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Formation and Structure of 1:1 Complexes between Aryl Hydroxamic Acids and Vanadate at Neutral pH

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Although aryl hydroxamic acids are well-known to form coordination complexes with vanadate (V^V), the nature of these complexes at neutral pH and submillimolar concentrations, the conditions under which such complexes inhibit various serine amidohydrolases, is not well established. A series of qualitative and quantitative experiments, involving UV/vis, ^{1}H NMR, and ^{51}V NMR spectroscopies, established that both 1:1 and 1:2 vanadate/hydroxamate complexes form at pH 7.5, with the former dominating at submillimolar concentrations. Formation constants for the complexes of several aryl and alkyl hydroxamic acids were determined; for example, for benzohydroxamic acid, the stepwise formation constants of the 1:1 and 1:2 complexes were 3000 and 400 M^{-1}, respectively. The ^{51}V chemical shift of the 1:1 4-nitrobenzohydroxamic acid complex was −497 ppm, and that of its unsubstituted analogue was −498 ppm. A ^{1}H−^{15}N HSQC spectrum of the 4-nitrobenzo-^{15}N-hydroxamic acid/vanadate complex indicated the presence of an N−H group with ^{15}N and ^{1}H chemical shifts of 115 and 5.83 ppm, respectively. A ^{13}C NMR spectrum of the complex of 4-nitrobenzo-^{13}C-hydroxamic acid with vanadate displayed a resonance at 170.1 ppm and thus a coordination-induced shift (CIS) of +3.8 ppm. In contrast, the CIS value of an established 1:2 complex, thought to contain chelated hydroxamic acid ligands, was +11.9 ppm. These spectral data led to the following structural picture of 1:1 complexes of vanadate and aryl hydroxamic acids. They contain penta- or hexa-coordinated vanadium. The ligand is in the hydroxamate rather than hydroximate form. The ligand is presumably bound to vanadium through the hydroxamic hydroxyl oxygen, but the hydroxamic acid carbonyl oxygen interacts weakly with vanadium. These species are the most likely candidates for the inhibitors of serine amidohydrolases found in vanadate/hydroxamic acid mixtures.

Introduction

The colored complexes that form between aryl hydroxamic acids and vanadate (V^V) have been well-known for many years. The earliest references largely deal with the application of these complexes to the qualitative and quantitative colorimetric and gravimetric analysis of vanadium.[1] More recently, a naturally occurring hydroxamic acid, amavadin, was discovered in the fungus *Amanita muscaris*, where it apparently sequesters vanadium (V^{IV} in this instance) in a pale blue complex.[2] Although the colorimetric analyses have usually been carried out in strongly acidic media to reduce interference by other metals,[3] the presence of vanadate/hydroxamic acid complexes has been observed over a wide pH range.[4−6]

Several crystal structures of vanadium(V) hydroxamate complexes have now been described.[7−11] These show that in mononuclear complexes the hydroxamates interact with the metal through the carbonyl and hydroxamate oxygen

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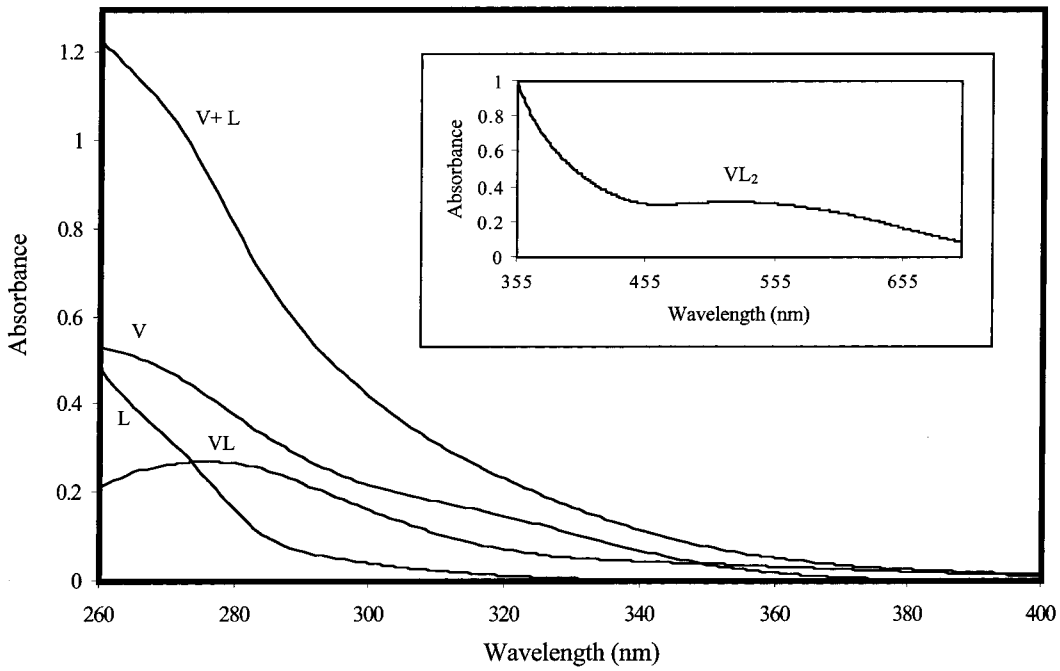

Figure 1. Absorption spectra of 0.3 mM vanadate (V), 0.3 mM benzohydroxamic acid (L), a mixture of the two (V + L), and the 1:1 complex (VL). The latter spectrum was obtained by subtraction of the calculated absorptions of V and L (Scheme 1) from the total (V + L) absorption. Inset is the absorption spectrum of 0.3 mM vanadate with 50 mM benzohydroxamic acid showing the long wavelength absorption band of the 1:2 complex VL_2.

atoms,[7,9,11] although additional coordination by nitrogen is also seen in a trinuclear complex.[8,10] The structure reported by Fisher et al.[7] is of particular interest since it is of the complex formed between vanadate and benzohydroxamic acid under the acidic conditions used in analytical chemistry. In general, these highly colored complexes have 1:2 vanadium:hydroxamate stoichiometry with the hydroxamic acid present chelated to the metal center.[12−14] The oxochlorabis(benzohydroxamato)vanadium(V) complex described by Fisher et al.[7] is pseudooctahedral (six-coordinated) around the vanadium center.

Although, as indicated above, the nature of the dominant vanadate/hydroxamate complexes in acidic media seems well-established, this is not true at neutral pH where interactions of these complexes with biological systems occur. Vanadium is suspected of being generally required for life, although no specific essential role has yet been assigned to it.[15] Nonetheless, vanadium compounds have a rich biochemical and physiological profile, and much research in this area is currently in progress.[16] Vanadium(V) hydroxamates have been employed as insulin mimics,[17] for example, and we have recently shown that combinations of vanadate with specific hydroxamic acids inhibit serine β-lactamases and proteases.[18] Consequently, it is important to develop a body of solid information on the nature of vanadate/hydroxamate complexes existing in aqueous solutions around neutral pH and at low, physiologically relevant, concentrations.

This paper describes our efforts to characterize the complexes found in solutions with β-lactamase inhibitory activity by means of a combination of UV/vis and ^{1}H, ^{13}C, and ^{51}V NMR spectroscopies. We find, perhaps surprisingly in view of the bulk of the literature on vanadium complexes, that at submillimolar concentrations at neutral pH the dominant complexes of vanadate with aryl hydroxamates are of 1:1 stoichiometry with a structure that involves only weak carbonyl coordination. These results complement very well some recent studies of the interactions of two alkyl hydroxamates with vanadate.[5,6]

Results and Discussion

Qualitative Observations. The addition of benzohydroxamic acid (final concentrations 0−50 mM) to vanadate solutions (≤1 mM total vanadate) gave rise to absorption increases with maxima around 272 and 520 nm as shown in Figure 1. Addition of 4-nitro- and 4-methoxybenzohydroxamic acids to separate samples of vanadate led to similar spectral changes. As indicated in the figure, the species with λ_{max} at 275 nm appeared at low benzohydroxamate concentration (≤1 mM) while that at 520 nm appeared only at higher concentrations. The latter absorption suggests the formation of the colored 1:2 chelate complexes well-known to form strongly in acidic solutions:[12−14] indeed, in acidic solutions, at pH 1 for example, purple solutions with λ_{max} at 520 nm are formed at low hydroxamic acid concentrations. This identification of the long wavelength absorption with 1:2 complexes is confirmed by the Job plot shown in Figure 2 for the complex of vanadate with 4-nitrobenzohydroxamic

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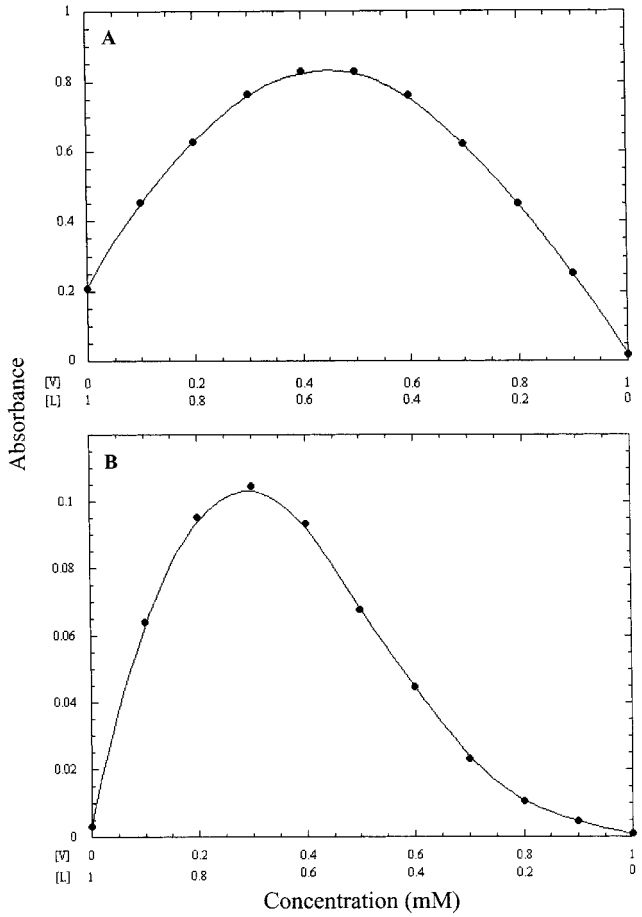

Figure 2. Job plots showing the effect of continuous variation of vanadate (V) and 4-nitrobenzohydroxamic acid (L) concentrations, both 0−1 mM, on the absorbance at 440 (A) and 560 nm (B). Smooth lines have been drawn through the points.

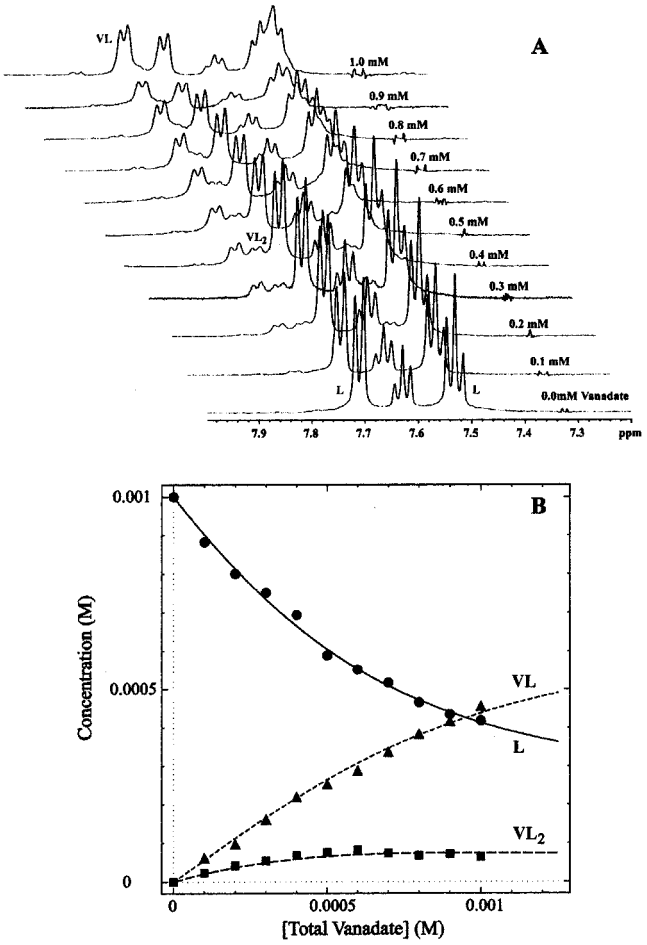

Figure 3. (A) ^{1}H NMR titration showing the formation of vanadate/benzohydroxamic acid complexes as a function of increasing total vanadate concentration at fixed benzohydroxamic acid concentration (1 mM). Resonance peaks believed due to benzohydroxamic acid (L), 1:1 (VL) complex, and 1:2 (VL_2) complex are labeled. (B) Quantitative fit of normalized integrals of the indicated peaks from the spectra in panel A to Scheme 1. The points represent the experimental data, and the solid lines represent the best fit to Scheme 1 calculated by Dynafit.[19]

acid. A Job plot of the data at lower wavelength is also shown in Figure 2. This plot suggests that the lower wavelength absorption, absorbing maximally at around 275 nm, derives largely from a 1:1 complex. This would certainly be in accord with its appearance at lower hydroxamic acid concentrations. We are unaware of any previous characterization of 1:1 complexes of vanadate and aryl hydroxamic acids in aqueous solution, in the absence of other ligands, except for a brief report by Grigor'eva and Slesar.[4] They show no spectra but, curiously, report that both 1:1 and 1:2 complexes absorb maximally at 460 nm. Yamaki et al. report the presence of 1:1 complexes of vanadate with alkyl hydroxamic acids, although their studies were generally carried out at higher vanadate concentrations.[5,6]

The evidence presented below, both qualitative and quantitative, confirms that 1:1 complexes of vanadate and aryl hydroxamic acids are the dominant associated species at submillimolar concentrations in aqueous solution at neutral pH.

Spectral Titrations. The following quantitative titrations were performed to confirm the stoichiometry of the complexes described above and to determine their formation constants. Figure 3 contains the results of a ^{1}H NMR titration of vanadate (0−1.0 mM) against benzohydroxamic acid (1.0 mM). As the vanadate concentration increased from zero, the formation of two complexes is evident from the appearance of two downfield doublets at 7.75 and 7.80 ppm. The other resonances of the complexes must lie under the multiplet at 7.5−7.6 ppm. As the concentration of vanadate increased, the doublet at 7.80 ppm increased in size with respect to that at 7.75 ppm. This observation suggests that the former resonance probably represents formation of a 1:1 complex and the latter a 1:2 complex. The integrals of these peaks, and that of the free hydroxamic acid at 7.71 ppm, which decreased in size as vanadate increased, were normalized with respect to the latter peak at zero vanadate concentration and plotted against vanadate concentration as also shown in Figure 3. These data were then fitted to Scheme 1 by means of the program Dynafit.[19] In this scheme, V and L represent free vanadate and hydroxamic acid, respectively, VL and VL_2 represent the 1:1 and 1:2 complexes of vanadate and hydroxamate, respectively, and V_2 and V_4 represent divanadate and tetravanadate, respectively. Values of K_3 and K_4 at pH 7.5 and 25 °C, 310 M^{-1} and 3.0×10^8 M^{-3}, respectively, were taken from the literature.[20] It

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Scheme 1

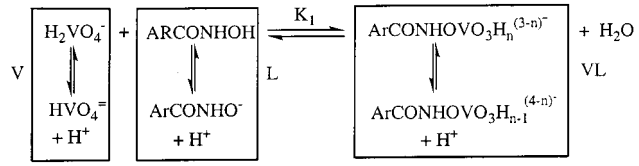
$$V + L \underset{}{\overset{K_1}{\rightleftharpoons}} VL$$

$$VL + L \overset{K_2}{\rightleftharpoons} VL_2$$

$$V + V \overset{K_3}{\rightleftharpoons} V_2$$

$$4V \overset{K_4}{\rightleftharpoons} V_4$$

Scheme 2



was assumed in the fitting procedure, on the basis of the above discussion, that VL corresponded to the 7.80 ppm resonance and VL_2 to the 7.75 ppm peak; the converse assumption did not allow a fit of Scheme 1 to the data and thus the assignment given above is correct. The solid lines of Figure 3B represent the best least-squares fit to the data, yielding values of the stepwise formation constants, K_1 and K_2, of (2820 ± 130) M^{-1} and (410 ± 35) M^{-1}, respectively. The larger value of K_1 than K_2 is consistent with the observation that a 1:1 complex, VL, accumulates at low hydroxamic acid concentration.

The association constants above are defined in terms of total vanadate and hydroxamic acid. It is recognized,of course, that at pH 7.5 vanadate exists partly in the monoanionic (pK_a around 8.3 in low ionic strength solutions[21]) and dianionic forms as may the hydroxamate complex (Scheme 2). The hydroxamic acid may also be significantly dissociated at pH 7.5, depending on the aryl substituents. For example, the pK_a of benzohydroxamic acid is 8.8 and that of 4-nitrobenzohydroxamic acid is 8.0.[22] An experiment (see Experimental Section) with benzohydroxamic acid showed that no measurable protons were taken up or released on formation of VL at pH 7.5 (<0.2 proton/VL formed). This suggests that $H_2VO_4^-$ and the protonated form of VL have very similar pK_a values and therefore the apparent association constants will not be a strong function of pH in the neutral region. Indeed, Yamaki et al. report little change with pH in the VL content of their solutions with aliphatic hydroxamic acids around neutral pH (7 ± 1).[5,6] The effects of more extreme pH changes were beyond the scope of this investigation.

The data from spectrophotometric titrations was also fitted to Scheme 1 with the assumption that the absorption at 520 nm was that of VL_2[12−14] and the absorption at lower wavelength (Figure 1) was that of VL; again, the converse assumption did not permit a fit of the data. The spectrophotometric titrations of vanadate against benzohydroxamate and

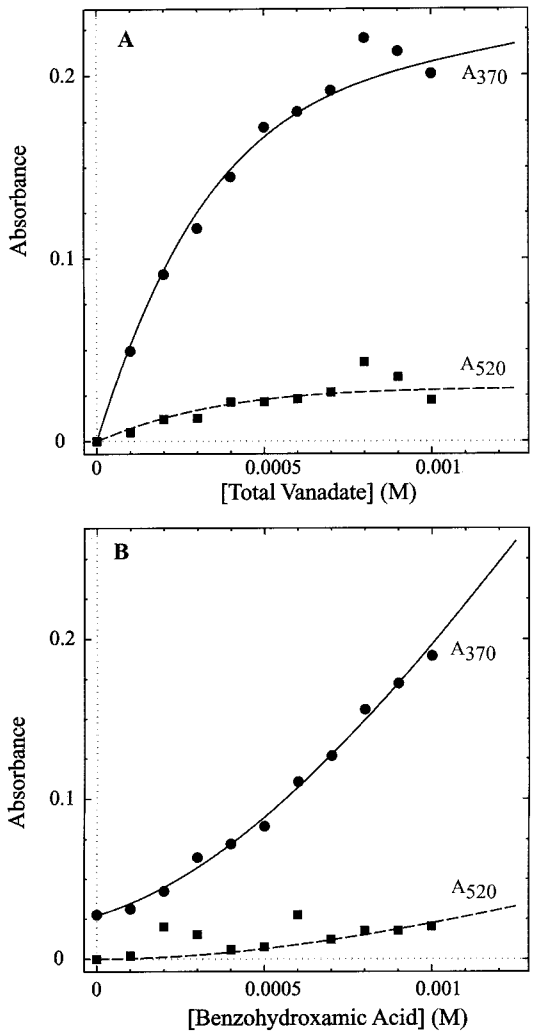


Figure 4. Spectrophotometric titrations of vanadate against benzohydroxamic acid (1 mM) (A) and vice versa (B). The absorbances at 370 and 520 nm correspond to the 1:1 and 1:2 vanadate/hydroxamic acid complexes, respectively. The points represent the experimental data, and the solid lines represent the best fit to Scheme 1 calculated by Dynafit.[19]

vice versa are shown in Figure 4. The solid lines of Figure 4A represent least-squares fits of the data to Scheme 1, where the values of K_1 and K_2 are (2950 ± 120) M^{-1} and (420 ± 40) M^{-1}, respectively. The solid line fits to the data in Figure 4B yielded K_1 and K_2 values of (3000 ± 300) M^{-1} and (430 ± 45) M^{-1}, respectively. The excellent agreement between these values and those derived from the ^{1}H NMR titration (Figure 3) provides strong support for Scheme 1 and the spectral assignments.

Similar titrations were carried out with 4-nitro- and 4-methoxybenzohydroxamic acid. These data are available as Supporting Information, and the fitting parameters are given, with those from benzohydroxamic acid, in Table 1.

Finally, spectrophotometric titrations were carried out with both acetohydroxamic acid and phenylacetohydroxamic acid and vanadate. The absorption spectra of these complexes differed from those of the aromatic complexes in that the maximal absorption of both the 1:1 and 1:2 complexes occurred at 310 nm (spectra are presented in the Supporting Information). This lower wavelength absorption of the 1:2 complex presumably reflects the absence of a contribution

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Table 1. Stepwise Formation Constants and Extinction Coefficients for Complexes between Hydroxamic Acids and Vanadate at pH 7.50

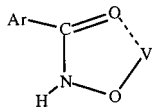
hydroxamic acid	K_1 (M^{-1})	K_2 (M^{-1})	ϵ_{VH} (M^{-1} cm^{-1})	ϵ_{VH_2} (M^{-1} cm^{-1})
PhCONHOH	2820 ± 20	410 ± 35[a]		
	2950 ± 120	420 ± 40[b]	2690 ± 45 (370 nm)	400 ± 25 (520 nm)
	3010 ± 300	430 ± 45[c]		
4-NO_2PhCONHOH	30 500 ± 1000	13.6 ± 0.8[a]		
	29 000 ± 1200	9.9 ± 0.9[b]	3070 ± 90 (440 nm)	12 300 ± 500 (560 nm)
	31 200 ± 500	12.7 ± 0.8[c]		
4-MeOPhCONHOH	990 ± 20	≤100[a]		
	990 ± 40	120 ± 1[b]	1130 ± 40 (370 nm)	1000 ± 20 (530 nm)
	990 ± 20	120 ± 8[c]		
$PhCH_2CONHOH$	440 ± 10	3400 ± 300[b]	740 ± 40 (310 nm)	3320 ± 70 (310 nm)
	430 ± 20	3380 ± 500[c]		
	445 ± 10	3450 ± 50[d]		
$CH_3CONHOH$	2100 ± 100	430 ± 10[b]	765 ± 10 (310 nm)	3000 ± 50 (310 nm)
	2100 ± 50	410 ± 10[c]		
	2100 ± 100	420 ± 15[d]		

[a] ^{1}H NMR titration. [b] Spectrophotometric titration; 1 mM hydroxamic acid, 0−1 mM vanadate. [c] Spectrophotometric titration; 1 mM vanadate, 0−1 mM hydroxamic acid. [d] Spectrophotometric titration; 0.3 mM vanadate, 0−50 mM hydroxamic acid.

of the aromatic rings to charge transfer. The data from these titrations at 310 nm were fitted to Scheme 1 (titration curves and fitted lines are shown in Supporting Information), and the parameters yielding these fits are reported in Table 1.

Quantitative Overview. The formation constants of Table 1 indicate first that the formation of VL_2 from VL seems generally thermodynamically less favorable at pH 7.5 than the formation of VL from V. The exception to this generalization is phenylacetohydroxamic acid, where the formation of VL_2 is relatively more favorable. Electron-withdrawing substituents appear to favor formation of VL from the aryl hydroxamic acids. The limited literature available on this subject, with alkoxide and aryl oxide ligands, suggests that K_1 values may not vary greatly with the electronic properties of substituents,[23,24] although some variation seems to occur in the case of the latter ligands.[23]

It is noticeable that the formation constants of VL are considerably larger for hydroxamic acids than for alcohols and phenols.[21,23−25] This may partly derive from the greater stability of products derived from α-effect nucleophiles[26] such as hydroxamates. Hydroxamates are unusually reactive with phosphate esters,[27] and this is thought to arise, in part at least, from product stability.[28] Hydroxylamine and hydrogen peroxide also form very stable vanadate complexes.[29] Another contribution to the stability of the current complexes may be that of chelate formation as in **1** or **2**. Crystal



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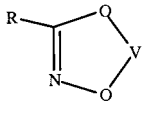

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structures of hydroxamic acid complexes of vanadate certainly show structures of this type.[7−11] Nonetheless, 1,2-diols do not seem to form stronger 1:1 complexes with vanadate than do simple alcohols.[30] The structure of the present complexes is discussed in more detail below. The formation constants of the 1:2 complexes VL_2 in Table 1 are much smaller than those reported in acidic solution,[4,12] This presumably reflects the effect of protonation of the vanadate oxygens to create a more electronegative center (H_3VO_4 or VO_2^+).

Structure of VH: ^{51}V Spectra. A ^{51}V NMR titration of 0−1 mM 4-nitrobenzohydroxamic acid against 1 mM vanadate was performed. Representative spectra are shown in Figure 5A. As the 4-nitrobenzohydroxamic acid concentration increased, peaks representing the vanadate monomer (−559 ppm), dimer (−571 ppm), and tetramer (−576 ppm) decreased in size, and a new resonance at −497 ppm arose. In view of the quantitative titration data presented above, the new peak should correspond to VL. The decrease in vanadate concentration with hydroxamate concentration was taken from the normalized integrals of these spectra (Figure 5B). These data could be fitted to Scheme 1, and from the best fit (solid line) K_1 and K_2 values of (30 000 ± 1200) M^{-1} and (13.9 ± 1.4) M^{-1} were obtained. The agreement between these values and those from the ^{1}H NMR and spectrophotometric titrations described above (Table 1) is clearly very good.

The chemical shift of VL, in Figure 5A, is indicative of either five- or six-coordination of vanadium but not four-coordination.[31,32] The ^{51}V NMR spectra of oxohydroxybis(benzohydroxamato)vanadium(V) and its 4-nitrobenzo analogue in methanol, where the (presumably hexacoordinated) complexes do not dissociate,[14] exhibited resonances at −510 and −514 ppm, respectively. In MOPS buffer at pH 7.5, at a concentration of 20 mM, the spectra of the bis(benzohydroxamato) complex showed peaks corresponding to vana-

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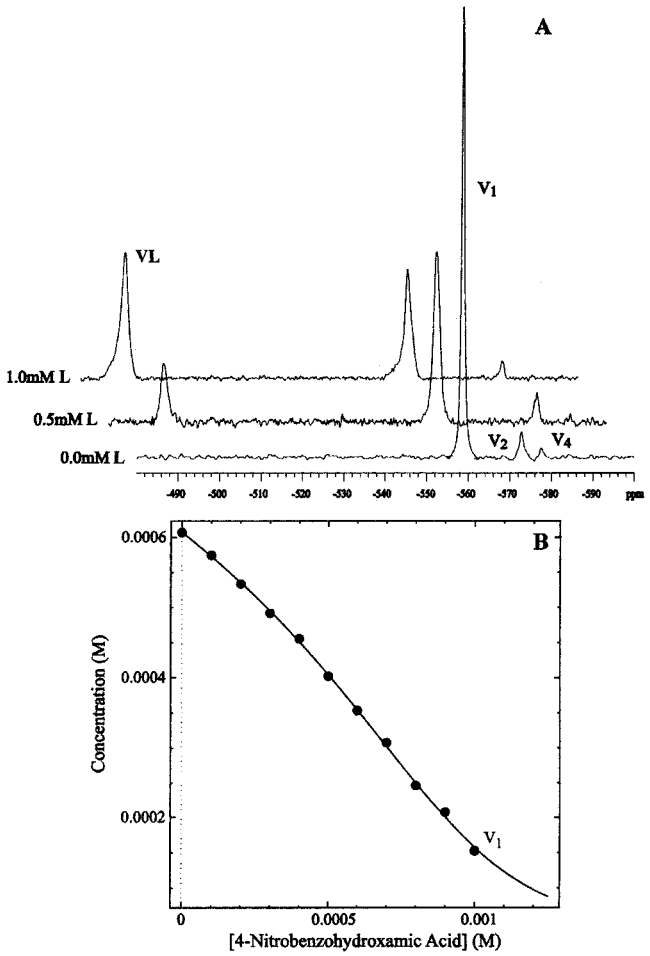


Figure 5. (A) Representative ^{51}V NMR spectra from a titration of 4-nitrobenzohydroxamic acid against vanadate (1 mM). Labeled peaks derive from vanadate monomer (V_1), dimer (V_2), tetramer (V_4), and the 1:1 vanadate/hydroxamic acid complex (VL). (B) Plot of vanadate monomer concentration, taken from the ^{51}V NMR spectra, as a function of 4-nitrobenzohydroxamic acid concentration. The points represent the experimental data, and the solid lines represent the best fit to Scheme 1 calculated by Dynafit.[19]

date, divanadate, and tetravanadate as well as peaks at −498 and −511 ppm. The latter presumably represents VL_2, and the former has the same chemical shift as that assigned to VL. The bis(4-nitrobenzohydroxamato) complex spectrum in buffer showed similar peaks at −497 and −514 ppm. It is clear that the species obtained from mixing vanadate and hydroxamic acid are identical to those obtained on dissolution of preformed complexes. All chemical equilibria in solution are therefore rapidly established.

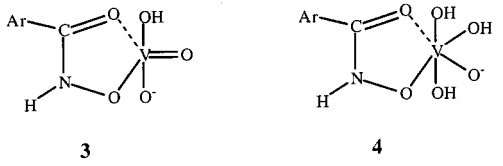
Structure of VL: ^{1}H−^{15}N HSQC Spectra. The presence of a correlation peak in the ^{1}H−^{15}N HSQC spectrum (presented as Supporting Information) of 1 mM 4-nitrobenzo-^{15}N-hydroxamic acid in the presence of 1 mM vanadate, i.e., of VL, shows that ^{15}N is bound to a proton. Further, the ^{15}N resonance at 115.2 ppm coupled to a proton to 5.83 ppm indicates[33] that the complexed hydroxamate nitrogen is amide-like (as in **1**) rather than imine-like (as in **2**[8,34]).

Structure of VL: ^{13}C Spectra. The carbonyl chemical shift of free 4-nitrobenzo-^{13}C-hydroxamic acid was found to be 166.30 ppm. The corresponding chemical shift of oxohydroxybis(4-nitrobenzo-^{13}C-hydroxamato)vanadium-(V) was 178.22 ppm. Coordination-induced chemical shifts, CIS, defined as $\delta_{bound} - \delta_{free}$, give information about whether a functional group is coordinated to a metal center.[32] Coordination of O or N directly bound to a carbon atom appears to give a ^{13}C CIS of 10 ppm or greater. In the instance above, the CIS is 11.92 ppm, which appears sufficient to indicate direct coordination as in **1**. This is in accord with all precedent since 1:2 complexes of vanadate and hydroxamic acids are known to have chelated structures.[7]

On the other hand, the ^{13}C NMR spectrum of a mixture of 1 mM 4-nitrobenzo-^{13}C-hydroxamic acid and vanadate at pH 7.5 showed a small peak at 166.30 ppm, representing free hydroxamic acid, and a peak at 170.08 ppm, which must represent VL. The CIS for this complex is therefore +3.78 ppm. Such a small CIS indicates weak interaction between the hydroxamic acid carbonyl group and vanadium. Also indicative of little interaction are the absorption spectra of these complexes, where there is little change in the absorption wavelength from those of vanadate itself (Figure 1). Simple vanadate monoesters, which are thought to contain tetracoordinated vanadium, have similar spectra, as do pentacoordinated species.[35] It is noticeable, even in the chelated 1:2 complexes, that crystal structures show that the V−ON bond is considerably shorter than the V−OC bond. Small variations in the extent of carbonyl coordination may lead to the observed differences in formation constants (Table 1).

Conclusions

At millimolar and lower concentrations at neutral pH, aryl hydroxamic acids form 1:1 complexes with vanadate in aqueous solution. At higher hydroxamate concentrations, the better known 1:2 complexes appear. The ^{51}V NMR spectra of the 1:1 complexes suggests that they contain penta- or hexacoordinated rather than tetracoordinated vanadium. ^{1}H−^{15}N HSQC spectra employing a ^{15}N-labeled hydroxamic acid showed that these complexes are hydroxamates rather than hydroximates. Finally, ^{13}C NMR spectra of a ^{13}C-labeled hydroxamic acid suggests that the carbonyl oxygen atom is only weakly coordinated to the vanadium in the 1:1 complexes. A pentacoordinated (**3**) or hexacoordinated (**4**)



3 **4**

structure therefore seems most likely. These species then are presumably the most likely candidates for the inhibitors of serine amidohydrolases.[18] Details of the inhibition of a class C β-lactamase, employing the results reported above, are described in a separate publication.[36]

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Experimental Section

Materials. Benzohydroxamic acid and acetohydroxamic acid were purchased from Aldrich Chemical Co. and used as received. Phenylacetohydroxamic acid was prepared by Mr. Michael Ernst in this laboratory following a literature procedure.[37] 4-Nitro- and 4-methoxybenzohydroxamic acid were synthesized from the respective methyl benzoates and hydroxylamine hydrochloride by the procedure of Stolberg et al.[38] 4-Nitrobenzo-^{15}N-hydroxamic acid was prepared in the same fashion, although starting with ^{15}N-hydroxylamine hydrochloride (Cambridge Isotope Labs). 4-Nitrobenzo-^{13}C-hydroxamic acid was prepared by the following procedure. Thus, 4-nitrobenzo-^{13}C-nitrile was first obtained by reaction of 4-nitrodiazonium tetrafluoroborate (Aldrich) and ^{13}C-KCN (Isotec).[39] The nitrile was hydrolyzed to the carboxylic acid by heating it under reflux in phosphoric acid for 20 h.[40] The resulting acid was then converted directly to the hydroxamic acid by reaction with hydroxylamine hydrochloride in the presence of 1,1′-carbonyldiimidazole and triethylamine.[41] The synthesized hydroxamic acids were purified by recrystallization, displayed the anticipated ^{1}H and ^{13}C NMR spectra, and exhibited the expected strong color reaction with $FeCl_3$.

Oxohydroxybis(benzohydroxamato)vanadium(V) and its 4-nitrobenzo analogue were prepared as described by Banerjee et al.[14] The former compound exhibited an identical absorption spectrum (λ_{max} = 445 nm, log ϵ = 3.41) to that reported;[14] the latter compound had a similar spectrum (λ_{max} = 460 nm, log ϵ = 3.67).

Methods.

Absorption Spectra. All absorption spectra and spectrophotometric titrations were routinely performed in 20 mM MOPS buffer at pH 7.5 and at 25 °C, using a Hewlett-Packard 8453A diode array spectrophotometer. This buffer was chosen because, at the concentration employed, it does not form complexes with vanadate.

Vanadate stock solutions (10 mM) were prepared by dissolution of sodium orthovanadate, Na_3VO_4 (99.99%, Aldrich) into the appropriate buffer and then dilution of this as needed. Vanadate stock solutions with identical spectral characteristics and behavior toward hydroxamic acids were obtained from ammonium metavanadate,[42] NH_4VO_3 (99.99%, Aldrich). The ^{51}V NMR spectra of these solutions were identical and closely similar to published spectra,[16] where the ratio of vanadate to divanadate and tetravanadate was the criterion. No acid was used to adjust the pH of these solutions since this procedure leads to the refractory decavanadate.[16] On dilution of these stock solutions into buffer, the required pH was obtained. Hydroxamic acid stock solutions were prepared by dissolution of the solid acid into the appropriate buffer. Solutions for NMR spectroscopy were prepared in the same way.

^{1}H NMR Spectra. ^{1}H NMR titrations were performed on a Varian Unityplus 500 spectrometer operating at 499.32 MHz. Data acquisition parameters were 10 kHz spectral width, 1.2 s acquisition time, 64.1° pulse, 15 s delay, and 1000 transients accumulated at 25 °C. The samples were prepared in 5 mM MOPS buffer in D_2O at p^{2}H = 7.5. Stock solutions of vanadate and hydroxamic acid were prepared in this buffer and diluted appropriately as described above.

^{13}C NMR Spectra. ^{13}C spectra were obtained from either a Varian Unityplus 500 spectrometer operating at 125.7 MHz or a Varian Gemini 300 instrument operating at 75.5 MHz. In the former case, the operating parameters were 31.1 kHz spectral width, 1.04 s acquisition time, 90° pulse, and 1.0 s delay time, and in the latter they were 200 ppm spectral width, 1.04 s acquisition time, 90° pulse, and 700 ms delay time. Chemical shifts were measured with respect to the internal standard dioxane (66.23 ppm).

^{15}N HSQC Spectroscopy. Two-dimensional (2D) ^{15}N HSQC spectra were taken at 5 °C using the Varian Unityplus 500 spectrometer of 4-nitrobenzo-^{15}N-hydroxamic acid (1 mM) in the presence and absence of 1 mM total vanadate in a 5% ^2H_2O aqueous solution. The data acquisition parameters were 6 kHz spectral width, 5.9 kHz 2D spectral width, 1.334 s acquisition time, 2.0 s delay time, 2 × 128 increments, and 496 transients accumulated. Chemical shifts were measured with respect to ^2H_2O (4.80 ppm) as an internal standard.

^{51}V NMR Spectra. ^{51}V spectra and spectral titrations were obtained at 25 °C from a Varian Unity plus 400 spectrometer operating at 105.1 MHz. Data acquisition parameters were 20 kHz spectral width, 4K data points, 0.05 s acquisition time, 7.8 μs (35.1°) pulse, and 0.3 s delay time. Stock solutions of vanadate and hydroxamic acid in 5 mM MOPS buffer in D_2O at p^{2}H = 7.5 were prepared as described above. Chemical shifts were measured with respect to the external standard $VOCl_3$ (neat).

Proton Release Measurement. An aliquot (0.2 mL) of a 0.1 M solution of sodium orthovanadate was added to a stirred, thermostated (25 °C) vessel containing 19.6 mL of 20 mM MOPS buffer, pH 7.50. The pH was then restored to 7.5 by addition of 1 M HCl. An aliquot (0.2 mL) of a 0.1 M solution of benzohydroxamic acid was then added. Final concentrations of vanadate and hydroxamic acid were thus both 1 mM. The measured pH did not change (±0.01 units). The addition of 0.02 mL 1 M HCl caused the pH to drop 0.10 unit. Addition of benzohydroxamic acid (1 mM) alone to the buffer produced no pH change (±0.01 unit).

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Supporting Information Available: Figures S1−S4 show spectral titrations of 4-nitrobenzohydroxamic acid and 4-methoxybenzohydroxamic acid with vanadate. Figure S5 shows absorption spectra of complexes between vanadate and aceto- and phenylacetohydroxamic acid. Figures S6 and S7 show spectral titrations of aceto- and phenylacetohydroxamic acid with vanadate, and Figure S8 shows the ^{1}H−^{15}N HSQC spectrum of the vanadate complex with 4-nitrobenzo-^{15}N-hydroxamic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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