it too has a low resistance at room temperature but other physical data could not be obtained because of experimental difficulties.

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Supplementary Material Available: Tables of anisotropic thermal parameters for Y_4I_5C and $Y_6I_7C_2$, extended Hückel input parameters, and overlap populations (3 pages); tables of observed and calculated structure factors for Y_5I_5C and $Y_6I_2C_2$ (6 pages). Ordering information is given on any current masthead page.

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Spontaneous and Reversible Interaction of Vanadium(V) Oxyanions with Amine Derivatives

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The interaction between vanadate and tri- or tetradentate ethanolamine derivatives has been studied by using ^{51}V NMR spectroscopy. The reactions occur spontaneously in aqueous solutions, at ambient temperatures and in the physiological pH range. In addition to one amine group and one hydroxyl group, the ethanolamine derivative should contain a third and/or fourth functionality that is an alcohol, a carboxylic acid, a phosphonium acid, or an amine. The reactions are highly dependent on pH, concentrations of monomeric vanadate, and amine. The stability constants for the complexes are minimum orders of magnitude greater than those found for vanadate derivatives of corresponding ether derivatives, and the high stability is associated with the central nitrogen. Only one vanadium complex is formed in substantial amounts in the reaction of ethanolamine derivatives with vanadate, and that complex is mononuclear in vanadium. Several of the ethanolamine derivatives that form complexes are commonly used buffers in biological and biomedical studies in vitro.

Introduction

Vanadium is a trace element whose role in biological systems is obscure. Several organisms including sea squirts, mushrooms, algae, chickens, and rats require vanadium as an essential element.[1] Human dietary intake averages 10–60 μg/day, and although vanadium appears beneficial at low levels, high levels of this element are toxic.[1] Vanadate ($H_2VO_4^-$) induces many biological effects, including cardiovascular activity and hormonal action, but little is known about the chemical and biochemical function of vanadium. We now describe chemical reactions that can occur under physiological conditions between vanadate and various amine derivatives. These reactions can lead to serious (and unappreciated) complications when biological studies are conducted in vitro and in vivo with vanadate.

Vanadium(V) has the potential to act as a phosphorus analogue.[2] The three pK_a's of vanadic acid are less than 1 pH unit from the pK_a's of phosphoric acid, and as a result the aqueous acid–base chemistry of vanadate is very similar to that of phosphate.[2] The ability of vanadate to substitute for phosphate in many biological systems demonstrates the similarity between these two ions biologically.[1] In addition to its other similarities to phosphate, vanadate also has the tendency to expand the coordination sphere of vanadium and spontaneously generate oligomers[3,4] or other derivatives.[5,6] These remarkable reactions occur at ambient temperatures in aqueous solutions and in the neutral pH range. Examples include the reactions of monomeric vanadate with oligomers of vanadate,[2-4] the reactions of vanadate with catechols and alcohols,[5] and the reactions of vanadate with phosphate or arsenate.[6] Similar reactions have not previously been reported for ligands with non-oxygen donor atoms; this paper describes such a group of reactions. We furthermore show that at least one non-oxo ligand is essential for the high stability of these vanadium complexes.

A vanadium(V) complex of EDTA has been characterized and studied by X-ray crystallography.[7] Related vanadium complexes of the ligand ethylenebis((*o*-hydroxyphenyl)glycine) have recently been studied.[8] Other derivatives of vanadium(V) containing vanadium–nitrogen bonds have been prepared, and in a few cases structural results are available.[9] A derivative of triethanolamine and vanadium(V) is used as a mild oxidation reagent for specific oxidation of α-ethylenic and α-acetylenic alcohols in organic synthesis.[10,11] In vitro studies of biological systems often use triethanolamine and derivatives as buffers.[1,12,13] It is, therefore, of interest to examine whether vanadium ethanolamine complexes form spontaneously in aqueous solutions.

We now describe the spontaneous production of mononuclear vanadium complexes of ethanolamine derivatives in aqueous solutions as observed by ^{51}V NMR spectroscopy.[3,4] The properties

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required of ethanolamine derivatives for the complexation of the vanadium(V) atom in vanadate have been examined, and the stability constants for such complexes have been determined. A convenient method is described to compare the stabilities of vanadium complexes. This method simplifies the problems encountered when amines with different proton affinities as ligands are compared. We have also examined a series of buffers commonly used in biochemical studies[12,13] and have shown that a large number of these will complex the vanadium(V) atom in vanadate. These buffers are therefore not suitable for biochemical or medical studies of vanadate function or activity.

Experimental Section

Reagents. The reagents used in this work were all reagent grade. The water was distilled and further deionized on an anion-exchange column. Vanadium pentoxide was purchased from Fisher Scientific Company and Sarcosine from Sigma. Diethanolamine (DEA) was purchased from Eastman Organic Chemicals and *N*-(hydroxyethyl)-β-alanine from B. F. Goodrich Chemical Co. Other chemicals were purchased from Aldrich and used without purification unless otherwise noted. In the case of sodium 2,2′-iminodiacetate, the commercial product contained two compounds; the 2,2′-iminodiacetate was recrystallized before use.

Spectroscopic Methods. Vanadium-51 is a NMR-active nucleus of 99.75% natural abundance. Although its spin is $^7/_2$, its line widths are relatively narrow and are easily resolved in the vanadium window. ^{51}V NMR is therefore a convenient and informative tool for studies of vanadium(V) reactions. The ^{51}V NMR spectra were recorded on a ^1H 200-MHz Bruker WPSY (4.7 T) spectrometer, a ^1H 360-MHz Nicolet (8.45 T) spectrometer, or a ^1H 500-MHz Bruker (11.7 T) spectrometer. We typically used spectrum widths of 8000 Hz, a 90° pulse angle, an accumulation time of 0.2 s, and no relaxation delay. No change in the integration of various peaks was observed if the relaxation delay was increased; the T_1's were shorter than we could measure using the recovery saturation method. The chemical shifts are reported relative to the external reference standard $VOCl_3$ (0 ppm). In practice we use a solution (pH 7.5) containing the complex of vanadate and diethanolamine as a reference (−488 ppm) since this complex has a ^{51}V NMR resonance in the chemical shift range of interest that only varies slightly as a function of pH and ionic strength.

The concentrations of various vanadium(V) species were determined by integration of the ^{51}V NMR spectra. The NMR sample contained a known total amount of vanadium, which allowed the concentration of each species to be determined from the fraction of the total integrated areas observed in the NMR spectra. In the reactions where monomeric vanadate concentrations ($[V_1]$) were low, small changes in $[V_1]$ will significantly alter this concentration, and the $[V_1]$ was determined from the $V_1 \rightleftarrows V_4$ equilibrium.[4] The $V_1 \rightleftarrows V_4$ equilibrium is also used to determine $[V_1]$ when other species in the reaction mixture have ^{51}V NMR resonances that are in the vicinity or are superimposed on the V_1 resonance. The pK_a for monomeric vanadate was determined to be 8.2 from the changes in the chemical shift in the ^{51}V NMR spectrum as the pH increased.

The UV spectroscopy was carried out on a Perkin-Elmer Lambda-4B spectrophotometer equipped with a constant-temperature cell.

Sample Preparation. NMR Samples. The vanadate solutions for ^{51}V NMR studies were prepared by mixing buffer, amine, potassium chloride, and deuterium oxide. Sufficient vanadate was added from a standard vanadate solution to yield the final concentration of vanadate. The pH and volume were adjusted to the final pH and volume. The standard vanadate solution was prepared by dissolving vanadium pentoxide with 2 equiv of sodium hydroxide to generate a vanadate solution of 0.25 M; this solution was stored at 4 °C. The concentrations of the standard solutions were monitored by UV spectroscopy (at wavelengths in the 260–270-nm range), and no changes in concentrations were observed over the course of 6 months. At no time was acid added to a solution containing vanadate, since vanadate in the presence of acid generates the orange decamer.[2] The NMR sample solutions were stable for more than 1 month, as judged by lack of changes in their ^{51}V NMR spectra. Unless it is otherwise specified, all NMR samples were prepared with an ionic strength of 0.4; the constant ionic strength was maintained with KCl such that contributions of vanadate, buffers, and other solutes were taken into account. Conductivity measurements were performed on each sample to assure the constant ionic strength. These precautions have been taken because the ionic strength influences the equilibrium reactions of vanadate, and therefore it is important to maintain a constant ionic strength when equilibrium studies are conducted.

The value of 0.4 was chosen for two reasons. First, the high ionic strength will prevent changes in ionic strength resulting from differences in protonated forms of the vanadate or the amines. Second, an ionic strength of 0.4 is commonly used to mimic marine environments and is not uncommon in other biological studies.

We have carried out most of the reactions of vanadate with ethanolamine derivatives in solutions containing imidazole. The presence of imidazole was not observed to change the complex or monomeric vanadate concentration within experimental uncertainty. It is possible that imidazole interacts weakly with vanadate; however, such a weak interaction does not affect the studies of the stable complexes examined in this work. Although the effective buffer range of imidazole is limited to the neutral pH range, we have chosen to add this buffer in our reaction solutions outside this pH range. If buffers appropriate for the particular pH range under examination are used, Table V shows significant deviations (up to 20%) in the stability of the complex.

UV Spectroscopy Samples. The vanadate solutions were prepared by first mixing the hydrion buffer and amine and adjusting the pH to the desired value. The vanadate was added from a standard solution (25 mM vanadate) to give a final solution of 0.1–0.25 mM vanadate.

Data Analysis. The equilibrium constants were calculated from (a minimum) duplicate measurements of vanadium species in the reaction solutions. The average values are shown in this paper. Least-squares fitting of lines to the experimental data was done by using Statworks, a program designed for statistical manipulations for the Apple computer. The correlation coefficients are noted in the figure captions. We estimate that the errors in our data were not above 10% and in most cases were significantly below this value.

Results and Discussion

Aqueous Vanadate Reactions. Vanadium(V) in the form of vanadate will readily add other vanadate species in aqueous solutions.[2] These spontaneous reactions will lead to oligomerization of vanadate species; vanadate solutions above 1 mM will contain monomer, dimer, tetramer, and higher oligomers[2-4] at neutral pH. The concentration of monomeric vanadate is dependent on both pH and the total vanadate concentration. We have studied the reaction of vanadate with various polydentate amines at 10 mM vanadate and at varying pH. This total vanadate concentration was used for four reasons in the detailed study of this reaction.

First, high total vanadate concentrations ensure that a small fraction of the vanadate can be converted to complex and still be observed. Second, 10 mM vanadate produces solutions in which most of the vanadate is present as oligomers;[4] the monomeric vanadate concentration is small and relatively constant as the complexing ligand is added during studies at a certain pH. An approximately constant monomeric vanadate concentration simplifies the understanding of the equilibrium considerations involving complex formation. Third, the use of 10 mM total vanadate produces approximately 0.5–0.7 mM monomeric vanadate in the pH range 7–8.[2-4] This is about the maximum concentration of monomeric vanadate one can generate at any vanadate concentration in this pH range. A 10 mM solution of total vanadate will therefore contain a similar concentration of monomeric vanadate as the solutions used in biological studies. Fourth, the use of a high concentration of total vanadate allows observation of weak complexes with buffers or other interfering compounds that would not have been observed if the total vanadate concentrations had been 1 or 2 orders of magnitude smaller.

In addition we have also examined these reactions at low vanadate concentrations (1 and 0.1 mM). Such studies were carried out to ensure the described reactions also occur at low vanadate concentrations, at which biological studies are conducted. To probe the monomolecular nature of the vanadium complex, a series of experiments (at constant pH) were carried out in which the vanadate concentrations were varied. Both the complex concentration and the monomeric vanadate concentration changed at least 1 order of magnitude in these experiments and as predicted for monomolecular vanadium complexes.

Aqueous Vanadate Reactions with Polydentate Amines. Using ^{51}V NMR and UV spectroscopy, we have observed the generation of a new vanadium species when adding diethanolamine to solutions of vanadate. Figure 1 shows the ^{51}V NMR spectra of aqueous solutions of diethanolamine and monomeric vanadate at 10 mM total vanadate concentration and various amine concentrations. These results suggest that diethanolamine (DEA) forms labile complexes (−488 ppm) with vandate in aqueous solutions in the neutral pH range. Upon addition of amine, a new peak

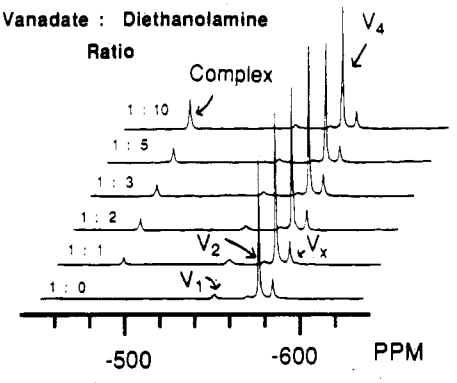


Figure 1. Spontaneously formed vanadium–DEA complex (complex at −488 ppm) observed by ^{51}V NMR spectroscopy as the concentration of DEA is increased. The vanadate concentration is maintained at 10 mM, and at pH 7.8 this generates a solution containing vanadate monomer (V_1 at −546 ppm), dimer (V_2 at −565 ppm), tetramer (V_4 at −572 ppm), and a higher oligomer (V_x at −580 ppm). The concentration ratios of vanadate to amine are marked. The reaction solutions contained 150 mM Tris at pH 7.8 and were made up to a total ionic strength of 0.4 with KCl.

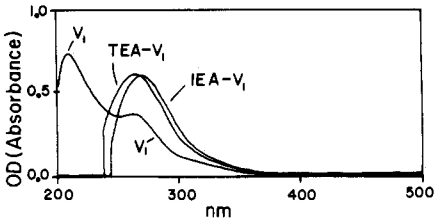


Figure 2. Absorbance spectra of vanadate, the vanadium complex with triethanolamine, and the vanadium complex with triisopropanolamine at pH 8.1. The total vanadium concentration is 0.15 mM, at which concentration the vanadate is exclusively present in the monomeric form. At 100 mM triethanolamine or 100 mM triisopropanolamine the vanadate will have completely formed vanadium complexes (this was observed experimentally by using ^{51}V NMR spectroscopy and can be deduced from the stability constant determined in this work). Both triethanolamine and triisopropanolamine absorb in the region 200–250 nm, so the absorption curves shown have been obtained by subtracting the spectra of the amines from the spectra of the vanadate complexes.

~60 ppm more shielded than monomeric vanadate (−550 ppm) appears in the ^{51}V NMR spectrum and increases in intensity as the concentration of added amine increases. The chemical shift of the new peak is considerably different from that of derivatives of vanadate such as vanadate oligomers,[3,4] vanadate esters,[5] and cyclic vanadate esters,[5] in which at most a bidentate ligand will be bound to vanadium. The chemical shift differences between vanadate oligomers (−530 to −580 ppm), cyclic and acyclic esters (−510 to −540 ppm), and the vanadate–DEA complex (−488 ppm) suggest the DEA–vanadate complex is a different type of complex. Since DEA is tridentate, it is reasonable that it forms a complex with vanadate in which all three functionalities are involved in binding to the vanadium atom. Most of the tri- and tetradentate ligands shown in Table I are found to form a complex with vanadate that produces a ^{51}V NMR peak in the −480 to −500 ppm range. Figure 2 shows the UV spectra of monomeric vanadate, the vanadium–triethanolamine complex and the vanadium–triisopropanolamine complex. The UV spectra were recorded at 150 μM vanadate concentrations and illustrate that the vanadate–triethanolamine complex and the vanadate–triisopropanolamine complex form at these low vanadate concentrations. Since such low vanadate concentrations often are used in biological studies, UV spectroscopy can also be used to probe whether a buffer complexes with vanadate. DEA will be used as a reference compound in this paper when vanadate reactions with amines are discussed.

When reactions with vanadate solutions are conducted in which many forms are in equilibrium with each other, it is not clear which vanadate species is (are) active in the DEA reaction(s). We presume that only one form of the vanadate species reacts with DEA, and since the vanadate oligomers can be described by the monomer, we will examine the equilibrium reactions and complex formation as a function of the monomer concentration.

Table I. Biological Buffers Forming High Concentrations of Complexes with Vanadate[a]

Tridentate Ligands

diethanolamine (−488 ppm)
methyldiethanolamine (−481 ppm)
(2-aminoethyl)ethanolamine[a] (−498 ppm)
iminodiacetic acid (−516 ppm)
N-methyliminodiacetic acid (−511 ppm)
N-(hydroxyethyl)glycine[b] (−506 ppm)
N-(tris(hydroxymethyl)methyl)glycine (Tricine; −504 ppm, pH 8)
N,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES; −485 ppm)

Ligands without Amine Lone Pair

diethanol ether[b] (−519 ppm)
diethanol sulfide[b]

Tetradentate Ligands

triethanolamine (−483 ppm)
1-[bis(2-hydroxyethyl)amino]-2-propanol (−483 ppm)
triisopropanolamine (−483 ppm)
N,*N*-bis(2-hydroxyethyl)glycine (Bicine; −506 ppm, pH 7; −495 ppm, pH 8; −486 ppm, pH 9)
bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bis-Tris; −516 ppm)
N-(2-hydroxyethyl)iminodiacetic acid (−496 ppm)

Tetradentate Ligands—Linear

ethylenediaminediacetic acid[b] (wide peak)[c]
N,*N*′-bis(2-hydroxyethyl)ethylenediamine (−480 ppm, pH 7; −490 ppm, pH 9)

Polydentate Ligands

N-(2-hydroxyethyl)ethylenediaminetriacetic acid[b] (two peaks: −493 and −515 ppm (major), pH 7; −490 (major) and −515 ppm, pH 9.0)
ethylenediaminetetraacetic acid (EDTA;[b] wide peak)[c]
ethylenebis(oxyethylenenitrilo)tetraacetic acid[b] (wide peak)[c]
ethylenediaminetetrakis(methylenephosphonic acid)[d,e] (wide peak)[c]
hexamethylenediaminetetrakis(methylenephosphonic acid)[d,f] (wide peak)
diethylenetriaminepentakis(methylenephosphonic acid)[d,g] (wide peak)[c]

[a] The ^{51}V NMR chemical shifts of the ligand–vanadate complexes are indicated in parentheses following the ligand. In cases where the chemical shifts vary with pH, the pH is indicated following the chemical shift value. The vanadate concentrations were 10 mM, and the reaction with the ligand was examined at pH 7, 8, and 9 at about 100 mM ligand concentration. The buffers used at these pHs were imidazole (pH 7), Tris (pH 8), and taurine (pH 9); these studies were carried out at an ionic strength of 0.4 with KCl. [b] These compounds are not actually used as buffers. Some are however used in biological studies for various reasons, and others have been included to illustrate the extent of the vanadate reaction. [c] The ligand generates complexes with ^{51}V resonances wider than 15 ppm. [d] This sample was generously given to us by Dr. Mark A. Adams courtesy of the Monsanto Chemical Co. [e] Trade name Dequest 2041. [f] Trade name Dequest 2051. [g] Trade name Dequest 2061.

Reaction Scope. The structural requirements for the organic ligands to react with vanadate have been determined with respect to the number of chelating functionalities, the nature of the functionalities, and the central atom in the ligand. The ligands forming complexes with vanadate are listed in Table I, and the ligands that did not form a similar type of complex are listed in Table II.

The first eight ligands in Table I are ethanolamine-derived tridentate ligands. The structural alterations of the DEA ligand were replacements of hydroxyl groups with carboxylic acid groups and amine groups and alkylations of the nitrogen and the alkyl arm. Replacing one ethanol functionality in DEA with one or two acetate arms increased the intensity of the new signal observed in the ^{51}V NMR spectrum, suggesting that the ability of the ligand to form complexes is increased. Alkylating the amine nitrogen also increases the ability of this ligand to form vanadium com-

Table II. Biological Buffers and Other Ligands That at Low Concentrations Do Not Form High Concentrations of Complexes with Vanadate[a]

Tridentate Ligands
diethylenetriamine
Bidentate Ligands
ethanolamine[a]
tris(hydroxymethyl)aminomethane (Tris)[a]
Bis-Tris-Propane[a]
ethylenediamine
glycine[a]
sarcosine (*N*-methylglycine)[a]
N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES)[a]
2-aminoethanethiol
2-aminoethanesulfonic acid (taurine)[a]
Ligands without Amine Lone Pair
tetraethanolammonium chloride
acetyldiethanolamine
1,5-pentanediol
Ligands with Long Arms
bis(3-hydroxypropyl)ethanolamine
N-(hydroxyethyl)-β-alanine

[a] Commonly used buffer in biological studies. It should be noted, however, that we observed vanadate forms weak complexes with most of these compounds, for example, Tris and Bis-Tris-Propane, so these buffers are not to be recommended for biological studies using vanadate if weak interactions of vanadate are important for the outcome of the study.

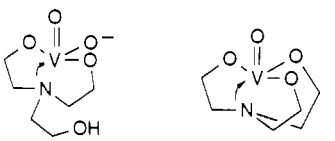
plexes. Replacing one ethanol arm with ethylamine maintains the ligand's ability to form complexes, and in this case two of the three functionalities interacting with the vanadium atom are amines! The chemical shift of the vanadium complex is −498 ppm and is within the range for tridentate ligand complexes (−480 to −500 ppm). Exchanging both ethanol arms with ethylamine gives the only examined tridentate ligand of this type, diethylenetriamine, that does not form these vanadate complexes as indicated by the ^{51}V NMR spectrum of this reaction mixture (Table II, Tridentate Ligands).

Whether bidentate ligands could react with vanadate in a fashion similar to that for DEA was examined for nine ethanolamine-derived bidentate ligands. Replacing DEA with any bidentate ligand eliminates or strongly reduces the intensity of the new signal in the ^{51}V NMR spectrum, suggesting that the ability of the organic fragment to bind to vanadate is reduced (Table II, Bidentate Ligands). Ethylenediamine does not bind to vanadate at any concentration level we have examined. Although somewhat unusual for other metal ions, this finding corresponds to the inability of the vanadyl cation to bind ethylenediamine.[14] Ethanolamine and alkylated ethanolamines do not generate cyclic vanadate complexes, but these compounds will at high concentrations form vanadate esters[15] as a result of the reaction between vanadate and the hydroxyl group in ethanolamine. Vicinal diols[5] form cyclic vanadate esters when the diol is present in large excess. Such cyclic vanadate esters differ from the vanadium complexes observed with DEA with respect to stability and spectroscopic properties.

We also probed the role of the central amine in the ligand in its reaction with vanadate. We tested five ligands in which the central amine functionality had been altered or substituted (see Table I and II, Ligands without Amine Lone Pair). When the lone pair of the nitrogen atom was tied up by alkylation of triethanolamine (TEA), the ligand lost the ability to strongly complex vanadate (e.g., tetraethanolamine). When the lone pair of the nitrogen atom was tied up by resonance delocalization by acetylation of DEA (e.g., acetyldiethanolamine), no vanadate complex was observed. If the amine was substituted by a methylene group (e.g., 1,5-pentanediol), the ligand no longer strongly complexed the vanadate (Table II, Ligands without Amine Lone Pair). If lone-pair-containing atoms such as oxygen or sulfur substituted the nitrogen (e.g., diethanol ether and diethanol sulfide), a complex between these derivatives and vanadate would form; however, such vanadate derivatives are much less stable than the complex formed between vanadate and diethanolamine (Table I, Ligands without Amine Lone Pair). The ^{51}V NMR chemical shifts of these derivatives are shielded by 20–50 ppm from the vanadium–DEA complex, which suggests that neither the oxygen nor the sulfur donates much electron density to the vanadium,[16] and the ligand might not even have all three groups firmly complexed to vanadium.

Changing the length of the chelating arm might have an effect on the ability of the ligand to react with vanadate. We examined three ethanolamine derivatives in which at least one arm contained a propylene fragment (e.g., bis(3-hydroxypropyl)ethanolamine). Compounds with ethanol and propanol arms (or carboxylic or phosphonium acid derivatives thereof) were not found to react with vanadate in the same manner as DEA (Table II, Ligands with Long Arms). A distance of ethylene is therefore crucial for the complexation reaction to occur between amine and vanadate. Methyldiethanolamine forms a complex with vanadate that in the ^{51}V NMR spectrum is 6 ppm more shielded than the signal from the complex formed with DEA. We interpret this perturbation in the chemical shift as a change caused by the stronger electron-donating effects of nitrogen in methyldiethanolamine compared to those of the nitrogen in diethanolamine.[16] This conclusion is supported by the fact that methyldiethanolamine generates complexes with vanadate that are more stable than the corresponding complexes with diethanolamine.

The addition of one more ethanol arm to the central amine in DEA generates a tetradentate ligand, and the six tetradentate ligands examined were all found to generate complexes with vanadate (Table I, Tetradentate Ligands). These ligands are of interest because they can form two types of complexes with vanadate, one anionic (T1) and one neutral (T2). Although vanadium–triethanolamine derivatives prepared under anhydrous conditions were not characterized by crystallography, a T2-like structure was proposed under anhydrous conditions.[10] We present the following spectroscopic observations to probe the form of complex that is generated in aqueous solutions.



T1 T2

Since triethanolamine and vanadate complexes are spontaneously formed in aqueous solutions, it is not likely necessary that they correspond to the species that have been prepared under anhydrous conditions. Electron densities and steric environment in the vicinity of vanadium are both factors known to influence the ^{51}V NMR chemical shifts of vanadium compounds.[16] The chemical shifts of vanadium complexes of the tetradentate ligand TEA and the tridentate ligand methyldiethanolamine are almost identical. The chemical shift of a T2-type vanadate–TEA complex would be very different from the chemical shift of the vanadate–DEA complex, because one negatively charged oxygen bound to vanadium is substituted with an –OR group. This expectation is supported by the chemical shift of −598 ppm for vanadate triethyl ester in methylene chloride[16] and the chemical shift of −551 ppm for vanadate diethyl ester in aqueous solution.[5] A 2 ppm difference is observed when the chemical shift of the vanadium complex formed by methyldiethanolamine is compared to that of the vanadium complex formed by triethanolamine. The tridentate amine DEA cannot form a T2-type complex but only an anionic complex of the T1 type. Since the tetradentate amine

(14) Chasteen, N. D. In *Biological Magnetic Resonance*; Berlind, L., Reuben, J., Eds.; Plenum: New York, 1981; Vol. 3, p 53.
(15) We call these vanadium alkoxides vanadate esters to emphasize the similarities of these systems with the phosphate esters.
(16) Rehder, D. *Magn. Reson. Rev.* **1984**, *9*, 125.

TEA is expected to form a type of complex similar to the vanadate–DEA complex, it is reasonable to expect that the time-averaged complex of TEA and vanadate has only two of the three ethanol arms bound to vanadium (T1) and the overall charge of the complex is −1. Ample precedence for such multidentate ligand complexation in which functionalities are unbound exist with other metal ions.[17]

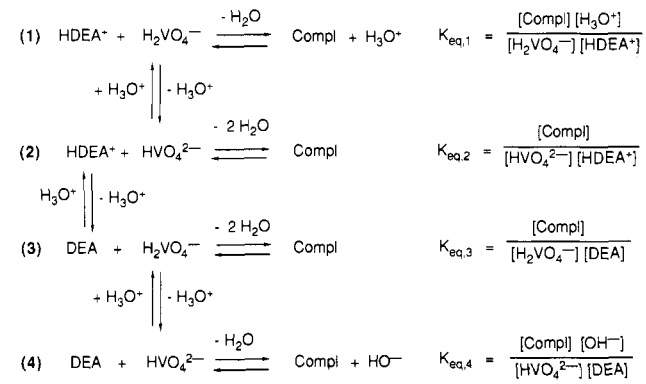
The expectation that an anionic complex forms between vanadate and triethanolamine in aqueous solution is also supported by the following observations of the vanadate complexes formed by *N*-(hydroxyethyl)glycine, iminodiacetic acid, *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine), and *N*-(hydroxyethyl)iminodiacetic acid. The presence of a complexing carboxylic acid in the ligand *N*-(hydroxyethyl)glycine shields the chemical shift by 18 ppm and increases the line width to 360 Hz of the ^{51}V NMR signal. Two complexing carboxylic acids in the ligands iminodiacetic acid shield the chemical shift by approximately 28 ppm and dramatically increase the line width. Bicine contains two ethanol arms and one acetic acid arm, and when it complexes with vanadate, its chemical shift becomes −497 ppm at pH 8.0, with a line width of 284 Hz.[18] *N*-(Hydroxyethyl)iminodiacetic acid contains two acetic acid arms and one ethanol arm. When it complexes with vanadate, its chemical shift is −498 ppm and the line width is 365 Hz. Since these complexes have almost identical chemical shifts, both time-averaged complexes are likely to involve one acetic acid arm and one ethanol arm bound to vanadium. Since both of these tetradentate ligands (Bicine and *N*-(hydroxyethyl)iminodiacetic acid) have three arms, only two of these arms will be bound to the vanadium at any point in time. A chemical shift of −497 to −498 ppm is therefore consistent with a complex formed from vanadate and a ligand where one acetic acid group has substituted the ethanol arm. The line broadening of the signals, on the other hand, may reflect the dynamic equilibrium involving the carboxylic acid arm in the complex. On the basis of the above observations, it seems reasonable to expect that most of the complexes involving vanadate and tetradentate ligands such as TEA, *N*-(hydroxyethyl)iminoacetic acid, and derivatives are expected to be anionic complexes of the T1 type. These are, however, speculations based on changes in chemical shifts, and cases exist in which the chemical shifts have resulted in erroneous conclusions.[19] These considerations presented cannot be experimentally substantiated for the vanadate–DEA-type (or –TEA-type) complexes by varying the pH of the reaction solution, since the first pK_a of the complex is expected to occur in the pH region where the vanadate–DEA complex does not form. We have, however, observed this first pK_a in complexes that show a pH dependence different from that of the vanadate–DEA-type complex discussed in this work.

Tetradentate ligands can also be linear if the new arm is added to a terminal functionality (e.g., ethylenediamine-*N*,*N*-diacetic acid). Two such ligands were examined and found to complex with vanadate (Table I, Tetradentate Ligands—Linear). Such vanadium complexes formed in lower concentrations than the equivalent nonlinear tetradentate amines.

Table I includes several polydentate ligands that have structural moieties described in the above discussion and these as expected also generate complexes with vanadate. Many of the ligands listed in Table II have the potential to form labile esters with vanadate through the hydroxyl groups in these ligands, and in several cases we have observed monoester formation. Monoesters, however, do not form in appreciable concentrations even at high ligand concentrations (1000-fold excess) and do not compete favorably with the complexes described in this work.

(17) Stephens, F. S. *J. Chem. Soc. A* **1969**, 1723.
(18) The chemical shift of the vanadium–Bicine complex at pH 7.0 is at −506 ppm, and the line width is 300 Hz. At pH 9.0 the line width decreased to 160 Hz and the chemical shift increased to −486 ppm, which is only 2–3 ppm different from the chemical shifts of both the vanadium–DEA and the vanadium–TEA complexes. The vanadium–Bicine complex may be formed by one ethanol and one acetic acid arm at pH 7–8, but at higher pH the complex will be formed from the two ethanol arms.
(19) Tracey, A. S.; Gresser, M. J.; Parkinson, K. M. *Inorg. Chem.* **1987**, *26*, 629. See also ref 4.

Scheme I. Various Equilibrium Reactions between Monomeric Vanadate and an Amine (DEA)[a]



[a] Experimental data relating to these equilibria are presented for the reaction of vanadate and DEA and analyzed in Figures 3–5.

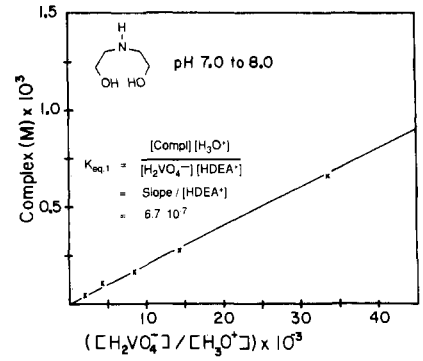


Figure 3. Concentration of the vanadium–DEA complex plotted as a function of $([H_2VO_4^-]/[H_3O^+]) \times 10^{-3}$ in the pH range 7–8. In this pH range $[H_2VO_4^-]$ approximately equals the total monomeric vanadate concentration ($[V_t]$) determined from the ^{51}V NMR spectra and diethanolamine is completely protonated ($[DEA]_t = [HDEA^+] = 30$ mM). From the slope (correlation coefficient 1.000) $K_{eq,1}$ is determined to be 6.7×10^{-7}. The reaction solutions contained 150 mM imidazole, 10 mM vanadate, and 30 mM diethanolamine and were made up to a total ionic strength of 0.4 with KCl.

In summary, ethanolamine derivatives will form complexes with vanadate when their third and/or fourth functionality is an alcohol, a carboxylic acid, a phosphonium acid, or an amine. The central functionality should be a nitrogen with an available lone pair, and the amine should be substituted with at least two ethyl arms. These structural features are crucial for the activity of the ligand, and if they are altered the compound will either lose the ability to complex with vanadate or generate complexes at several orders of magnitude lower concentration. Polyfunctional ligands will complex with vanadate when they contain the described structural fragments.

Equilibrium Considerations. In a description of the equilibrium reaction of monomeric vanadate with DEA derivatives, several ionic species must be considered. Monomeric vanadate can be monoprotonated or diprotonated, and the amine can be protonated or neutral. This will lead to the equilibria presented in eq 1–4 (Scheme I).

When examining a specific pH range, one can simplify the equilibrium considerations as follows. At low pH and below the pK_a of $HDEA^+$, the principal ionic forms are $HDEA^+$ and diprotonated vanadate (eq 1). The reaction of vanadate with diethanolamine follows this equilibrium expression in the pH range 7.0–8.0, and $K_{eq,1}$ is determined to be 6.7×10^{-7} (Figure 3).

As the pH increases, the concentration of monoprotonated vanadate will increase and will eventually become the major monomeric vanadate form. If the amine is still protonated, the equilibrium can then be described by reaction between protonated amine and monoprotonated vanadate (eq 2).

In the pH range close to the pK_a of $H_2VO_4^-$, both the mono- and the diprotonated forms of monomeric vanadate are present

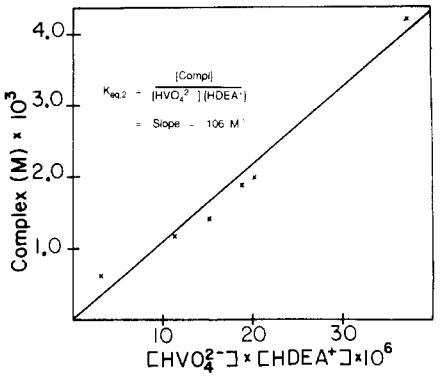


Figure 4. Concentration of the vanadium–DEA complex plotted as a function of $[HVO_4^{2-}][HDEA^+]$ in the pH range 8.25–8.8. $[HVO_4^{2-}]$ is calculated from the $[V_1]$ value determined from the ^{51}V NMR spectra and from $pK_{a,H_2VO_4^-}$ (8.2). $[HDEA^+]$ is calculated from the $[DEA]_t$ = 30 mM and the pK_a of $HDEA^+$ (8.88). From the slope (correlation coefficient 0.998) $K_{eq,2}$ is determined to be 106 M^{-1}. The reaction solutions contained 150 mM imidazole, 10 mM vanadate, and 30 mM diethanolamine and were made up to a total ionic strength of 0.4 with KCl.

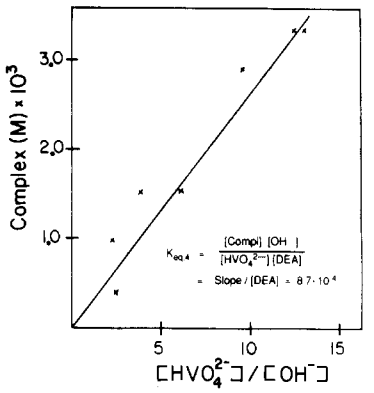


Figure 5. Concentration of the vanadium–DEA complex plotted as a function of $[HVO_4^{2-}]/[OH^-]$ in the pH range 9.5–10.5. In this pH range $[HVO_4^{2-}]$ approximately equals the total monomeric vanadate concentration ($[V_1]$) determined from the ^{51}V NMR spectra and the diethanolamine is almost completely deprotonated ($[DEA]_t$ = [DEA] = 30 mM). From the slope (correlation coefficient 0.972) $K_{eq,4}$ is determined to be 8.7×10^{-4}. The reaction solutions contained 150 mM imidazole, 10 mM vanadate, and 30 mM diethanolamine and were made up to a total ionic strength of 0.4.

in significant amounts and must both be considered. The concentrations of both mono- and diprotonated monomeric vanadate are obtained from $pK_{a,H_2VO_4^-}$ (=8.2) and $[V_1]$ in this pH region. We have plotted [Compl] as a function of $[HVO_4^-][HDEA^+]$ at pH 8.25–8.8 in Figure 4. In this pH region, $HDEA^+$ also deprotonates (pK_{HDEA^+} = 8.88), and $[HDEA^+]$ must be adjusted accordingly. As shown in Figure 4, a linear relationship between [Compl] and $[HVO_4^-][HDEA^+]$ is observed. The slope in this plot determines $K_{eq,2}$ to be 106 M^{-1}.

We conduct the following analysis to examine whether the data are internally consistent. The combination of eq 1 and eq 2 shows $K_{eq,1}$ is related to $K_{eq,2}$ (eq 5). Calculating $K_{eq,2}$ by using $K_{eq,1}$ and $K_{a,H_2VO_4^-}$ also gives 106 M^{-1}. This value is identical with the $K_{eq,2}$ value determined directly from Figure 4 and shows that the determinations of $K_{eq,1}$ and $K_{eq,2}$ are internally consistent.

$$K_{eq,1} = K_{eq,2}K_{a,H_2VO_4^-} \tag{5}$$

In cases where the pK_a of the amine is low, there may be a pH range in which the equilibrium expression must include amine (neutral) and diprotonated monomeric vanadate (eq 3).

At high pH, diethanolamine will not be protonated and the equilibrium involves only monomeric vanadate and neutral amine (eq 4). The reaction of vanadate with diethanolamine follows this equilibrium expression in the pH range 9.2–10.5, and $K_{eq,4}$ is determined to be 8.7×10^{-4} (Figure 5). Combining eq 1 and eq 4 shows that $K_{eq,1}$ is related to $K_{eq,4}$ as described in eq 6.[20]

$$K_{eq,4} = \frac{K_{eq,1}K_w}{K_{a,H_2VO_4^-}K_{A,HDEA^+}} \tag{6}$$

When $K_{eq,4}$ is calculated from $K_{eq,1}$, a value of 8.1×10^{-4} is obtained.[19] $K_{eq,4}$ determined from Figure 5 deviates only 6% from the $K_{eq,4}$ value determined from eq 6. Our data are therefore internally consistent in describing the equilibria reactions of vanadate.

Stoichiometry of Vanadium in the Complexes. Although only one resonance is observed in the DEA–vanadate reaction, this resonance might be a mononuclear or a binuclear vanadate complex. Alternatively, the resonance could be a combination of both mono- and binuclear vanadate complexes if such complexes have chemical shifts that fortuitously overlap. We have therefore carried out a series of experiments in which the vanadate concentration is changed and the pH and diethanolamine concentration are maintained constant. These experiments were conducted at pH 9.5, under which conditions that monomeric vanadate is monoprotonated and diethanolamine is neutral. The reaction of vanadate with diethanolamine should follow the equilibrium

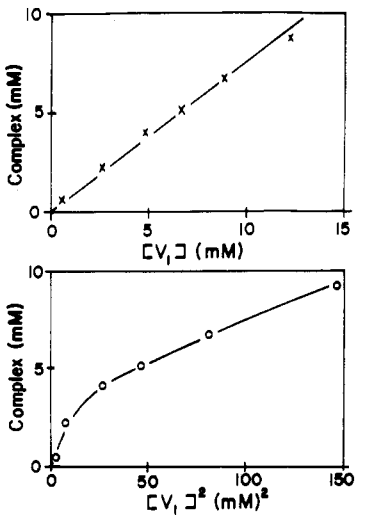


Figure 6. [Compl] plotted as a function of (top) $[HVO_4^{2-}]$ (correlation coefficient 0.999) and (bottom) $[HVO_4^{2-}]^2$. The reactions were carried out in 30 mM DEA and 150 mM imidazole and at pH 9.5. The total ionic strength was maintained at 0.4 by adding KCl. $[HVO_4^{2-}]$ varied from 0.5 to 15 mM while the total vanadate concentration varied from 1 to 50 mM.

shown in Scheme I, eq 4. If the mononuclear complex condenses to a binuclear complex (eq 7 or eq 8), the equilibrium constants of such a reaction should be expressed as eq 9 or as eq 10.

$$2\text{Compl} \rightleftarrows \text{BiCompl} + H_2O \tag{7}$$

$$\text{Compl} + H_2VO_4^{2-} \rightleftarrows \text{BiCompl} + H_2O \tag{8}$$

$$K_{eq,7} = \frac{[\text{BiCompl}]}{[\text{Compl}]^2} \tag{9}$$

$$[\text{BiCompl}] = \frac{[H_2VO_4^{2-}]^2K_{eq,7}K_{eq,4}[\text{DEA}]^2}{[OH^-]^2}$$

$$K_{eq,8} = \frac{[\text{BiCompl}]}{[\text{Compl}][HVO_4^{2-}]} \tag{10}$$

$$[\text{BiCompl}] = \frac{[HVO_4^{2-}]^2K_{eq,8}K_{eq,4}[\text{DEA}]}{[OH^-]}$$

Substituting [Compl] from eq 4 into eq 9 and 10 gives [BiCompl] as a function of $[H_2VO_4^{2-}]^2$, and plotting the concentration of

(20) The K_w value used in our calculations is equal to 10^{-14}.

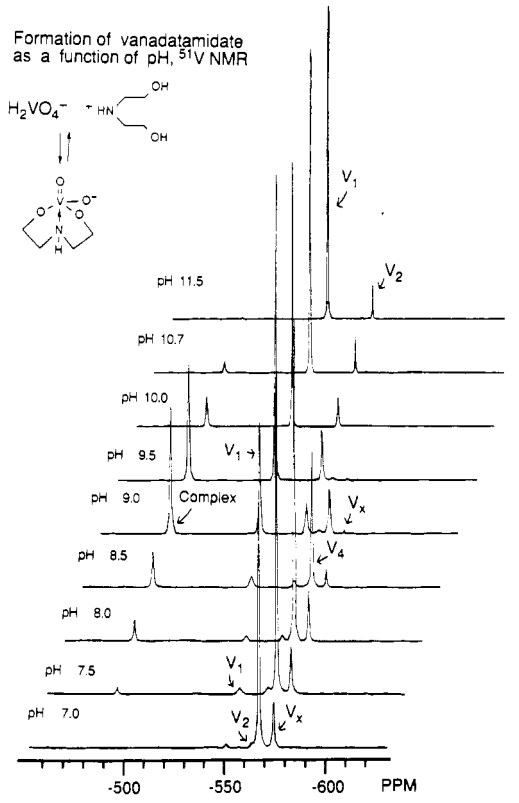


Figure 7. ^{51}V NMR spectra for 10 mM vanadate and 30 mM diethanolamine as the pH increases. The DEA–vanadate complex is marked Complex, the vanadate monomer V_1, the vanadate dimer V_2, the vanadate tetramer V_4, and the higher vanadate oligomer V_x. The reaction solutions contained 150 mM imidazole, and a total ionic strength of 0.4 was obtained by adding KCl.

complex as a function of $[H_2VO_4^{2-}]^2$ should give a straight line. If the formed complex is mononuclear, [Compl] will be proportional to $[H_2VO_4^{2-}]$ (eq 4). The experimental data are shown in Figure 6. Since [Compl] is proportional to $[H_2VO_4^{2-}]$, we conclude the complex formed in the vanadate reaction with diethanolamine is mononuclear.

pH Dependence of Complex Formation. The concentrations of various vanadate species are dependent on pH, and it is therefore expected that the reaction of vanadate and DEA is dependent on pH. The generation of complex was examined at a constant [amine]/[total vanadate] ratio as a function of pH, and the corresponding ^{51}V NMR spectra are shown in Figure 7. The chemical shift of the vanadate–DEA-type complexes did not change with pH. In the case of the vanadate–TEA complex the chemical shift did not change down to pH 5.5.

As the pH increases, the concentration of complex initially grows and after reaching a maximum begins to decrease at high pH. As the pH increases, the concentration of monomeric vanadate also increases, and because the concentration of the chelate is proportional to the concentration of monomeric vanadate, the increase in the signal intensity cannot directly be interpreted as an increase in the stability of the complex. When the ratio [complex]/[monomeric vanadate] is plotted, a curve with a maximum at pH 8.0–8.5 is obtained; this curve presents a true measure for the complex stability as a function of pH (Figure 8). The maximum in this curve is close to the pK_a for $H_2VO_4^-$ and slightly lower than the pK_a for the amine (8.88).

To determine the exact position of this maximum, we have carried out the following analysis. Using eq 2 and 3, we obtain eq 11.

$$\frac{[H_2VO_4^-]}{[\text{Compl}]} + \frac{[HVO_4^{2-}]}{[\text{Compl}]} = \frac{1}{K_{eq,2}[HDEA^+]} + \frac{1}{K_{eq,3}[DEA]} \quad (11)$$

Since the concentration of amine tied up in complex is very small compared to total amine concentration (eq 12), and

Table III. Reaction between 30 mM Methyldiethanolamine and 10 mM Vanadate in 150 mM Imidazole with KCl Adjusted to a Total of 0.4 Ionic Strength

pH	[Compl], mM	$[V_1]$, mM	$[\text{Compl}]/[V_1]$
6.94	0.41	0.64	0.64
7.46	1.32	0.53	2.5
8.04	4.01	0.94	4.3
8.59	7.05	1.23	5.7
9.00	7.42	1.87	4.0
9.46	6.21	3.22	1.9
10.02	4.21	4.42	0.95
10.49	1.85	6.37	0.29
10.94	0.58	8.64	0.067
11.94	0.055	9.14	0.0060

Table IV. Reaction between 30 mM TEA and 10 mM Vanadate in 150 mM Imidazole with KCl Adjusted to a Total of 0.4 Ionic Strength

pH	[Compl], mM	$[V_1]$, mM	$[\text{Compl}]/[V_1]$
6.99	0.41	0.41	1.0
7.51	1.28	0.32	4.0
8.00	2.54	0.51	5.0
8.55	3.72	0.86	4.3
9.08	3.99	1.84	2.2
9.52	3.71	3.67	1.0
9.94	2.09	5.44	0.38
10.51	1.00	7.41	0.14
11.05	0.53	7.86	0.067

$[HDEA^+]$ or [DEA] can be expressed in terms of the equilibrium constant, eq 11 is converted to eq 13. The inverse ratio

$$[\text{DEA}]_t = [HDEA^+] + [\text{DEA}] \quad (12)$$

$$\frac{[V_1]}{[\text{Compl}]} = \frac{1 + \dfrac{K_{HDEA^+}}{[H_3O^+]}}{K_{eq,2}[\text{DEA}]_t} + \frac{1 + \dfrac{[H_3O^+]}{K_{HDEA^+}}}{K_{eq,3}[\text{DEA}]_t} \quad (13)$$

$[\text{Compl}]/[V_1]$ is plotted in Figure 8. When $K_{eq,2}$ and $K_{eq,3}$ are expressed in terms of $K_{eq,1}$ and substituted into eq 13, eq 14 is obtained.

$$\frac{[\text{Compl}]}{[V_1]} = \frac{[H_3O^+][\text{DEA}]_t K_{eq,1}}{[H_3O^+]^2 + [H_3O^+](K_{a,H_2VO_4^-} + K_{HDEA^+}) + K_{a,H_2VO_4^-}K_{HDEA^+}} \quad (14)$$

To determine the maximum of this equation, the first derivative of $[\text{Compl}]/[V_1]$ was taken with respect to $[H_3O^+]$. It thereby follows that the maximum occurs when

$$2pH = pK_{a,H_2VO_4^-} + pK_{a,HDEA^+} \quad (15)$$

We present the experimental data of vanadate reacting with methyldiethanolamine ($pK_a = 8.52$) and triethanolamine ($pK_a = 7.75$) in Tables III and IV, and the expected shifts in the $[\text{Compl}]/[V_1]$ ratio are observed.

Relative Stabilities of Vanadium Complexes. We will compare the relative abilities of various amine ligands to form vanadate complexes in order to understand the structural features of ligands that stabilize vanadium–amine complexes. The differences in pK_a's of various amines complicate such a comparison since a large pH range must be used to determine stability constants for these complexes. A simple and convenient method involving one experimental measurement is the determination of $[\text{Compl}]/[V_1]$ ratios; in the event a particular amine is to be used in biological studies, the $[\text{Compl}]/[V_1]$ ratio determination will clearly indicate how much of the vanadate will be tied up as a vanadate–amine complex during the biological studies. The $[\text{Compl}]/[V_1]$ ratio is maximum at pH determined by $2pH = pK_a + pK_{a,H_2VO_4^-}$, so we conducted an exploratory study of $[\text{Compl}]/[V_1]$ ratios in the pH range 8.0–8.5. This pH range was chosen since the pK_{HA^+}

Table V. Relative Comparison of the Abilities Ethanolamine Derivatives Have To Form Complexes with Vanadate[a]

compd	[Compl]/[V_1]	pK_{HA^+}[d]
diethanolamine	1.9	8.88
methyldiethanolamine	5.5	8.52
triethanolamine	4.6	7.76
1-[bis-(2-hydroxyethyl)amino]-2-propanol	4.8	ND
triisopropanolamine	9.9	7.86
bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (Bis-Tris)	>20	6.46
diethanol ether[b]	0[c]	
diethanol sulfide[b]	0[c]	
(2-aminoethyl)ethanolamine[b]	0.16	ND
Tricine[b]	17.0	8.15
Bicine[b]	2.3	8.35

[a] The ratios [Compl]/[V_1] were determined by using 30 mM ligand, 10 mM vanadate, and 150 mM imidazole (the buffer), and KCl was added to the solution to give a total ionic strength of 0.4. The pH was 8.4. [b] These ligands are not carrying the same charge as DEA and therefore do not show the same pH dependence; these ratios are only to be considered as an indicative measure for the relative complex stability. [c] These ligands form complexes with vanadate, but at the 3/1 ligand to vanadate stoichiometry that has been used to study the vanadium–DEA-type complexes, these complexes cannot be observed. [d] These pK_A values were taken from ref 12. ND = not determined.

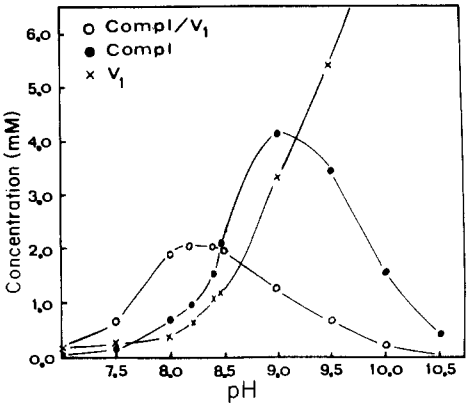


Figure 8. Concentration of the vanadium complex (●) and monomeric vanadate (×) and the ratio of the concentration of vanadium complex to the concentration of monomeric vanadate (O) plotted as a function of pH. These reactions were examined at 10 mM vanadate, 150 mM imidazole, and 30 mM DEA and made up to a total ionic strength of 0.4 with KCl.

values of many interesting amines are within this range, and it is also a physiologically important pH range. With use of the ratios shown in Table V, the fraction of vanadate complexed by various amines at pH 8.4 can be compared in a relative manner.

Increasing the numbers of alkyl groups on the amine (thereby increasing the availability of the nitrogen lone pair) increases the stability of the complex by a factor of 2–3. Both triethanolamine and methyldiethanolamine have similar effects. Decreasing the availability of the lone pair in diethanol ether and diethanol sulfide reduces the stability of the complex by 2 orders of magnitude; in fact, the complexes of both these ligands are not observable under the conditions DEA complexes are studied. The stability of vanadium chelates are therefore very dependent on the interaction with a nitrogen lone pair. In addition to increasing the substitution on the nitrogen, increasing the hydrophobicity of the alkyl groups on the nitrogen stabilizes the complex by a factor of 4–5. This effect suggests that hydrophobic environments might favor the stability of the vanadium complex.[21] Substituting one ethanol arm by an acetic acid arm increases the stability of the complex by a factor of 10. Alternatively, substituting one hydroxyl group with an amine decreases the stability of the complex by a factor of 10.

Effect of Buffers on Vanadium Complex Stability. Although the vanadate reactions are carried out at constant ionic strength, the reaction is still sensitive to the environment, i.e., the buffer system used in the reaction solution. Previously the spontaneous vanadate reactions were studied in Tris.[5] However, Tris forms a weak complex with vanadate[22] and possibly thereby influences the equilibria with DEA. Table VI illustrates that up to 20% deviation in the [Compl]/[V_1] ratios is observed when a different buffer is added to the reaction solution, and this is far above the experimental error limits. However, the overall pattern is not altered by variation in buffers and formation of labile weak complexes between buffer and vanadate and rapidly exchanging monoesters. We note that when weak vanadate complexes such as those between proteins and vanadate are in competition with weak buffer–vanadate complexes, the weak buffer–vanadate interactions are likely to interfere to a greater extent than observed in this work.

Table VI. Diethanolamine–Vanadate Complex Stability in Various Buffer Solutions[a]

[Compl]/[V_1]	pH	buffer	[Compl]/[V_1]	pH	buffer
1.8	7.8	Tris	1.1	8.8	taurine
2.0	8.0	imidazole	1.2	9.0	imidazole
1.8	8.0	Tris	0.87	9.4	taurine
1.9	8.1	Tris	0.63	9.5	imidazole
2.1	8.4	Tris	0.32	9.8	ethanolamine
2.4	8.5	imidazole	0.20	10.0	imidazole

[a] The ratios [Compl]/[V_1] were determined by using 30 mM ligand, 10 mM vanadate, and 150 mM imidazole, and KCl was added to the solution to give a total ionic strength of 0.4.

Comparison of the Reactions of Vanadate with DEA and Diethanol Ether. In this section we will compare the reactions of vanadate with alcohols and the reaction of vanadate with DEA to determine the quantitative stabilization effect of the central amine. The reactions of vanadate with itself,[2-4] with phosphate,[6] and with alcohols[5] have previously been studied in aqueous solution. It is apparent that the vanadate–DEA complex forms at much higher concentrations than previously reported vanadium compounds. The tridentate nature of DEA is, however, expected to generate vanadate derivatives of stability higher than those formed from mono- and bidentate ligands. In this regard we utilized the tridentate ligand diethanol ether to carry out a quantitative comparison. The reaction of vanadate with this tridentate ligand has not previously been studied and will therefore be described here.

Diols generate complicated mixtures of vanadate monoesters, diesters, and cyclic esters.[5] Diethanol ether is no exception. As the concentration of diethanol ether increases, the concentration of several vanadate esters increases (from −575 to −550 ppm) (Figure 9). The overall reaction yielding the cyclic vanadate ester from diol and vanadate can be written as shown in Scheme II.

(21) The hydrophobicity of buffers or solvents in the solutions will affect the complex stability. We are examining the question of how hydrophobicity affects these equilibria by studying reactions carried out in solutions with varying polarities. Unfortunately, the stability of complexes is not simply proportional to the polarity of the media. Some solvents will stabilize the complex, whereas others will destabilize it. Ottmar, L.; and Crans, D. C., unpublished results.

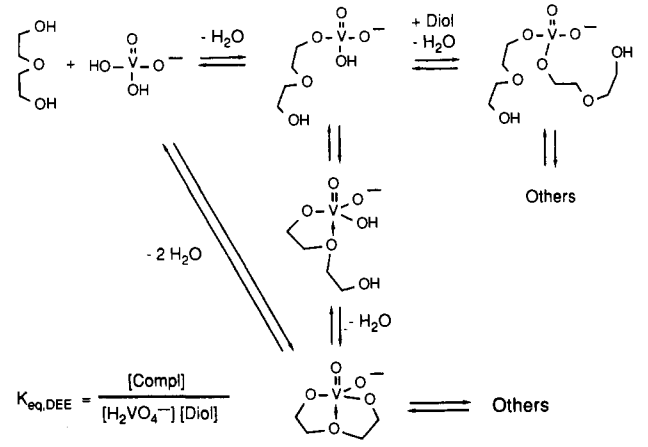
(22) Vilter, H.; Rehder, D. *Inorg. Chim. Acta* **1987**, *136*, L7.

Table VII. Summary of Equilibrium Constants Determined in This Work[a,b]

reactants	products	K_{eq}	conditions
$HDEA^+ + H_2VO_4^-$	Compl + H_3O^+	$K_{eq,1} = 6.7 \times 10^{-7}$	pH 7–8
$HTEA^+ + H_2VO_4^-$	Compl + H_3O^+	$K_{eq,1} = 2.7 \times 10^{-6}$	pH 7–7.5
$HDEA^+ + HVO_4^{2-}$	Compl	$K_{eq,2} = 110\ M^{-1}$	pH 8.25–8.8
$HTEA^+ + HVO_4^{2-}$	Compl	$K_{eq,2} = 420\ M^{-1}$	from $K_{eq,1}$
$DEA + H_2VO_4^-$	Compl	$K_{eq,3} = 510\ M^{-1}$	pH 7–8
$TEA + H_2VO_4^-$	Compl	$K_{eq,3} = 2000\ M^{-1}$	from $K_{eq,1}$
$DEA + HVO_4^{2-}$	Compl + OH^-	$K_{eq,4} = 8.7 \times 10^{-4}$	pH 9.5–11
$TEA + HVO_4^{2-}$	Compl + OH^-	$K_{eq,4} = 10 \times 10^{-4}$	pH 9.5–11
$DEE + H_2VO_4^-$	Compl	$K_{eq} = 1.2\ M^{-1}$	pH 7.3
$HDEA^+ + H_2VO_4^-$	monoester + H_3O^+	$K_{eq,M} \ll K_{eq,2}$	pH 7.8, 9.5

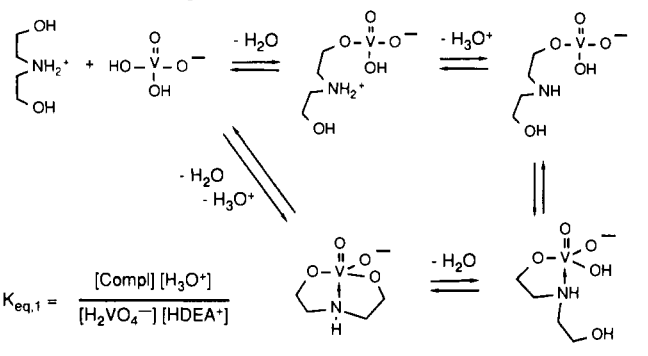
[a] The reactions were studied at 10 mM vanadate, 30 mM amine, and 150 mM imidazole, and KCl was added to give a total ionic strength of 0.4. The equilibrium constants were calculated by using least-squares fitting of lines; a minimum of four data points (determined in duplicates or triplicates) were used, although the average number of data points for each K_{eq} determination was six data points. The error in our data is not above 10%. [b] The [Compl]/[V_i] ratios shown in Table V can be used to calculate $K_{eq,1}$ by using eq 14. Since $K_{eq,2}$, $K_{eq,3}$, and $K_{eq,4}$ are easily obtained from $K_{eq,1}$, Table V gives access to a series of additional equilibrium constants on similar systems.

Scheme II. Equilibrium Reaction between Monomeric Vanadate and DEE[a]



[a] The overall equilibrium expression between the cyclic vanadate ester and DEE is shown ($K_{eq,DEE}$). Experimental data are presented and analyzed in Figure 9.

Scheme III. Equilibrium Reaction between Diprotonated Monomeric Vanadate and Monoprotonated DEA[a]



[a] The overall equilibrium expression between DEA and vanadate for this reaction is shown ($K_{eq,1}$).

The intermediate mono- and diesters are stable and do not rapidly convert, such that they will accumulate as the diethanol ether concentration increases. When one focuses on the overall reaction generating cyclic ester from diol and vanadate, the equilibrium constant is determined as shown in Scheme II. The experimental data for this reaction are shown in Figure 10, and the equilibrium constant is found to be 1.2 M^{-1}.

The reaction of vanadate with DEA is different from that of diethanol ether with respect to both complexity and reaction mechanism. A reasonable route for the reaction of diprotonated monomeric vanadate with protonated DEA is shown in Scheme III. Three similar routes using the monoprotonated monomeric vanadate and/or the neutral diethanolamine can be written. The

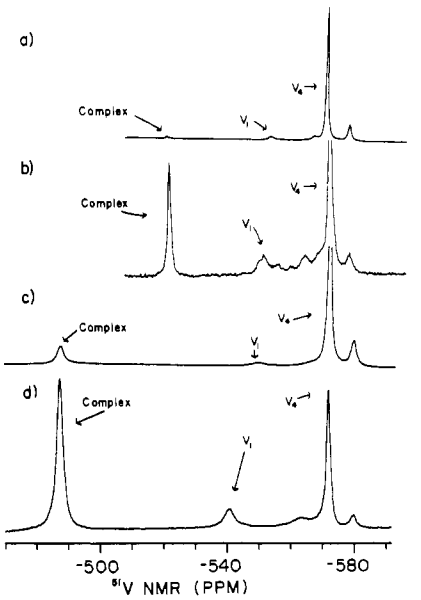


Figure 9. ^{51}V NMR spectra of the vanadium–DEE complex at 10 mM vanadate and (a) 330 mM and (b) 3.3 M diethanol ether (DEE) and of the vanadium–DEA complex at 10 mM vanadate and (c) 10 mM and (d) 100 mM DEA. The reaction solutions contained 150 mM imidazole and were made up to a total ionic strength of 0.4 (adding KCl). The reaction of vanadate with DEE was carried out at pH 7.3, and the reaction of vanadate with DEA was carried out at pH 7.8.

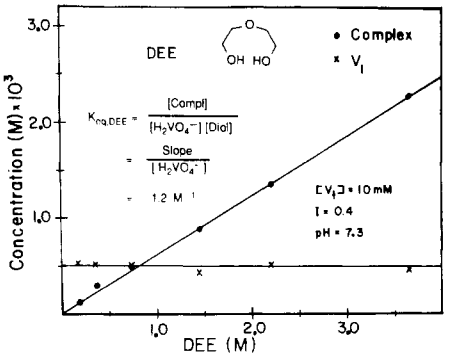


Figure 10. Concentration of the vanadate–DEE complex plotted as a function of DEE. The reaction solution contained 150 mM imidazole and 10 mM vanadate at pH 7.3, and a total ionic strength of 0.4 was obtained by using KCl. $K_{eq,DEE}$ was determined from the slope (correlation coefficient 0.989) to be 1.2 M^{-1}.

^{51}V NMR spectra of the vanadate–DEA reaction show one resonance presumably from the T1-type vanadate complex (−488 ppm) and several resonances from various vanadate oligomers (Figure 9). The vanadate monoesters (anticipated at approximately −550 to −555 ppm) of DEA cannot directly be observed

by ^{51}V NMR in these spectra. However, at neutral pH the chemical shift of the monomeric vanadate increases slightly as the concentration of ligand increases. Chemical shift changes of this type have previously been used to describe rapidly converting vanadate–phosphate derivatives.[6] Usually vanadate esters are not rapidly exchanging with vanadate, but the small chemical shift changes observed in the DEA reaction are presumably an example of rapidly converting vanadate monoesters. The observed change in chemical shift is very small (less than 2 ppm) and was barely observed by using a 500-MHz NMR spectrometer. The shift changes were used to estimate an equilibrium constant for formation of the monoester, and $K_{eq,M}$ was found to be several orders of magnitude smaller than $K_{eq,2}$. The rapidly converting monoester will not affect the overall equilibrium reaction of these buffer systems with vanadate, so we will not examine this phenomenon in greater detail in this work. It is, however, of importance to notice that such presumably acyclic vanadate monoesters also form with ligands such as DEA. In biological studies such rapid interconversion may be important and explain some behavior of vanadate in interaction with proteins in DEA-type buffers. When the amine concentrations are increased in 100- and 1000-fold excess to the vanadate concentration, the presence of vanadate monoesters emerged at pH 9.5 (−526 ppm; V_1 is observed at −534 ppm at this pH).

Since the reaction of vanadate with diethanol ether (DEE) is not dependent on pH, it is appropriate to compare the equilibrium constant of the DEE–vanadate reaction with $K_{eq,2}$ or $K_{eq,3}$. $K_{eq,3}$ can be expressed by $K_{eq,1}$ ($K_{eq,3} = K_{eq,1}/K_{a,HDEA^+}$) and accordingly is calculated to be 508 M^{-1}, whereas $K_{eq,2}$ was determined to 106 M^{-1}. Whether the DEE–vanadate reaction is compared to $K_{eq,2}$ or $K_{eq,3}$, the vanadate–DEA reaction is favored by at least 2 orders of magnitude over the DEE–vanadate reaction. It is therefore clear that the central amine drastically stabilizes a vanadate complex although this does indeed involve reducing the number of interactions of vanadium with oxygen donor ligands.

Conclusion

This paper describes a general reaction between monomeric vanadate and ethanolamine derivatives. The reaction occurs spontaneously in aqueous solutions, at ambient temperatures, and in the physiological pH range. The complexes form between vanadate and ethanolamine derivatives, in which the third and/or fourth functionality can be an alcohol, a carboxylic acid, a phosphonium acid, or an amine. The complexes are mononuclear in vanadium, and at relatively low ligand to vanadate ratios (up to 10/1) only one complex is observed. At higher ligand to vanadate ratios small changes in the chemical shift of monomeric vanadate indicate that acyclic monoesters might be in rapid equilibrium with monomeric vanadate. The equilibrium favors by 2 orders of magnitude the complexes formed by ligands containing a central amine over the ligands containing a central ether or sulfide. The stability of chelates containing tridentate DEA derivatives are at least 100-fold more favorable than those involving previously described vanadate derivatives formed by diols or trichelating ether ligands.

The reaction of vanadate with ethanolamine derivatives is highly dependent on pH and the concentration of monomeric vanadate. The number of protonated forms in the reaction mixture is large; however, various pH ranges lend themselves to determination of equilibrium and stability constants (Table VII). In the case of a neutral amine the greatest stability of the vanadate complex is observed at $(pK_{a,H_2VO_4} + pK_{a,HA^+})/2$. In characterizing the important structural features of the ligand in the reaction between diethanolamine and vanadate, we have used the $[Compl]/[V_1]$ ratio of various amines to quantitatively compare the relative abilities of amines to complex vanadate in the pH range 8.0–8.5.

The vanadium complexes with DEA derivatives are colorless, but when they form, they may interfere with the biological activities of vanadate. Therefore, the use of ethanolamine-derived buffers for biological studies with vanadate is not without potential problems. The number of biological buffers in question is very large, and since several of these, e.g. triethanolamine (TEA), Tricine, Bicine, Bis-Tris, and BES, are commonly used, care must be taken when studies are conducted with vanadate in biological systems.

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