Articles

Aqueous Chemistry of Ammonium (Dipicolinato)oxovanadate(V): The First Organic Vanadium(V) Insulin-Mimetic Compound

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The aqueous speciation, formation constants, and solution structure were determined for a new insulin-mimetic organic vanadium(V) compound (ammonium (dipicolinato)oxovanadate(V)). The solution properties of the system were characterized by using potentiometry, ^{1}H, ^{13}C, and ^{51}V NMR 1D and 2D spectroscopy, and UV/visible spectroscopy. These studies were conducted using the crystalline compound as well as combinations of the free ligand and the metal salt. The major complex is most stable in the acidic pH range, although it does protonate at low pH. It protonates at pH ∼1 and decomposes below pH 0. The dipic ligand is coordinated in a tridentate manner throughout the pH range studied. Protonation at low pH takes place on one of the oxo groups. Dynamic processes were explored using ^{1}H and ^{13}C EXSY NMR spectroscopy. VO_2dipic^- was found to exchange between the complex and the ligand at high and at low pH values. In the intermediate-pH range, no evidence for exchange processes was obtained, documenting the inertness of the complex at pH 3−4. The high stability and inertness in the pH 3−4 region may be of biological significance since the combination of high stability and low lability suggests the complex will be more resistant to hydrolysis at the pH of the stomach.

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is the most common form of diabetes in adult humans,[1,2] and human studies with an organic vanadium compound, KP-102,[3] in diabetic patients document the pharmaceutical potential of these types of compounds as oral therapeutic agents. The American Diabetes Foundation identified hypo- and hyperglycemic episodes as the main source of diabetic complications and recommended strict glycemic control for all diabetic patients.[4,5] Vanadium compounds are therefore of particular interest because a range of these compounds with insulin-like effects in the rat model of streptozotocin-induced diabetes support glycemic control. These compounds include simple salts (sodium ortho- and metavanadate, vanadyl sulfate)[6] and organic vanadium compounds, including bis(pyrrolidine-*N*-carbodithioato)oxovanadium(IV),[7] bis(cysteine methyl ester)oxovanadium(IV),[8] *N*,*N*′-ethylenediaminediacetate and *N*,*N*′-ethylenediaminedi-(*S*)-methionine oxovanadium(IV),[9] bis(acetylacetonato)oxovanadium(IV),[10,11] bis(picolinato)oxovanadium(IV),[12] and bis(maltolato)oxovanadium(IV).[13] Although potent effects due to peroxovanadium(V) complexes have been reported, these effects are observed in cultures or in live animals when oral administration is circumvented. All of the insulin-mimetic organic vanadium compounds reported to date contain vanadium in oxidation state IV. Despite the efficacy of oral vanadium compounds in lowering blood glucose, the exact mechanism of action and the target(s) are less obvious.[14] Evidence suggests that vanadate, and presumably also other vanadium complexes, bypasses the insulin receptor and activates glucose metabolism within the cell, presumably through inhibition of protein tyrosine phosphatases.[15] Toxicity as observed by weight loss, poor appetite, vomiting, and diarrhea has been associated with ingestion of vanadium compounds; therefore, the therapeutic index of some vanadium complexes (orthovanadate, metavanadate, and vanadyl sulfate) can be quite narrow.[16] Nevertheless, recent efforts have

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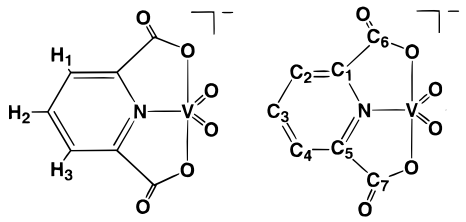

Figure 1. Structure and numbering scheme for VO_2dipic^-.

focused on identifying vanadium compounds with increased potency and decreased toxicity.[7−13] The recent successes achieved with organic transition metal complexes suggest that modifications of the metal ion chemistries by the organic ligands not only increased efficacy but also decreased toxicity.

The most successful vanadium complexes contain organic ligands that are reasonably solubile in both organic and aqueous environments and which are compatible with human metabolism. Most of the compounds reported contain bidentate ligands and have a 1:2 metal-to-ligand stoichiometry. The maltol ligand (3-hydroxy-2-methyl-4-pyrone)[17] is particularly desirable because, in addition to its favorable chemical properties, it is already an approved food additive and is sold in health food stores. Picolinic acid (2-pyridinecarboxylic acid) is a desirable ligand because it alone has a low level of insulin-like properties and is formed in the body as an intermediate in the tryptophan degradation pathway. Furthermore, it is an approved food supplement and Chromax chromium picolinate (tris(picolinato)-chromium(III)) is currently being used in human studies with diabetic patients.[18] Recent organic vanadium complexes also contain polydentate ligands and have a stoichiometry of 1:1, which should reduce the potential for side product formation.[9,19,20] One recent successful ligand is dipicolinic acid.[19,20] This ligand is desirable because of its low toxicity and its amphophilic nature. 3-Pyridinecarboxylic acid (commonly known as niacin or vitamin B_3), which is closely related to dipicolinic acid, is a precursor for the coenzyme NAD and is required in the human diet. Dipicolinic acid (2,6-pyridinedicarboxylic acid) is furthermore related to 2,3-pyridinedicarboxylic acid (quinolinic acid), which also is an intermediate in the tryptophan degradation pathway and is a precursor for NAD. We discovered that the vanadium(V) dipicolinate complex was a more potent inhibitor for phosphatases than the corresponding vanadium(IV) and peroxo complexes.[21] This study led us to pursue the activity of the VO_2dipic^- complex in live animals, and we discovered that it is effective as an oral agent.[19,20,22]

The synthesis and structure of VO_2dipic^- were reported previously (Figure 1).[23,24] Vanadium is five-coordinate in a mononuclear complex anion.[24] Studies probing the reactivity with H_2O_2 in solution suggested that an important species in solution was a 3:3 complex.[23] The purpose of this study is to determine the speciation and the properties of the complex in aqueous solution. All known effective insulin-mimetic organic vanadium compounds have a neutral charge; however, VO_2dipic^- is absorbed better than $VO(malto)_2$. This paper describes the speciation, stability, and lability of this complex at various pH values.

Experimental Section

Materials. All chemicals used were of reagent grade. Water was distilled and deionized on an ion-exchange column. Ammonium oxovanadium(V) 2,6-pyridinedicarboxylate (NH_4VO_2dipic) was prepared according to a literature method[23,24] and recrystallized from water. Its purity was assessed by ^{51}V and ^1H NMR spectroscopy and elemental analysis. A stock solution of 4.0 M KCl was prepared from distilled and deionized water.

NMR Sample Preparation. The ^1H NMR samples were prepared by dissolving the crystalline solid complex (or the crystalline solid complex and free ligand) in deuterium oxide. The pH was adjusted with a stock solution of DCl or NaOD. The ^{51}V NMR samples for the stoichiometry studies were prepared by mixing various amounts of stock solutions of vanadate, free ligand (2,6-pyridinedicarboxylic acid), and potassium chloride. ^{13}C NMR samples contained 33% (v/v) deuterium oxide. The pH values were adjusted with 0.2 M HCl or 0.2 M NaOH. The indicated pH is that measured unless the pH is below 0.5; then the pHs indicated are calculated values.

NMR Spectroscopy. 1D ^1H, ^{13}C, and ^{51}V spectra were recorded on a Varian INOVA-300 spectrometer (7.0 T) at 300 MHz for ^1H, 75.4 MHz for ^{13}C, and 78.9 MHz for ^{51}V. Routine parameters were used for ^1H NMR spectroscopy. ^{13}C spectra were acquired with a 20 000 Hz spectral window, a 90° pulse width, an acquisition time of 0.8 s, and a relaxation delay of 1.0 s. A 5 Hz exponential line broadening was applied prior to Fourier transformation. DSS (3-(trimethylsilyl)-propanesulfonic acid sodium salt) was used as an external reference for ^1H and ^{13}C chemical shifts. ^{51}V NMR spectra were acquired with a spectral window of 83 600 Hz, a pulse angle of 60°, and an acquisition time of 0.096 s with no relaxation delay. ^{51}V NMR chemical shifts were referenced against an external sample of $VOCl_3$. A 15 Hz exponential line broadening was applied before Fourier transformation. Complex and oligomeric vanadate mole fractions were measured using the Varian integration software. Assuming that all vanadium present in solution was in the form of vanadium(V), the mole fractions of vanadium complex and oligomers gave concentrations of complex and oligomeric vanadates.

Variable-temperature ^1H, ^{13}C, and ^{51}V NMR spectra also were recorded. The temperatures for the variable-temperature experiments were calibrated using an 80% ethylene glycol sample in DMSO-d_6 to an accuracy of ±2 K.[25] The NMR spectra were recorded first at 298 K and then at gradually increasing temperatures. Each sample was allowed to equilibrate for 10 min at every temperature before the spectrum was recorded. At the end of the temperature series, the sample was cooled back to 298 K to ensure that no changes in the sample had taken place during the variable-temperature experiment.

2D ^1H and ^{13}C EXSY experiments were run on a Varian INOVA-500 spectrometer (11.7 T) at 500 MHz for ^1H and 125.7 MHz for ^{13}C at 20 °C. The typical 2D ^1H EXSY spectra were recorded with a sweep width of 1120 Hz, an accumulation time of 0.229 s, a delay time of 3.0 s, a mixing time of 0.5 s, and 128 increments of four scans each. The 2D ^{13}C EXSY spectra were recorded with a sweep width of 7620 Hz, an accumulation time of 0.134 s, delay times of 1.5−2.0 s, mixing times of 0.2−0.5 s, and 128 increments of four scans each. To increase sensitivity, the spectral window was narrowed to include only the signal region of interest. This reduced the size of the data matrix required in the F1 domain and decreased accumulation times.

Solution Preparation for Potentiometric Studies. Solutions were prepared by dissolving H_2dipic and $NaVO_3$ in distilled water under an argon atmosphere. The vanadium in the stock solution was reduced to V(IV) with HCl, and after removal of the Cl^- ions, the concentration was standardized by permanganate titration as described earlier.[26] The hydrogen ion concentration in this solution was determined by the pH-

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metric method.[26] The purity of the ligand and the exact concentration of the stock solution were determined by the appropriate Gran plot.[27] Individual samples were prepared by combining the appropriate calculated amounts of ligand, vanadate, and KCl. The ionic strengths of all solutions were adjusted to 0.40 M KCl.

Potentiometric Studies. The stability constants of the vanadium(V) complexes were determined at 25 °C by pH-metric titration of 25 mL samples. The pHs were measured with an Orion 710A pH-meter equipped with an Orion Ross 8103BN-type combined glass electrode calibrated for hydrogen ion concentration.[28] The concentrations of the ligand were 0.0040 and 0.0020 mol/L, and the metal ion to ligand molar ratios were 0:4, 1:1, 1:2, and 1:4. Titrations were performed with KOH solutions of known concentrations (ca. 0.4 M) under a purified argon atmosphere to avoid interference from the oxygen and carbon dioxide in air. The pH range studied was 1.8−10.0 for the metal−ligand systems. The reversibility of the complexation reactions was checked by back-titration, i.e., by titrating samples from basic pH (∼10) with HCl solutions of known concentrations. Equilibrium was reached in all these solutions within 5 min. Experiments were performed in duplicate, and in all cases, the reproducibility of the titration curves was within 0.005 pH unit. A pK_w value of 13.75 was determined and used for the titrations at 25 °C and $I = 0.40$ M (KCl).

The stability constants calculated in this work are defined as shown in eq 1, where M^- refers to $H_2VO_4^-$ and L^{2-} refers to $dipic^{2-}$. The concentration stability constants $\beta(pqr)$ are defined in eq 2 and do not

$$pM^- + qL^{2-} + rH^+ \rightleftharpoons M_pL_qH_r^{(2p-)+(2q-)+(r+)} \quad (1)$$

$$\beta(pqr) = [M_pL_qH_r]^{(p-)+(2q-)+(r+)}/[M^-]^p[L^{2-}]^q[H^+]^r \quad (2)$$

consider the contributions of activity coefficients. The constants were calculated using the computer program PSEQUAD.[29,30] The speciation of vanadate into monomeric, dimeric, tetrameric, and pentameric species was considered in the calculation of the overall speciation in this system.[31] When necessary in the speciation calculations, the stability constants used from previous reports were corrected for different ionic strengths by using the Davies equation. The errors indicated in Table 1 refer to 3 SDs.

UV/Visible Spectroscopy. A series of studies were conducted with a combination of NMR and UV/vis spectroscopy at 298 K. The UV/vis spectra were recorded on a Perkin-Elmer Lambda 4 spectrometer equipped with a variable-temperature bath.

Results and Discussion

NMR Spectroscopic Studies of the Vanadium(V)−H_2dipic System. Studies were conducted to characterize the pH dependence and stoichiometry of the VO_2dipic$^-$ complex. The ^{51}V NMR spectra show only one signal at −533 ppm for solutions of dissolved crystalline NH_4VO_2dipic (10 mM) from pH 1.6 to 5.2 (Figure 2). However, when the acidity increases and the pH decreases below 1, the complex hydrolyzes to form VO_2^+, as seen in Figure 2, where both complex and VO_2^+ are observed at pH 0.4, 0, and −0.3. At pH 6.5 and above, less of the complex is observed and signals for vanadate oligomers are present. In Figure 3 are shown the ^{1}H NMR spectra of solutions containing 37 mM crystalline complex and 31 mM free ligand from pH 1.6 to pH 6.7 and, for comparison, a spectrum of only the complex at pH 5.2. The signals for the H1 and H3 protons in the complex have identical chemical shifts and show a doublet centered at 8.32 ppm. The signal for the H2 proton in the complex exhibits a triplet centered at 8.64 ppm. As the pH increases above 6, the complex hydrolyzes to form vanadate and mainly dianionic dipicolinate ($dipic^{2-}$). The corresponding signals for the free ligand at pH 2.3 are 8.38 and 8.47 ppm. As the pH increases, no change is observed in the chemical shifts for complex. The free ligand, however, shows significant changes consistent with deprotonation of H_2dipic (signals at pH 5.28: H2, 8.18 ppm; H1 and H3, 8.10 ppm). In summary, the ^{51}V (Figure 2) and the ^{1}H NMR data show that the complex is most stable in the acidic pH range.

Table 1. Compositions, Notations, Formation Constants (log β), and Acidity Constants (pK_a) for the H^+−$dipic^{2-}$, H^+−$H_2VO_4^-$, and H^+−$H_2VO_4^-$−$dipic^{2-}$ Systems [$I = 0.40$ M (KCl), 25 °C][a]

(r,q,p)	notation	log β (±3SD)	pK_a	ref
(1,1,0)	$Hdipic^-$	4.49 (1)	4.49	this work
(2,1,0)	H_2dipic^0	6.52 (1)	2.03	this work
(3,1,0)	H_3dipic^+	7.1 (2)	∼0.5	this work
(3,1,1)	$H(VO_2dipic)^0$	16.3 (2)	∼0.5	this work
(2,1,1)	VO_2dipic^-	15.79 (1)		this work
(2,0,1)	VO^{2+}	6.74		*b*
(−1,0,1)	HVO_4^{2-}	−8.13		*b*
(0,0,1)	$H_2VO_4^-$	0	8.13	*b*
(−2,0,2)	$V_2O_7^{4-}$	−16.03		*b*
(−1,0,2)	$HV_2O_7^{3-}$	−5.77	10.26	*b*
(0,0,2)	$H_2V_2O_7^{2-}$	2.67	8.44	*b*
(−2,0,4)	$V_4O_{13}^{6-}$	−9.64		*b*
(−1,0,4)	$HV_4O_{13}^{5-}$	−0.41	9.23	*b*
(0,0,4)	$V_4O_{12}^{4-}$	9.36	9.77	*b*
(0,0,5)	$V_5O_{12}^{5-}$	11.37		*b*
(4,0,10)	$V_{10}O_{28}^{6-}$	50.50		*b*
(5,0,10)	$HV_{10}O_{28}^{5-}$	57.00	6.50	*b*
(6,0,10)	$H_2V_{10}O_{28}^{4-}$	61.07	4.07	*b*
(7,0,10)	$H_3V_{10}O_{28}^{3-}$	62.85	1.78	*b*

[a] The data in this table give a constant of 3.9×10^2 M^{-1} for $[VO_2dipic^-]/[V_1][dipic^{2-}]$ (where, at pH 6.6, $[V_1] = [H_2VO_4^-]$). This value is obtained from the table by using the constant for the 2,1,1 species (15.79) and the reaction $2H + H_2VO_4^- + dipic^{2-} \rightarrow VO_2dipic^- + 2H_2O$ (formation constant: $[VO_2dipic^-]/[H^+]^2[H_2VO^{4-}][dipic^{2-}]$). At pH 6.6, the $[H^+]$ concentration is $10^{-6.6}$ M and $[H_2VO^{4-}] = [V_1]$. Appropriate substitution gives a constant of 3.9×10^2 M^{-1} for $[VO_2dipic^-]/[V_1][dipic^{2-}]$. [b] Calculated from data reported in ref 31.

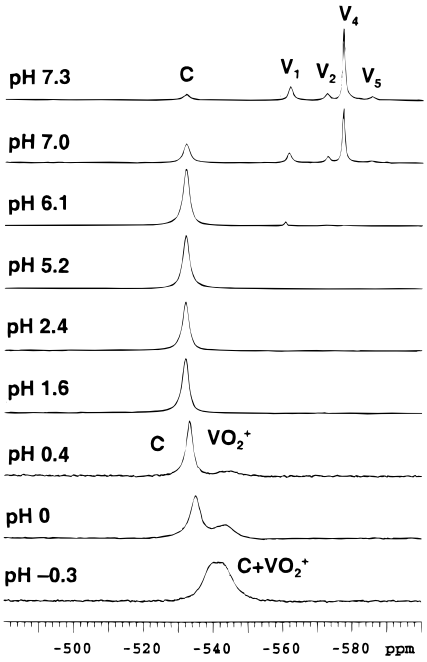

Figure 2. ^{51}V NMR spectra for solutions of dissolved crystalline NH_4VO_2dipic (10 mM) from pH −0.30 to +7.3.

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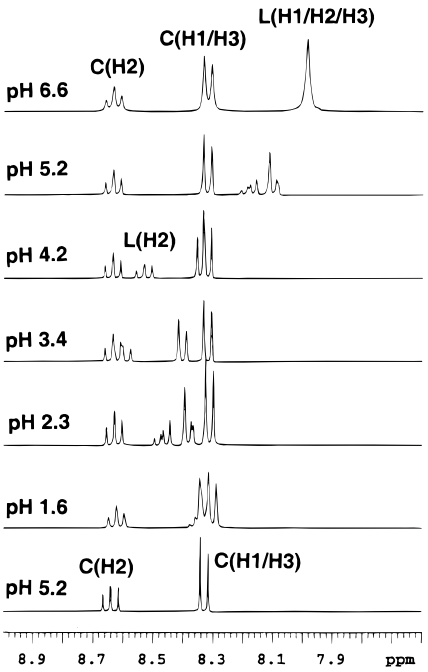

Figure 3. ^{1}H NMR spectra of solutions containing 37 mM crystalline complex and 31 mM free ligand from pH 1.6 to 6.6 and a spectrum of the crystalline complex at pH 5.2.

The stoichiometry was determined by measuring the NMR spectra for a series of aqueous solutions containing 1−10 mM vanadate and 1−10 mM H_2dipic at pH 6.6 in the presence of 0.40 M KCl. Plotting [VO_2dipic$^-$] as a function of [V_1][dipic$^{2-}_{free}$] gave a straight line, whereas plotting [VO_2dipic$^-$] as a function of [V_1]^3[dipic$^{2-}_{free}$]^3 (or any other pertubation of vanadium and ligand) gave no linear relationship (data not shown). [V_1] represents the concentration of total observed mononuclear vanadium(V), which at low pH corresponds to [VO_2^+] and at higher pH to the sum of [$H_2VO_4^-$] and [HVO_4^{2-}]. This information is only consistent with the possibility that the major solution complex observed at −533 ppm has a stoichiometry of 1:1. The equilibrium constant at pH 6.6 (defined as [VO_2dipic$^-$]/[V_1][dipic$^{2-}_{free}$]) for the VO_2dipic$^-$ complex was found to be [1.2 (±0.1 (3SD))] × 10^3 M^{-1}. The stability constant is known to be sensitive to ionic strength and a slightly lower formation constant was observed at pH 6.6 in the presence of 0.50 M KCl ([8.5 (±0.1 (3SD))] × 10^2 M^{-1}).

UV/Visible Spectroscopy Studies. The optical properties of this complex were previously characterized in detail.[23,32] We carried out a few studies combining NMR and UV/vis spectroscopy. In agreement with previous studies, we found that the UV/visible spectra have one local maximum with one or two shoulders in the region 250−300 nm. The fine-structure features of the spectra are very sensitive to pH,[32] and the extinction coefficients near λ_{max} are in the range (2−5) × 10^3 M^{-1}cm^{-1} (depending on ionic strength).[32] Since both the ligand and vanadate absorb in the same wavelength range and because the concentrations at which the UV/vis spectra were run were significantly lower than those used in most of the NMR studies described above, those ^{1}H NMR studies were carried out directly on the samples that were examined by UV/vis spectroscopy. In general, it was found that, at low pHs and at low concentrations, the complex remained intact. Specifically, no evidence for the free ligand was observed in the samples at pH 2−4 in the concentration range 0.07−0.4 mM; however, some free ligand was observed in samples at pH 4−6. Free ligand only was observed in a sample with added 0.07 mM complex at pH 6.6.

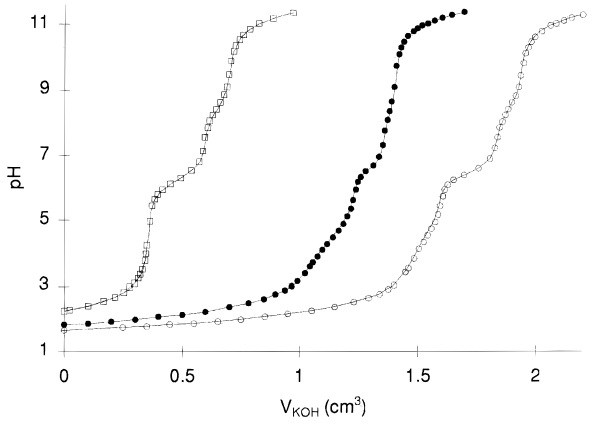

Figure 4. Experimental pH-metric titration points and calculated titration curves (full lines) for the H^+−$H_2VO_4^-$−dipic^{2-} system. The experimental points for concentrations of M^- and L^{2-} are 0.0020 and 0.0040 M (○), 0.0010 and 0.0040 M (●), and 0.0020 and 0.0020 M (□). (The experimental points are spaced out for better visibility.)

Although most of the NMR and UV/vis studies were conducted using the crystalline compound, identical spectra were obtained for solutions containing equimolar amounts of vanadate and H_2dipic. This indicates that the solutions rapidly reach equilibrium and potentiometric studies would be useful in further exploring the detailed speciation of this system.

Potentiometric Studies of the Vanadium(V)−H_2dipic System. A joint evaluation of the normal and back-titrations of the H^+−$H_2VO_4^-$−dipic^{2-} system yielded the concentration stability constants given in Table 1. Experimental pH-metric tritration points and calculated titration curves are shown in Figure 4. A separate evaluation of the two types of titrations provided very similar stability constants within the range of 3 SDs given in Table 1. This indicates the complete reversibility of the complexation reaction and the exclusion of any redox reaction between the reactants. The two log protonation constants (log K_a(Hdipic$^-$) = 4.49 (±0.01) and log K_a(H_2dipic) = 2.03 (±0.01)) are in good agreement with the results reported previously.[32] The third log protonation constant characterizing the protonation of the second carboxylate group is log K_a(H_3dipic$^+$) = 0.5 (±0.2) and was measured with lower accuracy.

The metal−ligand system was well described by a single species, VO_2dipic$^-$, which was predominant in the pH range 2−6. The speciation curves of the HVO_4^{2-}−dipic^{2-} system are depicted in Figure 4**.** The speciation and the stability constants obtained for the species VO_2dipic$^-$ are in fairly good agreement with those obtained by the ^{51}V NMR spectroscopic studies described above. Using the potentiometric data, the H^+-dependent constant defined as [VO_2dipic$^-$]/[V_1][dipic$^{2-}_{free}$] was calculated to be 3.9 × 10^2 M^{-1} (see Table 1). Since the NMR data are not as carefully pH-controlled as the potentiometric measurements, the results obtained by these two methods are in general agreement. The stability constants determined here by both potentiometry and NMR spectroscopy are slightly higher than those determined earlier with an ionic strength of I = 1.0 M ($NaClO_4$).[32] The log equilibrium constant obtained for the reaction VO_2^+ + dipic^{2-} ⇌ VO_2dipic$^-$ was 8.65 (K_a = 4.47 × 10^8 M^{-1})[32] and that determined in this work is 9.05 (K_a = 1.12 × 10^9 M^{-1}). Since an increase in ionic strength decreases the stability of vanadium(V) complexes in solution,[33] the constants reported in this paper are higher than those previously reported.

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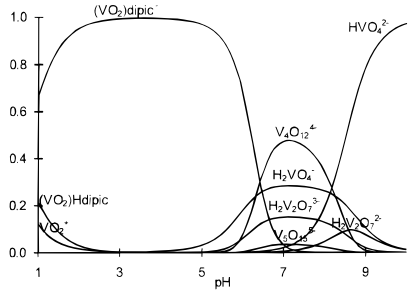

Figure 5. Speciation diagram for a titration of the total vanadium(V) and H_2dipic concentrations of 2.00 mM each in 0.40 M KCl at 25 °C.

In addition to the major species, two minor species, a protonated complex, $H(VO_2dipic)^0$, and a mixed hydroxo complex, $VO_2(OH)dipic^{2-}$ (also described as $VO_2dipicH_{-1}$), were assumed to check for a better fit. The fitting between the experimental and calculated titration curves improved only by ~2% when these species were assumed. The log protonation constant $\log K_a(H(VO_2dipic)^0) = 0.5 \pm 0.2$ characterizes the protonation reaction $VO_2dipic^- + H^+ \rightleftharpoons H(VO_2dipic)^0$ in accord with the literature reports on the existence of this species in acidic and organic solutions[23] and the spectroscopic studies described below. A log dissociation constant of $-\log K_a(VO_2dipicH_{-1}) = 8.4 \pm 1.7$ characterizes the deprotonation reaction $VO_2dipic^- + H_2O \rightleftharpoons VO_2(OH)dipic^{2-} + H^+$. The high uncertainty of the latter log stability constant is indicative of the uncertainty with which this hydroxo species is characterized. Furthermore, this is not observed with NMR spectroscopy because of the low stability of this species. The maximum concentrations of both of these species did not exceed 5% in the measured pH range (2−10) and are at the detection limit of these studies.

As seen in Figure 5, VO_2dipic^- complex formation is complete at acidic pH and practically no uncomplexed VO_2^+ ion is present at pH ~1.8, where the titrations begin. Despite this fact, a reasonable and accurate overall stability constant can be obtained for the major species VO_2dipic^- from the pH-metric data since the equilibrium system is fixed to the high-pH range of uncomplexed $H_2VO_4^-$ and HVO_4^{2-} species. The VO_2dipic^- complex does protonate at pH <1 and deprotonates at pH >8. The $VO_2(OH)dipic^{2-}$ complex, if it exists at all, forms in negligible concentration and is less significant than previously suggested.[32] pH-potentiometry alone cannot alone determine if the mononunclear species VO_2dipic^- or its oligomeric forms $(VO_2dipic^-)_n$ are formed, since the equilibrium $nVO_2dipic^- \rightleftharpoons (VO_2dipic^-)_n$ has no direct effect on pH. However, speciation calculations together with the spectral results (see above) gave no indication for the existence of the 3:3 species reported previously.[23] Spectroscopic structural characterization of the species formed was carried out as described below.

Solution Structure of the VO_2dipic^- Anion. ^1H and ^{13}C NMR spectra were recorded to obtain information on the solution structure and ligand coordination of the vanadium. For the aromatic ring, ^1H NMR spectra showed a large shift for the H2 proton (8.64 ppm) and small shifts for the H1 and H3 protons (8.31 ppm) in the complex at pH 4.2. The large shift for the ligand H2 (8.51 ppm) is consistent with direct coordination of the pyridyl nitrogen to the vanadium, and the smaller shifts observed for the ligand H1 and H3 (8.38 ppm) are consistent with both carboxylate groups (more distant from H1 and H3) being coordinated through a single oxygen. The ^{13}C NMR spectra of solutions (Figure 6) showed that all of the carbon atoms are affected upon complexation: the carboxylate carbon

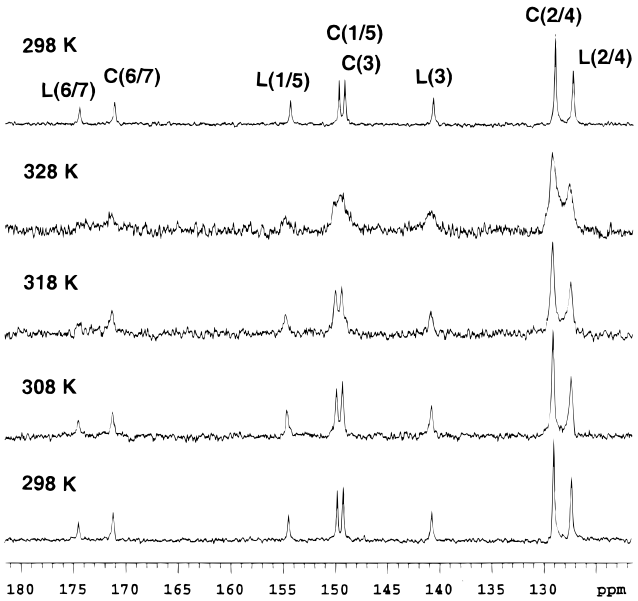

Figure 6. Variable-temperature ^{13}C NMR spectra of a solution with 730 mM added NH_4VO_2dipic and 520 mM H_2dipic at pH 6.6 (±0.1). The spectra were recorded at 298, 308, 318, and 328 K and then again at 298 K.

atoms (C6/C7, 171.8 ppm; L6/L7, 174.6 ppm), the carbons adjacent to the pyridine nitrogen (C1/C5, ~150 ppm; L1/L5, 154.6 ppm), the meta carbon atoms (C2/C4, 129.7 ppm; L2/L4, 128.2 ppm), and the para carbon atom (C3, ~150 ppm; L3, 142.1 ppm) (see Figure 1 for the numbering system). This assignment is consistent with the slow relaxation times for carboxyl and ternary carbon atoms and with the inductive and resonance effects observed in heteroaromatic ring systems. Furthermore, the assignment was confirmed with a DEPT experiment which showed that the $dipic^{2-}$ ligand is covalently coordinated in a tridentate manner to the vanadium.

As the pH increased, no change was observed in the chemical shift. Lack of chemical shift change is consistent with no further deprotonation reaction taking place up until pH 7.6 (no more complex was observed above this pH). The free ligand however shows significant changes, consistent with deprotonation of $Hdipic^-$ (Figure 3). Corresponding ^{51}V NMR experiments confirm the lack of deprotonation of the VO_2dipic^- anion. However, as the pH decreased from 2 to 0.5, a small but consistent change was observed by both ^1H (<0.25 ppm for H2 and <0.05 ppm for H1/H3) and ^{51}V (2 ppm) NMR spectroscopy. The chemical shift changes at these low pH values may suggest protonation of the complex on the oxo group, generating a species with the formula $VO(OH)dipic^0$. Such a species is very acidic; only a small fraction (~5%) of the complex was detected at pH 2 in the speciation study. However, isolation of yellow $H(VO_2dipic)\bullet 2H_2O$ has previously been reported in both aqueous and ethanolic solutions.[23]

Motional Processes and Lability of the VO_2dipic^- Anion. Variable-temperature spectra were recorded for a solution with added crystalline VO_2dipic^- and for solutions with both crystalline VO_2dipic^- and excess ligand by ^1H, ^{51}V, and ^{13}C NMR spectroscopy. For all pH values examined, dynamic processes were observed. In Figure 6 are shown the ^{13}C NMR spectra of a solution containing 730 mM complex and 500 mM ligand at pH 6.6 recorded at 298−328 K. No decomposition reactions took place, as evidenced by the fact that the identical spectrum was obtained after completing the variable-temperature experiment. Exchange broadening was observed for all ^{13}C

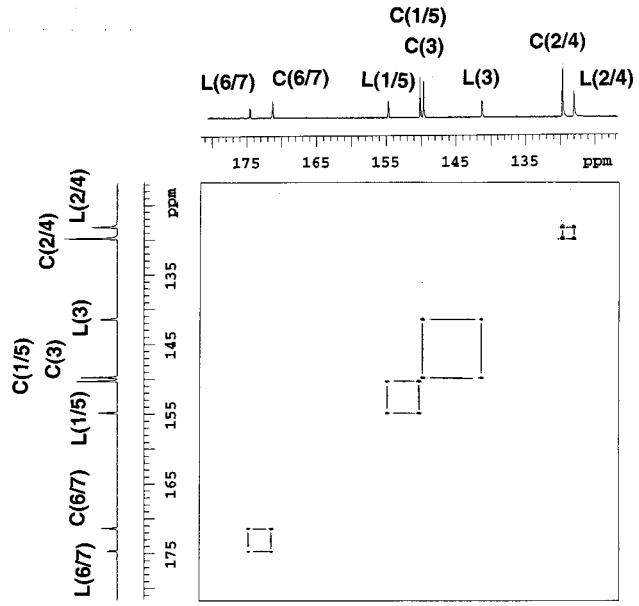

Figure 7. ^{13}C EXSY spectrum of the VO_2dipic^- complex (687 mM) in the presence of free ligand (581 mM) at pH 6.6 (±0.1).

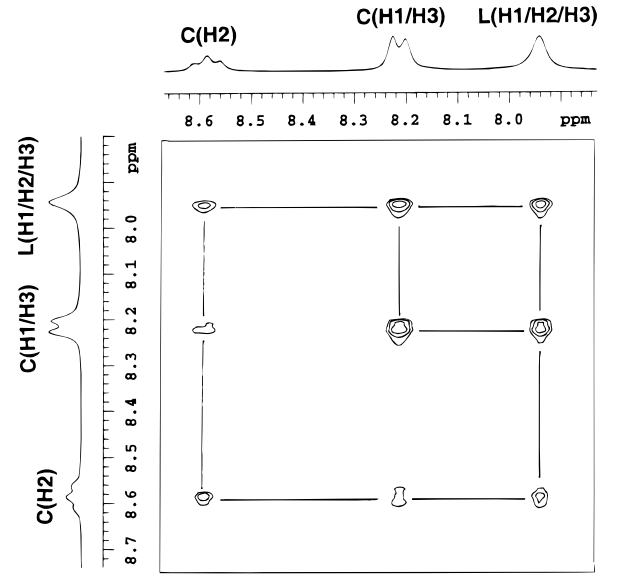

Figure 8. ^1H EXSY spectrum of the VO_2dipic^- complex (694 mM) in the presence of free ligand (572 mM) at pH 6.6 (±0.1).

signals as the temperature was increased, and the carbonyl signals at 174.6 and 171.8 ppm and the complex signal at 150.5 ppm each disappeared into the baseline. To obtain additional information regarding the nature of this process, homonuclear ^1H and ^{13}C EXSY NMR spectra were recorded.

EXSY Spectroscopy. The initial focus was on determining whether intermolecular processes such as ligand exchange take place in solutions containing VO_2dipic^-. Both ^1H and ^{13}C EXSY spectroscopy were used to examine complex lability. The ^{13}C EXSY spectrum of the VO_2dipic^- complex (687 mM) in the presence of free ligand (581 mM) at pH 6.6 (±0.1) is shown in Figure 7. The cross-peaks between ligand signals and complex signals are indicated by solid lines in this figure. These cross-signals signify that the $dipic^{2-}$ ion in the VO_2dipic^- anion is exchanging with the free $dipic^{2-}$ ion in solution on the time scale of the NMR experiment. The corresponding ^1H EXSY NMR spectrum is shown in Figure 8 for a solution of 694 mM VO_2dipic^- and 572 mM free complex at pH 6.6 (±0.1). Interpretation of ^1H NMR EXSY spectra is complicated by the fact that other types of processes are observed in the ^1H NMR EXSY experiment, including cross-signals attributed to NOEs and scalar couplings. Thus, although Figure 8 shows a clean spectrum with cross-signals between pairs of ligand and complex protons, lower intensity cross-signals were observed between complex H2 (C2(H2)) and complex H1/H3 (C(H1/H3)). These cross-peaks signify scalar couplings or NOEs between complex protons and are not related to the dynamic process(es) being investigated here.

Using the ^1H NMR EXSY spectra, the exchange process was examined as a function of pH. However, since this process is inherent to the complex, it should be less sensitive to changes in solution pH and was initially used as a reference for comparison. ^1H NMR EXSY spectra were recorded at pH 0.9, 1.6, 2.2, 3.2, 3.9, 4.2, 5.3, 6.1, 6.6, and 7.1. In Figure 9 are shown the ^1H NMR EXSY spectra recorded at pH 4.2 and 2.2. As the pH approaches neutral, the cross-peaks indicating intermolecular exchange between VO_2dipic^- and free ligand increase significantly compared to the cross-peaks between protons in the complex (compare Figures 8 and 9). Decreasing the pH to 4.2, 3.9, and 3.2 yields EXSY spectra in which the cross-peaks for the intramolecular system are equally as intense as or more intense than the cross-peaks for the intermolecular process. However, as the pH is decreased further to 2.2, 1.6, and 0.9, the intensity of the cross-peaks for the intermolecular process begins to increase again and surpasses the intensity of the cross-signals for the intramolecular system (Figure 9). At low pH, the cross-peaks for the intramolecular system are much smaller than the cross-peaks for the intermoleculer process.

As shown in Figure 2, the chemical shifts for the free ligand varied significantly with pH, and accordingly, on the basis of chemical shift differences, some changes in cross-signal intensities would be anticipated. However, the cross-peak volume intensities changed as a function of pH to a greater extent than would be justified on the basis of differences in chemical shifts. As indicated in Figures 8 and 9, complex and ligand exchange is observed as the cross-peaks between the H2 of the complex and the H2 of the ligand and between the H1/H3 of the complex and the H1/H3 of the ligand. In Figure 10 are shown the total exchange volume intensities as expressed by the ratios of total exchange volume integrals (for the complex to ligand exhange) to diagonal signals as a function of pH. Although most of the data shown were recorded for solutions with similar concentrations, the results obtained with higher and lower concentrations were similar, as indicated in Figure 10. Also, as clearly indicated in Figure 10, the exchange is least at pH 3−4 compared to either lower or higher pHs. To validate this observation, a similar comparison was carried out for the ratio of the total volume integrals between the complex and ligand to the total volume integrals between the complex H2 and the complex H1/H3. This ratio should change as a function of pH if the differences in the lability are genuine. Indeed, the ratio was found to be greatest at low and high pHs (about 35−40) and near unity at pH 3−4. On the basis of this combined information, we conclude that the complex is least labile in the pH range from 3 to 4.

In view of the complicating scalar couplings and NOEs, support for the results shown in Figure 10 was sought by recording the ^{13}C EXSY NMR spectra at both low and high pH values for comparison with the data obtained at pH 4.2. Given the lower sensitivity of ^{13}C NMR, such experiments require high concentrations, and as a result of the lower solubility at low pH, these spectra were recorded at lower concentrations than those shown in Figure 7. Although the

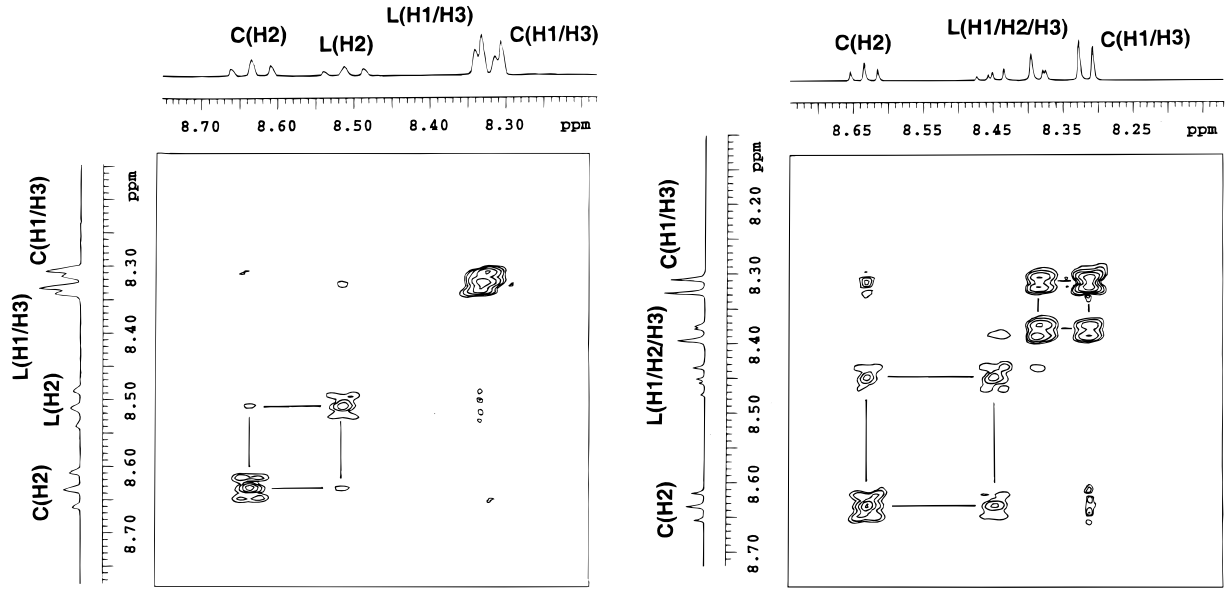

Figure 9. ^{1}H EXSY spectra of the VO_2dipic$^-$ complex (37 mM) in the presence of free ligand (31 mM) at pH 4.2 (±0.1) (left) and of the VO_2dipic$^-$ complex (37 mM) in the presence of free ligand (31 mM) at pH 2.2 (±0.1) (right).

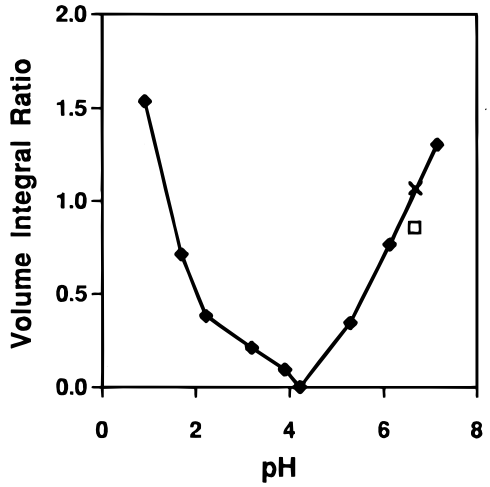

Figure 10. Ratio of total volume integrals of exchange cross-signals between VO_2dipic$^-$ and free ligand and the total diagonal signals obtained from ^{1}H EXSY NMR spectra plotted as a function of pH. The points depicted by closed diamonds represent data obtained from solutions containing 37 mM VO_2dipic$^-$ and 31 mM free ligand. The points depicted by the open square and the cross represent data obtained at approximately 10-fold lower and 10-fold higher concentrations, respectively.

accumulation times for these spectra were much greater, there is no evidence for exchange in the pH 3−4 region (the diagonal signals are very clear as anticipated). Decreasing the pH to 2 further limited the solubility of the ligand. However, it was still possible to observe exchange in the ^{13}C EXSY NMR spectrum when the slices were examined separately (data not shown). In summary, the ^{13}C NMR data support the ^{1}H NMR data in that the exchange at pH 3−4 was not observable, whereas exchange was observed at both higher and lower pHs.

Why would the complex lability decrease in the pH 3−4 regime? The explanation for the observed differences in rates of ligand-complex exchange is presumably associated with the differences in protonation states of the ligand and the fact that complex formation more readily may occur with some of the dipic species (H_3dipic$^+$, H_2dipic, Hdipic$^-$, or dipic^{2-}). This observation can be explained as a combination of the species nucleophilicity and the charge repulsion that develops between VO_2dipic$^-$ and the free ligand. Specifically, dipic^{2-} is more nucleophilic than Hdipic$^-$, which again is more nucleophilic than H_2dipic. However, as two negatively charged species approach each other, charge repulsion builds. This repulsion will be greater the larger the species charge. It is possible that only a highly nucleophilic dipic^{2-} species can effectively overcome the charge repulsion and consequently will continue to attack the charged complex. Although the monoanionic Hdipic$^-$ ion will replace the ligand in the complex, the rate will be slower because of reduced nucleophilicity. The neutral H_2dipic, on the other hand, will not generate such electrostatic repulsion upon approach, and as more of this species becomes present in solution, the rate of complex−ligand exchange increases again. As the solution becomes even more acidic, the presence of H_3dipic$^+$ and VO(OH)dipic^0 will begin to affect the reaction in this system. That is, H_3dipic$^+$ is a stable alternative to complex, and as the pH decreases, the complex stability begins to decrease again.

Biological Significance of the Chemical Properties of VO_2dipic$^-$. To date, the organic vanadium compounds that have been reported with insulin-mimetic properties contain vanadium(IV). In the case of VO(malto)$_2$ and kojic acid analogues,[34] the vanadium(V) complexes were also examined and found to be inferior to the vanadium(IV) complexes. Similar observations were made with VO(acac)$_2$ and its vanadium(V) analogue VO_2-acac (unpublished). The recent observation that the VO_2dipic$^-$ complex also is very effective suggests that this complex maintains the desirable properties of the vanadium(IV) complexes. Although it is commonly recognized that vanadium compounds act as protein phosphatase inhibitors, other factors such as compound absorption will be very important to the action of the compounds.

Since most of the reported active organic vanadium compounds are neutral, one important question with regard to VO_2dipic$^-$ chemistry is whether this species will be neutral in the environment of compound absorption. On oral ingestion, the complex passes through the acidic stomach (pH 2−4) after

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which it proceeds to the intestine, where the pH is neutral. Greatest absorption takes place when a complex is neutral. The speciation and structural chemistry described above show that the VO_2dipic$^-$ complex is very stable in the pH range of the stomach. Furthermore, the complex can protonate and form a neutral compound at low pH. Although pK_a(VO(OH)dipic^0) is ~0.5 and only a limited amount of the complex is in this form at pH 2−4, the results show that some will exist in the form that is most readily absorbed. These chemical properties point to the possibility that this type of compound is absorbed in the stomach or is very quickly absorbed after arriving in the intestine.

A series of organic vanadium(V) complexes[35,36] were recently shown to be labile.[37−41] These complexes include vanadium(V) compounds with polydentate O,N-donor-containing functionalities.[37,39−41] It was therefore of interest to examine the lability of the VO_2dipic$^-$ complex. The variable-temperature and EXSY spectroscopic studies described above show, perhaps not surprisingly, that the VO_2dipic$^-$ complex also is labile under some conditions. Interestingly however, the lability is significantly decreased in the pH range 3−4. It is possible that the decreased lability of the complex in the appropriate pH range is of significance with respect to its action in biological systems.

Chemical studies such as those presented in this work cannot alone elucidate the mechanism or the compound properties that can affect the insulin-mimetic action of these vanadium compounds. Furthermore, the studies presented here do not examine all aspects of the chemistry relevant to the insulin action of these compounds. However, our results do thoroughly describe the speciation and hydrolytic properties of our title complex, and this is necessary information for evaluation of the potential role that redox chemistry may have in the action of this complex.

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