,51}$; -0.22^{50}
R = HOOC(NH$_2$)CHCH$_2$ (Cysteine)	$-0.245^{a,51}$
R = $1/2$ H$_2$C(CHOH)$_2$CH$_2$ (Dithiothreitol)	$-0.327^{a,52}$

^{a} Standard apparent reduction potentials determined from thiol-disulfide equilibria in D$_2$O at pD $= 7.0$ and 298 K.

activation of a protein kinase(s), is involved.[15,43−46] The different modes of action of vanadate and peroxovanadate with protein tyrosine phosphatases (1B and Leukocyte Antigen Related (LAR)) have been characterized, the former acting as a reversible inhibitor and the latter as a redox active irreversible inhibitor.[46,47] Mechanistic studies are complicated by the rich hydrolytic chemistry of vanadium compounds and by the facile interconversion between oxidation states (especially between (V) and (IV)) under physiological conditions. Despite (or perhaps because of) the rapid interconversion between the two oxidation states, sufficient information to quantitatively evaluate both vanadium(V) and vanadium-(IV) chemistry with one thiol-containing ligand has not previously been reported.

The half reactions and standard apparent reduction potentials at pH $= 7.0$ for the systems relevant to this study are summarized in Table 1. On the basis of the values of the standard reduction potential for one electron reduction, thiols ($E° \sim -0.26$ V) should not be able to reduce vanadate ($E° = -0.341$ V). Since this reaction occurs, it is clear that concentrations of reaction components and pH play a critical role in whether redox reactions take place. In the case of thiols, the observed solution potential will be similar to the formal standard potential (-0.25 ± 0.01 V in case of 2-mercaptoethanol). This is not the case with the vanadium-(IV)/vanadium(V) redox couple because of the nature of the aqueous vanadium chemistry at neutral pH.[48] At neutral pH only low concentrations of monomeric vanadyl cation ($\sim 10^{-8}$ M) exist because of the low solubility product of vanadyl hydroxide. Furthermore, only low concentrations of vanadate monomer ($\sim 10^{-3}$ M) exist because of the high stability of vanadium(V) oligomers. In a solution containing 1 mM H$_2$VO$_4^-$ the reduction potential will equal 0.01 V

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when the vanadyl cation concentration is determined by the solubility product of [VO(OH)$_2$]. This suggests that thiols are readily oxidized by vanadate. However, the reaction quotient depends not only on the redox potentials describing reduction of vanadate and oxidation of thiol but also on the oligomerization and complex formation. All equilibria must be considered in the overall analysis of complex formation and redox chemistry. In the absence of complexation between vanadium(V) and reducing thiol, redox reactions are thermodynamically favored when a large excess of vanadium(V) over vanadium(IV) is present. Formation of vanadium(V)-thiol complexes can prevent redox chemistry, if the vanadium(V) complex is stabilized more than the vanadium(IV) complex.

We have characterized the stoichiometry, stability, and structure of the species that form in solution with 2-mercaptoethanol, a common reducing component in enzyme assays. The complexation chemistry of vanadate with 1,2-diols has been characterized both in solution and in the solid state.[53-55] While this work was underway, a crystal structure of [(VO$_2$)$_2$(OCH$_2$CH$_2$S)$_2$]$^-$ and some aqueous and organic solution properties were reported, and are used as structural benchmark data.[26] In this work, we study the interplay between complexes of different oxidation states and define the conditions under which vanadate and/or vanadyl cation form complexes with 2-mercaptoethanol. In aqueous solution, the vanadium(V)-thiolate complex (major 2:2 complex) is surprisingly stable. When the vanadium(V) is reduced, a vanadium(IV) complex with a 1:2 stoichiometry forms, attesting to the dramatic influence oxidation state has on complex stoichiometry and structure. Understanding the vanadium(V)-thiolate complexes and their redox chemistry is essential to understand the biological effects of vanadium compounds.

Experimental Section

Chemicals. Reagent grade compounds were purchased from Aldrich Chemical Co, Fisher Scientific, Mallinckrodt, and from Acros Organics (2-hydroxyethyl disulfide (95%)) and used without further purification. Deuterium oxide (Cambridge Isotope Laboratories, 99.9%), NaOD (Merck & Co., 98 atom % D) and DCl (Aldrich, 99.5 atom % D) and [^{17}O]H$_2$O (ICON, 10.43 atom % ^{17}O) were used as received.

Solutions. Deionized water (17.9 MΩ·cm) was used to prepare all the H$_2$O solutions. Stock solutions of sodium vanadate in D$_2$O or in H$_2$O were prepared by adding 1 equiv of V$_2$O$_5$ to 2 equiv of 2 M NaOD or NaOH. The suspension was heated until the solid was completely dissolved. After cooling to ambient temperature the solution was further diluted with D$_2$O or H$_2$O to the desired vanadium concentration (100 or 25 mM). When stock solution with lower pH was needed (Corning 140 pH meter equipped with a combination microelectrode), the

addition of DCl resulted in a bright yellow solution, which was then heated until the yellow color disappeared and the pH remained constant at room temperature (300 ± 1 K).[56] The series of 100 mM solutions of 2-mercaptoethanol in D$_2$O at various pH values were prepared, under N$_2$, just before use. Borate buffer stock solution (∼0.7 M) was prepared by dissolving of H$_3$BO$_3$ in D$_2$O (or H$_2$O) and adjusting pH with NaOD (or NaOH) solution as needed. Mixed aqueous-acetonitrile solutions of vanadate and 2-mercaptoethanol were prepared by adding an aqueous (D$_2$O) vanadate solution to a 2-mercaptoethanol solution in acetonitrile.

The solutions for spectroscopic studies were purged with N$_2$ for 15 min to prevent air oxidation of the thiol and kept under a N$_2$ atmosphere at 300 ± 1 K. Studies without buffers were monitored carefully, and no more than a 0.5 pH unit change was found over any 4 h experiment. All the spectroscopic measurements were made no more than 1 h after mixing the stock vanadate and thiol solutions.

NMR Spectroscopy. NMR spectra were recorded on a Bruker ACP-300 spectrometer operating at room temperature (300 ± 1 K) at 79 MHz for ^{51}V, 300 MHz for ^{1}H, 76 MHz for ^{13}C, and 41 MHz for ^{17}O. ^{51}V NMR spectra were recorded with an acquisition time of 0.04 s, no relaxation delay, 60° pulse-width, and 50 kHz spectral width. Chemical shifts are reported relative to external references of VOCl$_3$ 0 ppm for ^{51}V, TMS for ^{1}H and ^{13}C, and H$_2$O for ^{17}O. Samples for [^{17}O] NMR spectroscopy were enriched with [^{17}O]H$_2$O to 3% [^{17}O]H$_2$O. Parameters for an initial ^{17}O NMR spectrum with a 65,000 Hz spectral window, 0.10 s acquisition time, 90° pulse width, and no relaxation delay preceded a second acquisition using a narrower spectral window of ∼5,000 Hz resetting the transmitter frequency as necessary. Total collection time per spectrum was about 30 min. Baseline corrections were applied.

Quantitative analyses of all the ^{1}H and ^{51}V NMR spectra were performed using Bruker integration software. The concentrations of the vanadium and thiol species in each sample were calculated on the basis of the observed molar fraction of ^{1}H and ^{51}V signals, and the total added vanadium and thiol concentrations assuming reduction to the vanadium(IV) species was negligible. This assumption was confirmed by recording electron paramagnetic resonance (EPR) spectra of solutions with similar concentrations of thiol and vanadate where no more than 2% of a vanadium(IV) species was observed.

EPR Spectroscopy. EPR spectra were recorded using 1 mm o.d. capillary inserted in a 4 mm o.d. quartz EPR tube for ambient temperature (300 ± 1 K) spectra and 4 mm o.d. quartz tubes for low temperature (140 ± 1 K) spectra, on a Bruker ESP 300 spectrometer. To acquire ambient temperature spectra, the spectrometer was operated at 9.77 GHz in the X-band with a microwave power of 20 mW. Previous studies[57] have documented that these parameters avoid signal saturation in similar systems. To acquire the low temperature spectra, the spectrometer was operated at 9.465 GHz in the X-band with a microwave power of 2 mW. A modulation frequency of 100 kHz, a modulation amplitude of 7.95 G, a time constant of 20.48 ms, and a conversion time of 81.92 ms were employed at low temperature. The spectrometer was calibrated with 1,1-diphenyl-2-picrylhydrazyl (DPPH, g = 2.0037).[57]

Computer simulations of the fluid solution EPR spectra were performed with ASYM, which includes second-order corrections to the nuclear hyperfine interaction[58] and the dependence of the line width on vanadium nuclear spin.[59,60] The estimated

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uncertainties are (based on data collection in duplicate and triplicate on identical samples, as well as simulation of one set of spectral data): hyperfine couplings, $\pm 1 \times 10^{-4}$ cm^{-1}; g values, ± 0.002; and concentration of species, $\pm 2\%$. Concentrations of vanadium(IV) species were determined by double-integration of the first-derivative EPR signals and calculated based on a calibration curve generated from solutions of 0.045, 0.091, 0.45, 0.91, 1.8, 4.83, 6.0, and 10.0 mM VOSO$_4$ in 0.1 M H$_2$SO$_4$. Unless otherwise specified all spectra were recorded within 30 min of solution preparation.

UV−visible Spectroscopy. Absorption spectra were recorded on a Perkin-Elmer Lambda 4B spectrometer equipped with a HAAKE A81 circulating temperature bath (300.2 $\pm$ 0.1 K).

Kinetic Measurements. The rate measurements for the formation of disulfide and other species in solutions containing vanadate and 2-mercaptoethanol (from 50 to 300 mM) were done by ^{1}H and ^{51}V NMR spectroscopy. Each measurement was conducted in duplicate. Studies at pH 8.95 were carried out in 0.40 M KCl in 0.40 M borate buffer. All anaerobic samples were purged with nitrogen for 15 min before the solutions were mixed, then the solution pH was adjusted and the sample placed in a NMR tube under nitrogen atmosphere. A control sample was prepared for comparison, containing all reagents except vanadate. During the time of these experiments no more than 15% of the total 2-mercaptoethanol was oxidized to the disulfide.

Data Analysis. Data analysis was carried out using an Excel spreadsheet. All deviations in parameters given indicate the 95% confidence level. The error bars on the figures represent the 95% confidence limit of at least three independent measurements.

Speciation and Calculation of Formation Constants by NMR Spectroscopy. Vanadate and 2-mercaptoethanol deprotonate according to eqs 1 and 2, respectively, in the pH range 7.0 to 10.5. Given the dependence of the pK_a values on ionic strength, for vanadate[61,62] pK_a values were determined at both the ionic strengths used in this study. At low ionic strength (no KCl) the deprotonation constant of 2-mercaptoethanol is 9.7 $\pm$ 0.1 (determined by both ^{1}H NMR spectroscopy and potentiometric titration), and the second pK_a of vanadate is 8.9 $\pm$ 0.2, consistent with those previously reported.[61,63] At high ionic strength the pK_a of 2-mercaptoethanol did not change (9.7), but the second pK_a of vanadate decreased to 8.5 $\pm$ 0.2.

$$H_2VO_4^- \rightleftharpoons HVO_4^{2-} + H^+ \qquad (1)$$

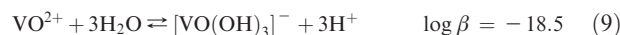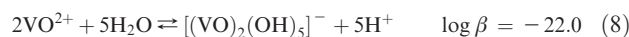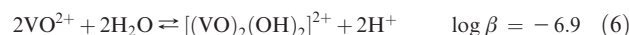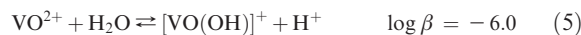$$HOCH_2CH_2SH \rightleftharpoons HOCH_2CH_2S^- + H^+ \qquad (2)$$
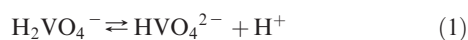
Vanadate, as previously described,[64] forms a series of oligomers including monomer (V$_1$), dimer (V$_2$), tetramer (V$_4$), and pentamer (V$_5$) that were measured by ^{51}V NMR spectroscopy. The concentrations of 2-mercaptoethanol and the oxidation product 2-hydroxyethyl disulfide were calculated from the integration of ^{1}H NMR spectra. Vanadate-2-mercaptoethanol complexes were determined using both ^{1}H and ^{51}V NMR spectra. Using the pK_a values for H$_2$VO$_4^-$ and HOCH$_2$CH$_2$SH measured above, the concentrations of H$_2$VO$_4^-$ (or HVO$_4^{2-}$) and HOCH$_2$CH$_2$SH (or HOCH$_2$CH$_2$S$^-$) can be calculated from eqs 3 and 4. Here [V$_1$] and [HOCH$_2$CH$_2$SH]$_T$ are the total concentrations of free vanadate monomer and [HOCH$_2$CH$_2$SH] + [HOCH$_2$CH$_2$S$^-$].

$$[H_2VO_4^-] = \frac{[V_1]}{1 + K_{a,H_2VO_4^-}/[H^+]} \qquad (3)$$

$$[HOCH_2CH_2SH] = \frac{[HOCH_2CH_2SH]_T}{1 + K_{a,H_2L}/[H^+]} \qquad (4)$$

Speciation and Calculation of Formation Constants by EPR Spectroscopy. The reactions to be considered around neutral pH in these solutions and the logarithms of the respective formation constants and solubility product are summarized in eqs 5 to 9.[65−68] The vanadyl cation as [VO(H$_2$O)$_5$]$^{2+}$ is observed only below pH 3. When the pH is increased, two hydrolysis products form: a paramagnetic [VO(OH)(H$_2$O)]$^+$ species (eq 5) and an EPR silent dimer [(VO)$_2$(OH)$_2$]$^{2+}$ (eq 6).[65,69] At neutral pH the precipitation of [VO(OH)$_2$] takes place (eq 7), which redissolves in basic media. The formation of insoluble hydroxides has previously been used to calculate the concentration of vanadyl cation in the neutral pH range.[66] Two soluble VO^{2+}:OH$^-$ stoichiometries are generally used in solution studies in the pH range from 6 to 13: the 2:5 stoichiometry (EPR silent species [(VO)$_2$(OH)$_5^-$]$_n$; eq 8 for n = 1), and the 1:3 stoichiometry (EPR active species [VO(OH)$_3^-$]$_n$; eq 9 for n = 1).[68−70] Speciation studies in dilute (1−10 mM) vanadium(IV) solutions provide results consistent with n = 1, which is commonly used.[71−73]

$$VO^{2+} + H_2O \rightleftharpoons [VO(OH)]^+ + H^+ \qquad \log\beta = -6.0 \quad (5)$$

$$2VO^{2+} + 2H_2O \rightleftharpoons [(VO)_2(OH)_2]^{2+} + 2H^+ \qquad \log\beta = -6.9 \quad (6)$$

$$VO^{2+} + 2OH^- \rightleftharpoons [VO(OH)_2]\!\downarrow \qquad \log K_{sp} = -22.0 \quad (7)$$

$$2VO^{2+} + 5H_2O \rightleftharpoons [(VO)_2(OH)_5]^- + 5H^+ \qquad \log\beta = -22.0 \quad (8)$$

$$VO^{2+} + 3H_2O \rightleftharpoons [VO(OH)_3]^- + 3H^+ \qquad \log\beta = -18.5 \quad (9)$$

Results and Discussion

Reaction of Vanadate with 2-Mercaptoethanol in Aqueous Solution. Mixing colorless aqueous solutions of 2-mercaptoethanol and vanadate at neutral or slightly alkaline pH immediately generates yellow or orange mixtures both in the presence and absence of oxygen. The color intensity is dependent on the concentration of reactants and solution pH. For example, mixing 12.0 mM mercaptoethanol and 12.0 mM vanadate at pH 7.5 produces a yellow solution, which changes color very slowly

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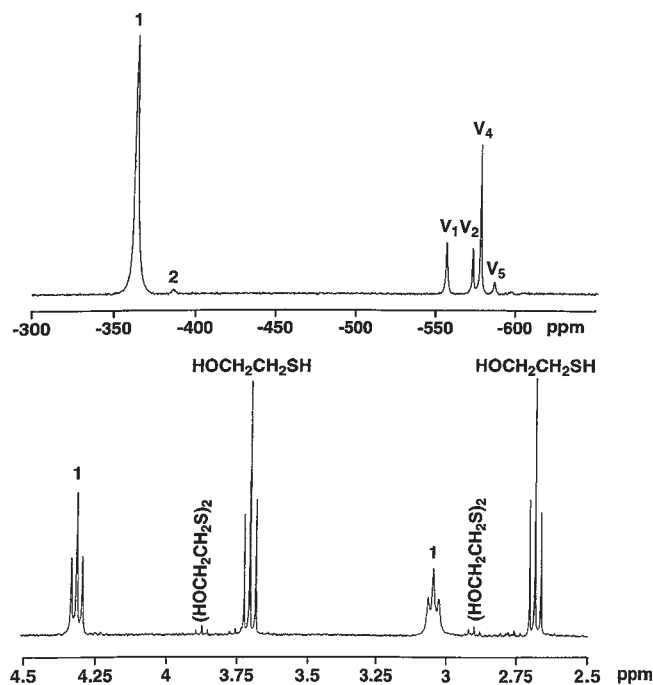(64) Crans, D. C. *Comments Inorg. Chem.* **1994**, *16*, 1–33.

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Figure 1. ^{51}V (top) and ^1H (bottom) NMR spectra of solutions containing vanadate (12.0 mM) and 2-mercaptoethanol (12.0 mM) at pH 8.20 ± 0.05. The spectra were recorded at 300 ± 1 K.

Figure 2. pH dependence of complex [1] and vanadate monomer [V_1] is shown. Data points were obtained by quantitative ^1H and ^{51}V NMR spectroscopy on solutions containing vanadate (4.0 mM) and 2-mercaptoethanol (12.0 mM) in the absence of KCl.

to pale green and eventually to a colorless solution over the course of 2 weeks. A deep orange color forms immediately upon mixing 300 mM mercaptoethanol and 300 mM vanadate solutions at pH 9.0. No ^{51}V NMR signal for decavanadate could be observed. This orange solution changed to dark green and to greenish-black over the course of a few hours. In contrast, mixtures above pH 10.0 remain colorless. A blue-black microcrystalline precipitate can be isolated from concentrated solutions that have turned greenish-black. The isolated blue-black material, upon dissolution in acidic solution, generated the blue vanadyl ion ($[VO(H_2O)_5]^{2+}$) with the characteristic eight line hyperfine pattern in the EPR spectrum. Elemental analysis and IR results confirmed that the blue-black microcrystals are vanadyl oxide VO_2.[74] These data clearly demonstrate that vanadate, in the presence of a thiol such as 2-mercaptoethanol, is reduced to vanadium(IV).

At low concentrations of vanadate and thiol the yellow vanadium(V)-thiolate complexes were observed at neutral pH and persisted for some time. The ^{51}V NMR spectrum of a yellow solution of 12.0 mM vanadate and 12.0 mM 2-mercaptoethanol is shown in Figure 1 (top). This spectrum contains a major signal at −362 ppm (complex **1**) and a minor signal at −385 ppm (complex **2**) in addition to signals due to the labile monomeric and oligomeric vanadate oxoanions. Two complexes are formed by vanadate and DTT at pH 7.5[27] but previously only a 2:2 vanadium(V) complex with 2-mercaptoethanol ($[(VO_2)_2(OCH_2CH_2S)_2]^{2-}$) at −362 ppm was reported.[26] In our system the ^{51}V NMR chemical shifts for both complexes are similar to those reported for vanadate and DTT mixtures.[27]
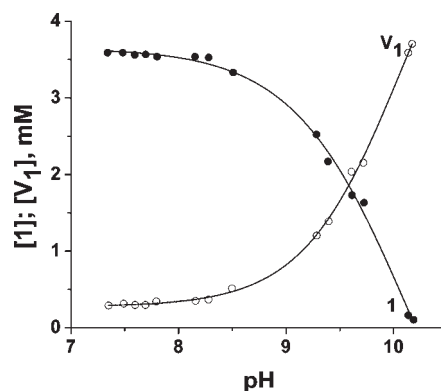
Solutions of 2-mercaptoethanol and vanadate generated ^1H NMR spectra containing signals due to 2-mercaptoethanol, one vanadium(V)-2-mercaptoethanol complex (complex **1**), and the oxidation product of 2-mercaptoethanol: 2-hydroxyethyl disulfide (Figure 1, bottom). The two signals for free 2-mercaptoethanol ligand are centered at 2.68 and 3.70 ppm (Figure 1, bottom). The signals at 2.90 and 3.87 ppm were readily assigned to 2-hydroxyethyl disulfide. The remaining two signals at 3.05 and 4.31 ppm are due to the major complex of vanadium(V) with 2-mercaptoethanol. The chemical shifts of the complex are consistent with the involvement of at least one S-donor in the coordination sphere of the vanadium atom.

Stoichiometry and Formation Constants of the Two Vanadium(V)-2-Mercaptoethanol Complexes. For determining the stoichiometry and formation constant of complex **1**, ^{51}V and ^1H NMR spectra were recorded of freshly prepared solutions containing various concentrations of vanadate (2.0 to 20.0 mM) and 2-mercaptoethanol (2.0 to 20.0 mM). The pH stability of this complex was examined in the pH region of 7.0 to 10.5 as shown in Figure 2 (for a solution containing 4.0 mM vanadate and 12.0 mM 2-mercaptoethanol). Spectra recorded outside this pH range lead to less reliable quantitative data because of the rapid reduction of the vanadium(V), rapid thiol oxidation and/or the low complex concentration.

The linear correlation shown in Figure 3a indicates that the complex **1**, at −362 ppm, has a stoichiometry of 2:2; other complex stoichiometries do not fit the data (Supporting Information, Figure S1). No protons are taken up or released during the reaction. Given the respective pK_a values for $H_2VO_4^-$ and 2-mercaptoethanol and the pH range studied, formation of a complex with a charge of (2−), as shown in eq 10, is consistent with the species present in the solution, their pK_a values, and the structure characterized in the solid state.[26] An analogous linear relationship between [1] and $[H_2VO_4^-]^2$-$[HOCH_2CH_2SH]^2$ was observed at low ionic strength (Supporting Information, Figure S2). The overall (H^+-independent) formation constant for complex **1** at low ionic strength was calculated using eq 11 to be $(3.7 \pm 0.1)\cdot10^8 \, M^{-3}$. The pH independent formation constant of complex **1** in 3.0 M KCl was found to be $(1.2 \pm 0.1)\cdot 10^8 \, M^{-3}$ (Figure 3a). The formation constant for complex **1** at high ionic strength is 3-fold smaller than that at low

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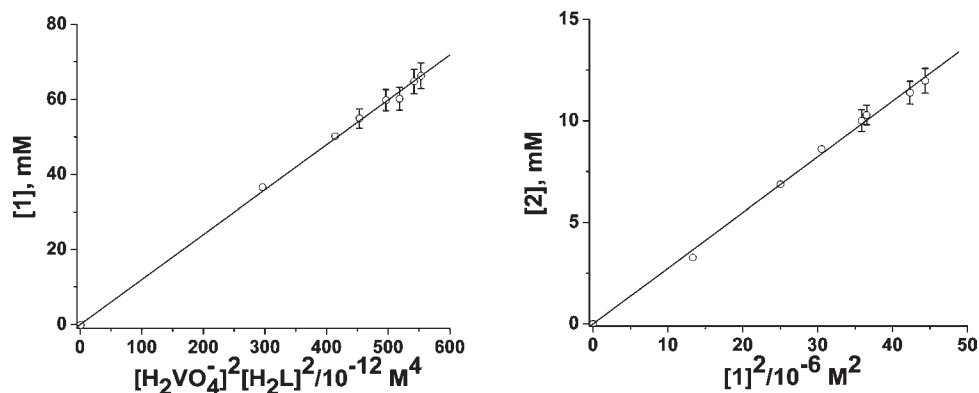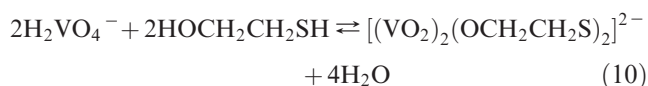
Figure 3. Plots of complexes [**1**] (signal at −362 ppm) and [**2**] (signal at −385 ppm) as a function of $[H_2VO_4^-]^2[H_2L]^2$ (here $H_2L = HOCH_2CH_2SH$) and $[\mathbf{1}]^2$, respectively. Data points were obtained by quantitative ^1H and ^{51}V NMR spectroscopy.

ionic strength as anticipated for a doubly charged dinuclear complex.[61,64]

$$2H_2VO_4^- + 2HOCH_2CH_2SH \rightleftharpoons [(VO_2)_2(OCH_2CH_2S)_2]^{2-}$$
$$+ 4H_2O \quad (10)$$

$$K_{f,1} = \frac{[(VO_2)_2(OCH_2CH_2S)_2^{2-}]}{[H_2VO_4^-]^2[HOCH_2CH_2SH]^2} \quad (11)$$

A minor component, complex **2**, was observed at high vanadate concentrations. As the solution ionic strength increased, the ^{51}V signal intensities for complex **2** (−385 ppm), V_4 (−577 ppm), and V_5 (−586 ppm) increased, whereas the signal intensities for complex **1** (−362 ppm), V_1 (−542 ppm), and V_2 (−566 ppm) decreased (data not shown). The greater sensitivity of complex **2** to ionic strength is consistent with this species being a higher oligomer than complex **1**. The low concentration of complex **2** at modest vanadate concentrations required that this species be studied at high ionic strength.[64] The ^{51}V chemical shifts for both complexes at −362 and −385 ppm do not change from pH 7.0 to 10.5 consistent with no change in the charge of either complex. Solutions containing vanadate (50−150 mM) and 2-mercaptoethanol (50−150 mM) in the presence of 3.0 M KCl at pH 8.4 (primarily) were used to determine the stoichiometry and formation constant of complex **2**. The expectation that **2** is an oligomeric species was confirmed by the linear relationship between [**2**] and [**1**]^2 shown in Figure 3b. Complex **2** has a stoichiometry of 4:4 (eq 12, 13). Other possible stoichiometries were ruled out (see Supporting Information, Figure S3). Experimental difficulties with variation of the solution pH at high vanadate concentrations limited the number of measurements made at other pH values. Using the pK_a values of vanadate and 2-mercaptoethanol in 3.0 M KCl, the H⁺-independent formation constant for the 4:4 species (eq 12, 13) was calculated to be $(3.9 \pm 0.1) \cdot 10^{16}$ M⁻⁷.

$$4H_2VO_4^- + 4HOCH_2CH_2SH \rightleftharpoons [(VO_2)_4(OCH_2CH_2S)_4]^{4-}$$
$$+ 8H_2O \quad (12)$$
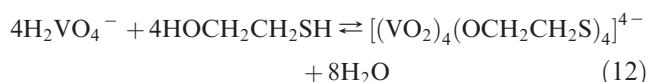
$$K_{f,2} = \frac{[(VO_2)_4(OCH_2CH_2S)_4^{4-}]}{[H_2VO_4^-]^4[HOCH_2CH_2SH]^4} \quad (13)$$

The structure of $(NEt_4)_2[(VO_2)_2(OCH_2CH_2S)_2]$[26] demonstrates an analogy with vanadate-1,2-diolate structures.[53−55,75−79] Higher oligomeric species akin to complex **2** are unprecedented in aqueous solution, but have been reported in studies in organic solvents.[80−82]

Solution Structures for the Vanadium(V) Complexes with 2-Mercaptoethanol. Using the Coordination Induced Shift (CIS, where $\Delta\delta = \delta_{complex} - \delta_{free\ ligand}$)[83,84] in ^{13}C and ^1H NMR spectroscopic studies, we deduced the solution structure for Complex **1**. The ^1H and ^{13}C NMR data for complexes **1** and **2**, free ligand, and CIS values at pH 8.9 are compiled in Table 2. The CIS value for the carbon adjacent to the oxygen is 15−19 ppm is in the range of CIS values commonly observed for carbon adjacent to bridging oxygen atoms.[83,84] The CIS shift for the carbon adjacent to the sulfur is 8.3 ppm. Since limited information is available for CIS shifts of vanadium(V)-thiol complexes, trithiatren [(HSCH$_2$CH$_2$)$_3$N][85] and its vanadium(V) complex [VO(SCH$_2$CH$_2$)$_3$N][31] were synthesized to determine its ^{13}C NMR CIS value. The CIS value for the carbon atoms adjacent to the sulfur in [VO(SCH$_2$CH$_2$)$_3$N] in CD$_3$CN was 14.5 ppm. Unfortunately, this species was not sufficiently soluble in aqueous solution to obtain the CIS value. However, solvent variations in CIS values for related oxygen derivatives show no more than 2−4 ppm variation because of changes in solvent.[83] Furthermore, a 1:1 complex of chromate and glutathione (GSH) has a CIS value, for the carbon atoms adjacent to sulfur, of 11.0 ppm.[19] Since the CIS value in complex **1** is 8.3 ppm the V−S bond in this system is weaker than those described above and suggest that the sulfur is not bridged.

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Table 2. NMR Data for 2-Mercaptoethanol, 2-Hydroxyethyl Disulfide and Complexes **1** and **2**

compound	^{51}V ppm	^{17}O ppm	^{1}H ppm (CIS)[a]		^{13}C ppm (CIS)[a]	
			H1	H2	C1	C2
HOCH$_2$CH$_2$SH			3.70	2.68	66.1	28.8
(HOCH$_2$CH$_2$S)$_2$			3.97(0.27)	2.90(0.22)	62.1(−4.1)	42.8(14.0)
1	−362	988	4.31(0.61)	3.05(0.37)	85.3(19.2)	37.1(8.3)
2	−385	802	4.23(0.53)	2.97(0.29)	83.1(17.0)	36.1(7.3)

[a] Numbers in parentheses indicate CIS (CIS $= \delta_{complex} - \delta_{free\ ligand}$).[83,84]
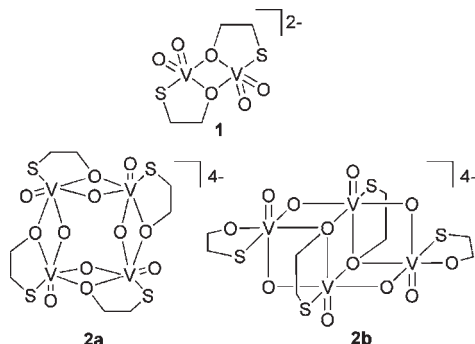
Figure 4. Solution structure for the 2:2 vanadium complex with 2-mercaptoethanol, **1**, and two likely structures for the 4:4 vanadium complex with 2-mercaptoethanol, **2**. Only one of several possible stereo-isomers is shown.

However, a CIS of 8.3 ppm is significant and consistent with formation of a covalent bond. These deductions yield a structure which is identical to that observed in the solid state[26] (Figure 4, **1**), and support the report that $[(VO_2)_2(OCH_2CH_2S)_2]^{2-}$ exists in solution.

Oxygen-17 NMR spectroscopy has been successfully used to distinguish between terminal (non-bridging) oxygen atoms and bridging oxygen atoms in vanadates[86,87] and in molybdates,[88] as well as to provide structural information in vanadate-1,2-diolates and other complexes.[53,89] The ^{51}V and ^{17}O NMR spectra of complex **1** were obtained from a concentrated solution of vanadate (300 mM) and 2-mercaptoethanol (600 mM) in 3.5 atom % ^{17}O enriched water at pH 8.6. The ^{51}V NMR spectrum (Supporting Information, Figure S4 (top)) shows one major peak for complex **1** (97.8%), one minor peak for complex **2** (1.2%), and two minor resonances (1.0%) attributed to vanadate monomer and oligomers. The major peak in ^{17}O NMR spectrum (Supporting Information, Figure S4 (bottom)) with a line width of 407 Hz and a chemical shift of 988 ppm, relative to $H_2[^{17}O]$, accounts for 98.2% of the ^{17}O signals observed (exclusive of $H_2[^{17}O]$) in the region from −100 ppm to +1500 ppm. No signals are observed for $H_2VO_4^-$ or HVO_4^{2-}/VO_4^{3-} which have ^{17}O shifts of 573 and 565 ppm.[87] We assign the resonance at 988 ppm to the oxovanadium oxygen atoms of complex **1**. Integration of a minor peak observed at 802 ppm resulted an intensity of less than 2%, consistent with this signal arising from a different species such as complex **2** and not from a different type of oxygen atom in complex **1**. The similarity of the ^{17}O NMR spectra of the vanadate-2-mercaptoethanol (988 ppm) and the vana-date-ethylene glycol complexes (1004 ppm)[53] support the solution structure being the same as that found in the solid state.[26]

The following consideration for complex **2** supports the proposed two structures **2a** and **2b** shown in Figure 4 with the caveat that the vanadium atoms in structure **2b** are rapidly exchanging as reported previously.[92] The ^{13}C CIS of 17.0 ppm for the carbon adjacent to the oxygen suggests the alkoxide oxygen to be bridging. The CIS of 7.3 ppm for the carbon adjacent to the sulfur indicates a covalent but non-bridging V−S bond. The ^{1}H NMR data support these conclusions (see Table 2). The ^{51}V chemical shift of complex **2** (−385 ppm) is only 23 ppm upfield of complex **1** (−362 ppm) consistent with one oxygen and one sulfur atom of the ligand coordinated to each vanadium atom in complex **2**. The ^{17}O NMR signal supports either a rapidly exchanging tetrameric structure such as **2b** or a structure in which the V=O groups are identical or sufficiently similar to have indistinguishable ^{17}O chemical shifts such as **2a**. Although there are many structural possibilities for complex **2**, the stoichiometry and the similarities in the CIS values between the two complexes rule out most of these possibilities, leaving structures **2a** and **2b** as likely candidates. Structures **2a** and **2b** are related to various tetra-nuclear vanadium alkoxides giving precedence for these types of structures as well as other alkoxides with the [V(OR)OV]-unit.[80−82,90−94]

Vanadium(V) Complexes with 2-Mercaptoethanol in Mixed Aqueous-Acetonitrile Solutions. Since the isolated complex $(NEt_4)_2[(VO_2)_2(OCH_2CH_2S)_2]$ dissolved in CH_3CN has a chemical shift of −385 ppm in between complexes **1** and **2**, spectra were recorded at different D_2O/CH_3CN ratios to reconcile the observation of only one complex in CH_3CN.[26] A series of ^{51}V spectra of solutions containing 50 mM vanadate and 50 mM 2-mercaptoethanol were recorded in D_2O and CH_3CN (from 0 to 87 wt %) and are shown in Figure 5.

The peaks and intensities for complexes **1** and **2** in D_2O (0 wt % CH_3CN) are perturbed when CH_3CN is added. Increasing the concentration of CH_3CN from 0 to 87 wt % results in ~10 ppm shift of the major signal for complex **1**.[26] The signals from complex **2** and vanadate oligomers

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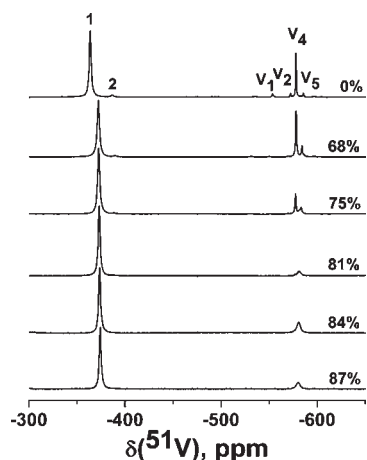(89) Crans, D. C.; Shin, P. K. *J. Am. Chem. Soc.* **1994**, *116*, 1305–1315.

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Figure 5. ^{51}V NMR spectra of solutions containing KVO$_3$ (50 mM) and 2-mercaptoethanol (50 mM) at pH 8.25 ± 0.05. CH$_3$CN is added up to 87 wt %, and spectra at 0, 68, 75, 81, 84, and 87 wt % are shown.

decrease in number and intensity upon addition of CH$_3$CN. Complex **2** did not shift with increasing concentration of CH$_3$CN, but its intensity decreased. These studies confirm that complex **1** forms in both aqueous and CH$_3$CN as reported previously,[26] but that complex **2** is only observed in our studies.

Complex **2** is not observed in aqueous solutions of vanadate and 2-mercaptoethanol when vanadate concentrations are below 8 mM. When complex **1** is formed from solid (NEt$_4$)$_2$[(VO$_2$)$_2$(OCH$_2$CH$_2$S)$_2$] its association, dissociation, and/or hydrolysis is slow, preventing complex **2** formation in these solutions. This hypothesis was confirmed using VT ^{51}V NMR spectroscopy in an aqueous solution of 50 mM of vanadate and 50 mM of 2-mercaptoethanol (pH 8.2) where complexes **1**, **2**, and vanadate were all present. At increasing temperatures the signals for complex **1** and **2** did not coalesce, consistent with no dissociation or association between the two complexes and vanadate. However, as the temperature was increased above 348 K, the concentration of complex **2** decreased below the detection limit. These studies document the kinetic inertness of complex **1**, and low concentration and narrow temperature range over which complex **2** exists.

Characterization of the Vanadium(IV)-2-Mercaptoethanol Complex: Stoichiometry and Formation Constant. Mixing a solution of VOSO$_4$ with 2-mercaptoethanol in the neutral or slightly alkaline pH range generates an EPR spectrum of a new 2-mercaptoethanol complex (complex **3**; parameters: g_0, 1.976 and A_0, 81 × 10^{-4} cm^{-1}). Solutions of VOSO$_4$ in this pH range are grayish-green and turn bright green upon addition of 2-mercaptoethanol. In Figure 6 we show EPR spectra of 10 mM VOSO$_4$ solutions with increasing concentrations (up to 200 mM) of 2-mercaptoethanol at pH 9.6 in borate buffer. In these solutions increasing concentrations of complex **3** are observed.

Formation of **3** is pH dependent as shown in Figure 7 from pH 3.2 to 13.4. At acidic pH only [VO(H$_2$O)$_5$]$^{2+}$ is observed (g_0, 1.968 and A_0, 107 × 10^{-4} cm^{-1}); however, as the pH is increased above 7 the 2-mercaptoethanol complex emerges. A single species, complex **3**, is shown at pH 10.4 in Figure 7.
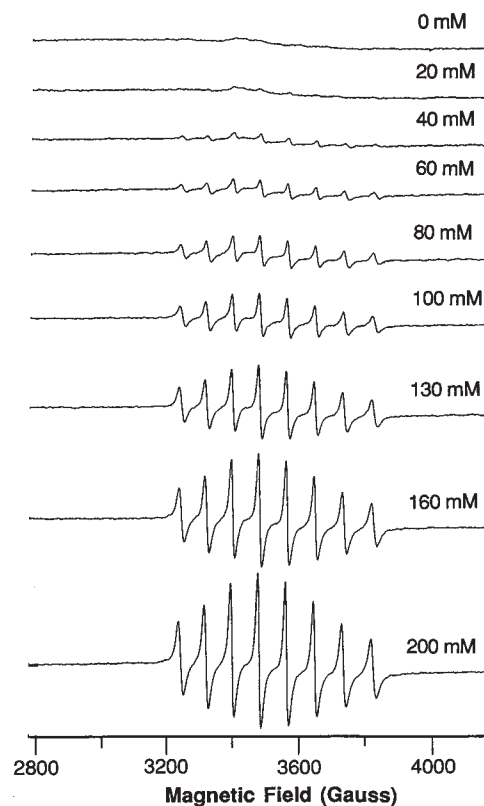

Figure 6. EPR spectra recorded in solutions containing VOSO$_4$ (10 mM) and increasing concentrations of 2-mercaptoethanol (from 0 to 200 mM) in 0.60 M borate buffer (pH 9.6).
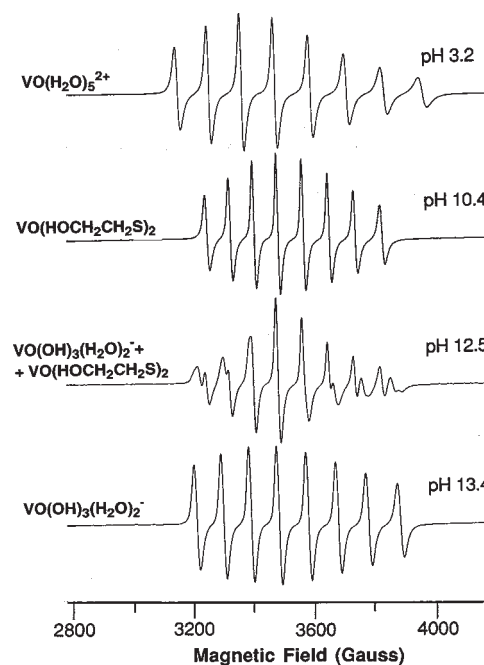

Figure 7. Normalized EPR spectra recorded in solutions with VOSO$_4$ (10 mM) and 2-mercaptoethanol (200 mM) at pH 3.2 to 13.4.

Complex **3** forms in the pH range near the pK_a value of 2-mercaptoethanol (9.7) consistent with the deprotonated 2-mercaptoethanol being the coordinating ligand. In Figure 8 the concentrations of complex **3** and [VO-(OH)$_3$(H$_2$O)$_2$]$^-$ (g_0, 1.973 and A_0, 82.9 × 10^{-4} cm^{-1})[66] are shown in solutions containing a total of 10 mM VOSO$_4$
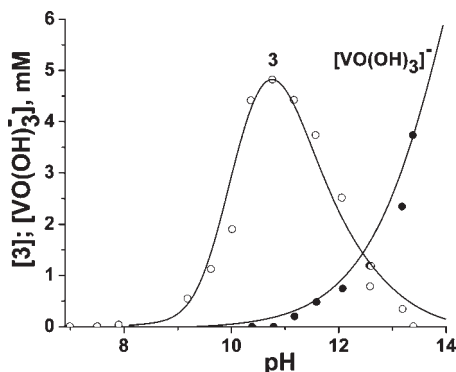
Figure 8. Concentration of complex **3** and $[VO(OH)_3]^-$ ($[VO(OH)_3(H_2O)_2]^-$) are shown as a function of pH. Data points were obtained by quantitative EPR spectroscopy in solutions with VOSO$_4$ (10 mM) and 2-mercaptoethanol (200 mM). No complex signal is observed outside the pH region 8−14. The smooth line combining the experimental points for [**3**] and $[VO(OH)_3]^-$ is calculated using the stability constants[95] and the reaction $VO^{2+} + 2\ HOCH_2CH_2S^- \rightleftharpoons [VO(OCH_2CH_2S)_2]^{2-} + 2\ H^+$.

and 200 mM of 2-mercaptoethanol over the pH range of 7 to 14.

The complex is most stable in the pH range 9.5 to 11.5 (Figure 8). Since high concentrations of $[VO(OH)_3(H_2O)_2]^-$ are observed above pH 10.8, evaluation of the EPR spectrum of **3** is best carried out from pH 9.2 to 10.8 where the spectrum is not complicated by the signal of $[VO(OH)_3(H_2O)_2]^-$. We therefore chose to study the complex at pH 9.6 in borate buffer. The maximum concentration of **3** was found to be 4.88 mM although a total of 10.0 mM of VOSO$_4$ was added to the solution. The additional species existing in solution are EPR silent vanadium(IV) (most likely a dimer $[(VO)_2(OH)_5]^-$) since, up to a pH of 8.5, less than 0.5% of the vanadium(IV) was oxidized within 2 h of addition of 2-mercaptoethanol, as determined by quantitative ^{51}V NMR spectroscopy. After 20 h at pH 8.5 the vanadium(V) complex content has increased to about 2%. At a higher pH, such as 10.3, 4% of the vanadium-(IV) was oxidized within 2 h and 15% within 20 h; however, a significant fraction of the vanadium added to the solutions is still present as EPR silent vanadium-(IV) complexes. As the pH was increased to 12.3 and 13.3, the fraction of oxidized vanadium(IV) increased further to 11% and 30% within 2 h. In the experiments shown in Figure 8, the amount of oxidized vanadium is negligible since the EPR were recorded within 30 min of preparation.

$$pVO^{2+} + rHL^- \rightleftharpoons [(VO)_p(L)_r(H)_{(r-q)}]^{(2p-r-q)} + qH^+ \quad (14)$$
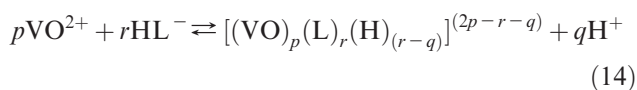
$$K_{f,3} = \frac{[(VO)_p(L)_r(H)_{(r-q)}^{(2p-r-q)}][H^+]^q}{[VO^{2+}]^p[HL^-]^r} \quad (15)$$

The stoichiometry of the complex was determined by quantitative EPR spectroscopy for freshly prepared solutions adding 10.0 mM VOSO$_4$ and from 20 to 200 mM 2-mercaptoethanol in 0.40 M borate buffer at pH 9.6. Equations 14 and 15 describe a general reaction of HL$^-$ (HL$^-$ = HOCH$_2$CH$_2$S$^-$) with $[VO(H_2O)_5]^{2+}$. The concentration of free $[VO(H_2O)_5]^{2+}$ was calculated from the
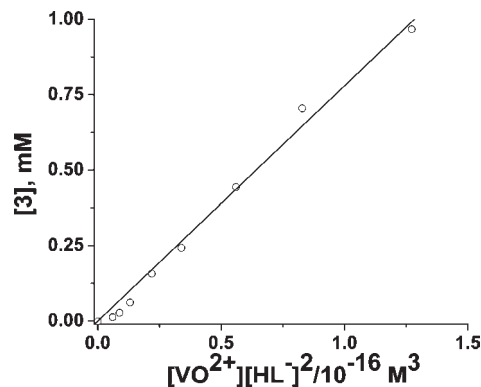
Figure 9. Plot of complex [**3**] in borate buffer at pH 9.6 as a function of $[VO(H_2O)_5^{2+}][HL^-]^2$ (here HL$^-$ = HOCH$_2$CH$_2$S$^-$). The linear relationship indicates a 1:2 complex stoichiometry for **3**. Other possibilities result in non-linear plots (Supporting Information, Figure S5).

concentration of $[VO(OH)_3(H_2O)_2]^-$ assuming three major species are present in the pH region of interest: complex **3**, $[(VO)_2(OH)_5]^-$, and $[VO(OH)_3(H_2O)_2]^-$. Plotting [**3**] as a product of $[VO(H_2O)_5^{2+}][HOCH_2CH_2S^-]^2$ gives a linear plot (Figure 9), which defines the *p* and *r* values (eq 14) as 1 and 2, respectively. Other *p/q* ratios such as 1:1, 2:2, and 1:3 were ruled out (Supporting Information, Figure S5). Only with *q* = 2 did the simulated curve of [**3**] as a function of pH give a maximum near pH 10 and model the complex concentration found experimentally. In Figure 8 the calculated curve is superimposed on the experimental points obtained from EPR studies. On the basis of these considerations, the complex has a (2−) charge, and its formation constant defined by eq 15 (*p* = 1, *q* = 2, and *r* = 2) is equal to 5.1 (±0.2) × 10^{-7}. Modeling studies using species with various *p/r* ratios (such as 1:1, 1:2, 2:1, 2:2, 2:3, and 2:4) and *q* ranging from −2 to 2 did not yield as good a fit to the experimental data. These calculations are consistent with complex **3** being $[VO(SCH_2CH_2O)_2]^{2-}$ in solution.

Proposed Solution Structures for the Vanadium(IV) Complexes with 2-Mercaptoethanol. We measured the ambient (300 K) and low (140 K) temperature EPR spectra of 10 mM vanadyl sulfate and 200 mM 2-mercaptoethanol at pH 9.6. The simulation of the EPR spectra at ambient temperature (Figure 7, pH 10.4) led to a g_0 of 1.976 and an A_0 of 81.0 × 10^{-4} cm^{-1}. From the low temperature spectrum (not shown), the anisotropic *g* factors and hyperfine splitting constants were obtained as $g_{||}$, 1.967; $g_\perp$, 1.980; $A_{||}$, 146 × 10^{-4} cm^{-1}; $A_\perp$, 41.0 × 10^{-4} cm^{-1}. The parameters for $[VO(H_2O)_5]^{2+}$ were g_0 of 1.968 and an A_0 of 107 × 10^{-4} cm^{-1} at room temperature (Figure 7, pH 3.2), and $g_{||}$ of 1.933, $g_\perp$ of 1.985, $A_{||}$ of 182 × 10^{-4} cm^{-1}, and $A_\perp$ of 68.4 × 10^{-4} cm^{-1} at 120 K (spectrum not shown) which are in agreement with previous reports.[66,96,97] The parameters for $[VO(OH)_3(H_2O)_2]^-$ species were g_0 of 1.973 and A_0 of 82.9 × 10^{-4} cm^{-1} at ambient temperature (Figure 7, pH 13.4), and $g_{||}$ of 1.951, $g_\perp$ of 1.978, $A_{||}$ of

(95) The parameters used in the simulation are: log β [VO(OH)$_3^-$] = −19.25; log β [(VO)$_2$(OH)$_5^-$] = −22.8; log β [VO(OCH$_2$CH$_2$S)$_2^{2-}$] = −5.68; pK$_a$ (HOCH$_2$CH$_2$SH) = 10.3.

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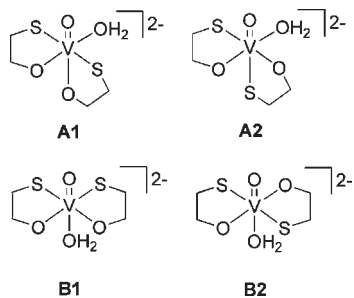
Figure 10. Four possible solution structures for complex **3**.

Figure 11. Absorption spectra of the vanadate- and vanadyl-2-mercaptoethanol complexes prepared under anaerobic conditions from vanadate (20 mM) and 2-mercaptoethanol (200 mM) at pH 8.9 at different times after the solution preparation: (a) 5 min; (b) 5 h; and (c) at pH 3.0 (not time dependent).

161×10^{-4} cm^{-1}, and $A_\perp$ of 46.9×10^{-4} cm^{-1} at low temperature (spectrum not shown).

Four possible structural isomers can be proposed for complex **3**, Figure 10. Since the equatorial ligands provide additive contributions to the $A_\parallel$ an estimation for each structure can be calculated.[98] The partial components in cm^{-1} for H$_2$O (45.7×10^{-4}), aliphatic alkoxide (35.3×10^{-4}), and aliphatic thiolate (31.9×10^{-4}), yields a $A_\parallel$ value of 145×10^{-4} cm^{-1} for A1, 148×10^{-4} cm^{-1} for A2, and 134×10^{-4} cm^{-1} for both B1 and B2, respectively. The observed $A_\parallel$ value of 146×10^{-4} cm^{-1} is consistent with complex **3** being the six-coordinate species A1 or A2 containing a cis-H$_2$O. However, examples of both cis-[99] and trans-adducts[57] have been reported. A detailed description of the vanadium(IV)- glutathione system confirms multiple species with a polydentate glutathione bound to the oxovanadium center.[100] Isolation and structural characterization of a range of oxo and non-oxo vanadium−sulfur complexes support the types of structures proposed in this system.[2,5−11]

UV−vis Spectroscopic Characterization of the Reduction of Vanadate to Vanadyl by 2-Mercaptoethanol. The reduction of vanadate by 2-mercaptoethanol was strongly dependent on solution pH, solute concentration, and the presence of molecular oxygen. The visible spectrum 5 min after preparation of the yellow solution containing 10 mM vanadate and 200 mM 2-mercaptoethanol at pH 8.9 is shown in Figure 11. This spectrum is attributed to the 2:2 complex, since the concentration of the 4:4 complex is negligible under these conditions. After 5 h the solution color had changed to green, with visible spectrum absorption maxima at 558 and 625 nm (Figure 11, pH = 8.9, 5 h). A solution of vanadyl sulfate (10 mM) and 2-mercaptoethanol (200 mM) gives the same absorption spectrum at pH 8.9 (data not shown), consistent with attributing the green color to a vanadium-(IV)-2-mercaptoethanol complex. When the pH of this solution was decreased below pH 4, a blue solution and the visible spectrum characteristic of vanadyl cation resulted. Identical spectra are obtained from solutions of vanadyl sulfate (10 mM) in the absence or presence of 2-mercaptoethanol (200 mM) at pH 3.0 (Figure 11, pH 3.0, 5 min). The maximum at 768 nm and the shoulder at 628 nm obtained from a solution of sodium vanadate in the presence of excess thiol indicate the formation of a
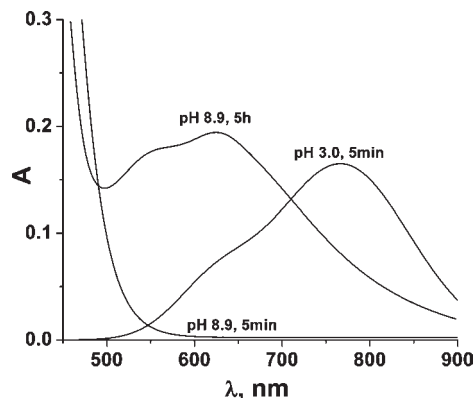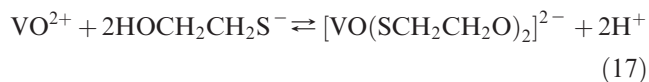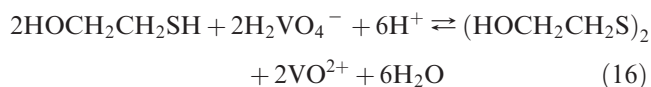
vanadium(IV) complex. A similar spectrum was also observed from a solution containing 2-mercaptoethanol, and the microcrystalline blue-black vanadium(IV) oxide precipitated from solutions at high pH. On the basis of these results, the formation of vanadium(IV)-2-mercaptoethanol complex in vanadate solutions follows the reduction of vanadate by 2-mercaptoethanol. The process is summarized in eq 16 and 17.

$$2HOCH_2CH_2SH + 2H_2VO_4{}^- + 6H^+ \rightleftharpoons (HOCH_2CH_2S)_2$$
$$+ 2VO^{2+} + 6H_2O \qquad (16)$$

$$VO^{2+} + 2HOCH_2CH_2S^- \rightleftharpoons [VO(SCH_2CH_2O)_2]^{2-} + 2H^+$$
$$(17)$$

The reduction of vanadate by 2-mercaptoethanol uses 3 equiv of H$^+$ consistent with the facile reduction in acidic media. At neutral pH or in the presence of excess vanadate, however, the gray suspension of soluble and insoluble vanadyl hydroxides will form and eventually generate the blue-black suspension of V$_2$O$_4$.[49] An excess of 2-mercaptoethanol will complex vanadyl cation in alkaline solution where sufficiently high concentrations of thiolate exist and can remain in solution for some time.

Observations by UV−vis and/or EPR spectroscopy have been reported with related systems.[12−15,101] In our hands vanadate(V) (20 mM) and glutathione (200 mM) at pH 8.9 produced a green solution in agreement with the reports that vanadate is reduced to vanadium(IV) in the presence of glutathione.[16,101] The solution of vanadate and glutathione yielded a similar UV−visible spectrum and EPR spectrum (data not shown) to the 2-mercaptoethanol complex. Interestingly the complex formed from cysteine and vanadyl cation[13] is purple, as is the complex between cysteine methyl ester and vanadyl cation.[102] Presumably this color is due to formation of a VO(S$_2$N$_2$)-type complex.

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Comparing the Stability of Vanadium(IV) and (V) 2-Mercaptoethanol Complexes. Given the fact that 2-mercaptoethanol forms complexes with both vanadium(V) and vanadium(IV), the question of what kind of vanadium species are present at neutral pH depends on the aqueous V(V) and V(IV) speciation chemistry. Unique species are present in both oxidation states, and more complex **1** is present than complex **3** based on the distribution diagrams (Figures 2 and 8). Calculating concentrations of vanadium species for two hypothetical cases at total vanadium concentrations of 1 mM and 1 μM at pH 7 confirmed that [**1**]$\gg$[**3**],[103] i.e., the amount of vanadium(V) bound to the ligand is greater than that of vanadium(IV) from 10^6-fold (at 1 mM) to 500-fold (at 1 μM). The formation of the V(V)-2-mercaptoethanolate and significantly less stable V(IV)-2-mercaptoethanolate complexes suggests that vanadate can coexist in solution with 2-mercaptoethanol because of the low vanadate oxidation potential. Our calculations carried out for a solution containing 1.0 mM vanadium(V), 1.0 mM vanadium(IV), 10 mM 2-mercaptoethanol, and 0.1 mM 2-hydroxyethyl disulfide show that the vanadium(V)/vanadium(IV) reduction potential reaches 2-mercaptoethanol/2-hydroxyethyl disulfide oxidation potential at pH 11.5 ($\pm$0.5). Below pH 11.5 vanadate oxidizes thiol to disulfide.

Rate of Complex 1 Reduction under Anaerobic Conditions. Having documented that 2-mercaptoethanol reduces vanadium(V) to vanadium(IV), it is an important question to determine how fast complex **1** decomposes. The redox reaction proceeds readily at low pH and high solute concentrations with consumption of 3 equiv of H$^+$ (eq 16). Because of H$^+$ consumption, kinetic studies should be carried out in buffered solution. To minimize thiol oxidation by oxygen (which is rapid in basic solution)[104] the reaction solution was nitrogen purged throughout the experiment. Borate was selected as buffer because of its buffering capacity (pK_a 9.24) at pH 8.9 and its lack of ^{1}H NMR signals. Although no new signal is observed in the ^{51}V NMR spectra of vanadate solutions containing borate, an increase in the linewidths of the V_1 and V_2 signals is indicative of an interaction. This interaction is likely to be weak, as previously observed for phosphate,[105] acetate,[106] chromate,[107] and other anions.[64] Given the lack of kinetic
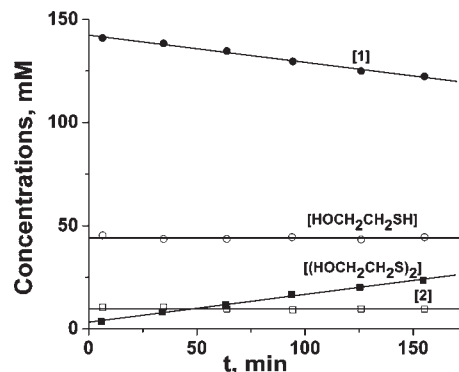

Figure 12. [**1**] and [**2**], [HOCH$_2$CH$_2$SH], and [(HOCH$_2$CH$_2$S)$_2$] during the first 3 h of reaction. The initial solution were added vanadate (200 mM), HOCH$_2$CH$_2$SH (200 mM), KCl (0.4 M), and borate buffer (0.4 M) at pH 8.95.

data on these types of reactions, even limited information on the reactivity of the system is valuable.

A series of ^{1}H and ^{51}V NMR spectra were recorded over 3 h on solutions 0.40 M borate and 0.40 M KCl with varying concentrations of vanadate and 2-mercaptoethanol. The vanadium(V) species rapidly equilibrate[108] with each other so that integrations of the ^{1}H and ^{51}V NMR spectra give the concentrations of each vanadium(V) species. Figure 12 shows the typical changes in concentration of complexes **1** and **2**, 2-mercaptoethanol, and 2-hydroxyethyl disulfide as a function of time. While the concentration of the disulfide increases, the concentrations of **1**, **2**, and the thiol decrease, albeit at different rates. In addition, the concentration of V_1 also decreases.

On the basis of initial reaction rates, we obtained a reaction half-life time $t_{1/2} > 16$ h in concentrated solutions (200 mM of each reactant) and $t_{1/2} > 87$ h (25 mM of each reactant). Under anaerobic conditions complex **1** decomposes only slowly which is in contrast to the decomposition observed in the presence of oxygen (data not shown). Further mechanistic details of the reaction are beyond the scope of this paper.

Conclusion

This work confirms the fact that vanadate can form complexes with thiols without undergoing reduction even though the same thiol under other conditions will induce redox chemistry. Although complex formation suppresses redox chemistry, these studies define conditions under which the reactants do undergo redox chemistry. In the specific case of 2-mercaptoethanol vanadate mainly forms a stable complex (**1**, $\delta(^{51}$V) -362 ppm) of 2:2 stoichiometry with 2-mercaptoethanol at neutral or alkaline pH. A minor 4:4 complex (**2**, $\delta(^{51}$V) -385 ppm) can be observed at high concentrations of vanadate and 2-mercaptoethanol. Vanadate is reduced in the course of oxidizing 2-mercaptoethanol to the disulfide, and rates are highest at low pH and high thiol concentrations. The reduction is sufficiently slow for observation of the coordination chemistry.

Complex **3** that forms between aqueous vanadium(IV) and 2-mercaptoethanol has a 1:2 stoichiometry and is sufficiently stable to be observable at high pH and high 2-mercaptoethanol

(103) The calculation for distribution of vanadium at pH 7.0 carried out for two cases (i) [V(V)]$_{TOT}$ = [V(IV)]$_{TOT}$ = 1.0 mM, [2-mercaptoethanol]$_{TOT}$ = 10 mM, and (ii) [V(V)]$_{TOT}$ = [V(IV)]$_{TOT}$ = 1.0 μM, [2-mercaptoethanol]$_{TOT}$ = 10 mM (Supporting Information, Table 1S) yielded the following results: (a) For the 1.0 mM solution, vanadium(V) species are 75 mol % complex **1**, 20 mol % V_1, 3.4 mol % V_2, and 1.2 % V_4. For the 1 μM solution the major species are 98 mol % V_1 and 2.0 mol % complex **1**. (b) In the 1 mM vanadium(IV) solutions the solubility is limited by the formation of insoluble [VO(OH)$_2$]. The major vanadium(IV) species at 1.0 mM are 28 mol % [(VO)$_2$(OH)$_5$]$^-$ and 0.21 mol % [VO(OH)$_3$]$^-$. At 1 μM the major species are 85 mol % [(VO)$_2$(OH)$_5$]$^-$ and 12 % [VO(OH)$_3$]$^-$. Complex **3** is only a minor species, present at the trace levels 7.6 $\times$ 10^{-5} mol % and 4.2 $\times$ 10^{-3} mol % in the 1 mM and 1 μM solutions, respectively. All percent values in this section represent molar percent of vanadium in oxidation states (IV) and (V).
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concentration. The structure of this complex was deduced from its EPR parameters. Both bis(2-mercaptoethanolate) complexes (V(IV) and V(V)) contain deprotonated ligands. However, the stoichiometries of the complexes in the two oxidation states are different. The amounts of vanadium(IV) and (V) complexes were measured in the same solution which is important to the evaluation of the action of vanadium complexes in biological systems.

Issues regarding literature reports and seeming discrepancies concerning whether or not a particular thiol will or will not reduce vanadate are clarified. We demonstrate that reactions, in which the components' standard reduction potentials are similar, are strictly governed by solution pH, solute concentration, and the stability of a potential complex. In this work we show that the high stability of a vanadium(V) complex can result in its formation, an observation that flies in the face of the common misconception that vanadium(IV) complexes are more stable.[11,107−109] The work described in this paper explains the apparent controversy regarding whether or not vanadium(V)

compounds oxidize thiols under physiological conditions. The redox behavior of vanadium complexes are likely to be important to their insulin-enhancing properties,[43,109−113] and this study provides some guidelines when complexes are reduced.

Supporting Information Available: Further details are given in Table 1S and Figures S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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