Inorg. Chem. **2006**, *45*, 1471–1479

Reduction of Vanadium(V) by L-Ascorbic Acid at Low and Neutral pH: Kinetic, Mechanistic, and Spectroscopic Characterization

Patricia C. Wilkins,[†] Michael D. Johnson,*[,†] Alvin A. Holder,[‡] and Debbie C. Crans*[,‡]

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003, and Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

Received May 11, 2005

L-Ascorbic acid interacts with vanadium(V) over the pH range of 0.4–7.0 to form three different coordination complexes. Both inner- and outer-sphere electron-transfer pathways are proposed to form vanadium(IV) complexes with L-ascorbate or dehydroascorbate, respectively. Effects of the pH on the coordination of L-ascorbic acid to the vanadium(V) center were observed and are presumably related to the speciation of the vanadium(V) ion. Three vanadium(IV) complexes were observed using ambient-temperature electron paramagnetic resonance spectroscopy. Two of these complexes are proposed to be vanadium(IV) L-ascorbate complexes, and one is consistent with a vanadium(IV) dehydroascorbic acid complex proposed earlier. These reduction reactions will occur under physiological conditions and could be important to the reduction of vanadium(V)-containing coordination complexes used as insulin-enhancing agents for treatment of diabetes.

Introduction

The investigations into the use of vanadium coordination complexes as therapeutic agents for diabetes underline the importance of this element's redox chemistry under physiological conditions.[1] The kinetics of ascorbic acid reduction for a wide range of metal complexes have been reported.[2−7] Particular attention has been paid to outer-sphere processes because these are most commonly observed. Creutz,[8] Macartney and Sutin,[9] and others have demonstrated unambiguously that reductions by ascorbic acid and its corresponding ions can be successfully described using Marcus theory.[10−12] A review by Davies[13] nicely summarizes the many issues involved in these reactions and the factors affecting the electron-transfer processes. Here, mechanistic studies of the vanadium L-ascorbate system are described and extend the studies originally reported by the Kustin and Toppen[14] group at acidic pH values.

While several transition-metal complexes of ascorbic acid form, only a few of these are known to undergo electron transfer through an inner-sphere mechanism. Perhaps not surprisingly, chromate(VI) has received the most attention in view of its known carcinogenicity.[15] The use of chromium(III) compounds as dietary supplements prevails regardless of their potential to be converted to chromium compounds in higher oxidation states. The potential for ascorbic acid to detoxify the high-oxidation-state chromium compounds[16−18] has stimulated many studies involving inner-sphere electron-transfer processes of these compounds.[19] Gould, Ghosh, and others have characterized in detail chromium(V) reductions by ascorbic acid, which are now recognized as possible intermediate species in the electron-transfer processes involving chromium(VI).[19]

Several groups have explored the complex reaction schemes observed with iron(III) and ascorbic acid.[20] Depend-

* To whom correspondence should be addressed. E-mail: johnson@nmsu.edu (M.D.J.), crans@lamar.colostate.edu (D.C.C.).
† New Mexico State University.
‡ Colorado State University.

(1) Crans, D. C.; Smee, J. J.; Gaidamauskas, E.; Yang, L. Q. *Chem. Rev.* **2004**, *104*, 849.
(2) Lappin, A. G.; Laranjeira, M. C. M. *J. Chem. Soc., Dalton Trans.* **1982**, 1861.
(3) Pelizzetti, E.; Mentasti, E. E.; Pramauro, E. *Inorg. Chem.* **1978**, *17*, 1181.
(4) Ghosh, S. K.; Gould, E. S. *Inorg. Chem.* **1989**, *28*, 1538.
(5) Akhtar, M. J.; Haim, A. *Inorg. Chem.* **1988**, *27*, 1608.
(6) Holder, A. A.; Im, S.-C.; Dasgupta, T. P. *Transition Met. Chem.* **1997**, *22*, 135.
(7) Macartney, D. H.; McAuley, A. *Can. J. Chem.* **1981**, *59*, 132.
(8) Creutz, C. *Inorg. Chem.* **1981**, *20*, 4449.
(9) Macartney, D. H.; Sutin, N. *Inorg. Chim. Acta* **1983**, *74*, 221.
(10) Marcus, R. A. *J. Phys. Chem.* **1963**, *67*, 853.
(11) Marcus, R. A. *Discuss. Faraday Soc.* **1960**, *29*, 21.
(12) Marcus, R. A. *Can. J. Chem.* **1959**, *37*, 155.
(13) Davies, M. B. *Polyhedron* **1992**, *11*, 285.
(14) Kustin, K.; Toppen, D. L. *Inorg. Chem.* **1973**, *12*, 1404.
(15) Cohen, M. D.; Klein, C. B.; Costa, M. *Mutat. Res.* **1992**, *269*, 141.
(16) Codd, R.; Dillon, C. T.; Levina, A.; Lay, P. A. *Coord. Chem. Rev.* **2001**, *216*, 537.
(17) Levina, A.; Codd, R.; Dillon, C. T.; Lay, P. A. *Prog. Inorg. Chem.* **2003**, *51*, 145.
(18) Levina, A.; Lay, P. A.; Dixon, N. E. *Inorg. Chem.* **2000**, *39*, 385.
(19) Ghosh, S. K.; Bose, R. N.; Gould, E. S. *Inorg. Chem.* **1987**, *26*, 2684.

10.1021/ic050749g CCC: $33.50 © 2006 American Chemical Society
Published on Web 01/20/2006

ing on the complex and conditions, iron(III) complexes can undergo reduction by either inner- or outer-sphere processes.[20] Davies summarized the outer-sphere electron-transfer reactions and noted the paucity of examples of inner-sphere electron transfer.[13] Few studies have focused on the spectroscopic characterization of the complexes that form between the oxidant and ascorbate or its corresponding oxidation product. One successful study was the reaction of pertechnetate with ascorbic acid, which after reduction was shown to produce what is believed to be a technetium(V) dehydroascorbic acid complex.[21]

In 1973, Kustin and Toppen[14] reported the first kinetic study of the reduction of vanadium(V) with ascorbic acid. At low pH, vanadium(V) exists as *cis*-dioxovanadium(V), VO_2^+; however, as the pH is increased, the most prevalent forms are vanadate, $H_2VO_4^-$, or decavanadate, $H_3V_{10}O_{28}^{3-}$.[1,22] At neutral pH, the metavanadates exist, which in the simplest form is $VO_2(OH)_2^-$ (commonly referred to as $H_2VO_4^-$). The reaction of ascorbic acid with vanadium(V) is likely to change significantly as the vanadium(V) species in solution convert from VO_2^+ to $H_2VO_4^-$ [21,22] and ascorbic acid deprotonates.[23] The initial study by Kustin and Toppen was carried out at acidic pH.

Since the original study by Kustin and Toppen,[14] other kinetic studies involving vanadium ions and ascorbic acid have been reported.[18] Studies involving both ascorbate and vanadate include speciation studies,[24] isolation of ascorbate-derived complexes,[25] and examination of the insulin mimetic effects of ascorbate when coadministered with some vanadium compounds.[26] Spectroscopic and kinetic characterizations of the reduction of vanadium(V) complexes by ascorbate at pH 7 conflict regarding the reduction of vanadium(V) or the lack thereof.[25,27] In light of the current interest in the effectiveness of vanadium complexes with different ligands and multiple oxidation states for use as insulin-enhancing drugs,[28] a more detailed understanding of the fundamental reduction reactions of aqueous vanadium(V) with ascorbic acid is needed. This work reports the reduction of vanadium(V) by ascorbic acid between pH 0.4 and 4.0 using kinetic methods, as well as spectroscopic characterization of complexes that form in this and in the neutral pH range.

Experimental Section

Materials. Ammonium metavanadate and L-ascorbic acid were purchased from Aldrich Chemical Co. Potassium chloride, hydrochloric acid, and potassium hydroxide were purchased from Fisher Scientific (Acros). All chemicals were used as obtained unless otherwise noted.

Solution Preparation for Kinetic and Spectroscopic Studies. The solutions of L-ascorbic acid were prepared fresh daily. Kinetic solutions were used immediately and without an argon purge of oxygen because no differences were observed for the rates under aerobic and anaerobic conditions. The pH was adjusted to the desired value using HCl or NaOH and checked both before and after the reaction; no significant changes were observed.

The solutions for the electron paramagnetic resonance (EPR) and NMR spectroscopic studies were prepared from double-deionized and distilled water. Stock solutions of vanadate and aqueous vanadium(IV) were prepared and adjusted to the desired pH before mixing with L-ascorbate. Stock solutions of L-ascorbic acid and monoprotonated L-ascorbate were freshly prepared just before preparation of the vanadium ascorbate solutions. The pH was adjusted prior to recording the spectra, and the reported pH values were those determined immediately after completion of the spectral analysis.

Kinetic Studies. The kinetic data were collected either on a Dionex stopped-flow instrument interfaced with an OLIS data acquisition/data reduction system or on an OLIS RSM1000 system. All reactions were carried out using absorbance changes at 430 nm at 25.0 °C and an ionic strength of 0.40 M KCl. Pseudo-first-order conditions were used with the L-ascorbic acid in at least 10-fold excess (5.0×10^{-4}−0.10 M) over the vanadium(V) concentration (5.0×10^{-5} M). Each rate constant represents the average of three to five trials and was obtained using the OLIS Kinfit routines for first-order behavior and with an error of no more than 10%. Analysis of the rate constants obtained used Origin linear fitting routines or nonlinear fits of the more complex dependencies.

EPR and NMR Studies. X-band EPR spectra were obtained on a Bruker EMX 300 spectrometer at ambient temperature. The solutions were placed in 0.2-mm capillary tubes, which were inserted into 4−5-mm quartz tubes. The spectra were recorded using parameters similar in part to those in reported studies[27,29] and to test for potential parameter artifacts while investigating different types of complexes. Most experiments were reported at 9.82-GHz and 20-mW microwave power with a modulation frequency of 100 kHz, a modulation amplitude of 5.00 G, a time constant of 164 ms, a sweep width of 1500 G, a sweep time of 168 s, a resolution of 2048 points, and four scans with a central field of 3238 G. These parameters were modified slightly (specifically, the microwave power was decreased from 50 mW) from those previously used in order to decrease heating and other artifacts when recording the spectra.[29] Parameters used by Ding et al.[27] were also used comprising a receiver gain of 5×10^4, a modulation amplitude of 0.8 mT, a scan time of 200 s, a time constant of 300 ms, a sweep width of 1500 G, and a sweep time of 200 s and with a central field of 3470 G. A powder sample of 2,2-diphenyl-1-picrylhydrazyl ($g = 2.0036$)[30] was used as an external standard. Data analyses were performed with a Bruker WINEPR System. The ^{51}V NMR spectra were recorded on a Varian INOVA-300 spectrometer using routine parameters.[31] The chemical shifts were recorded against an external reference of $VOCl_3$ with a ^{51}V chemical shift of 0 ppm.

(20) Inner-sphere electron transfer is most prevalent with aqueous iron(III) possessing substitution sites. Hiseh, Y.-H. P.; Hiseh, Y. P. *J. Agric. Food Chem.* **2000**, *48*, 1569. Gosh, S. K.; Gould, E. S. *Inorg. Chem.* **1989**, *28*, 1538. Hynes, M. J.; Kelly, D. F. *Chem. Commun.* **1988**, *13*, 849 and refs 41 and 42 cited therein.
(21) Grases, F.; Genestrar, C.; Amat, E. *J. Chem. Kinet.* **1986**, *18*, 899.
(22) Crans, D. *Comments Inorg. Chem.* **1994**, *16*, 1.
(23) Holder, A. A.; Brown, R. F. G.; Marshall, S. C.; Payne, V. C. R.; Cozier, M. D.; Alleyne, W. A.; Bovell, C. O. *Transition Met. Chem.* **2000**, *25*, 605.
(24) Sreedhara, A.; Susa, N.; Rao, C. P. *Inorg. Chim. Acta* **1997**, *263*, 189.
(25) Ferrer, E. G.; Baran, E. *J. Biol. Trace Element Res.* **2001**, *83*, 111.
(26) Sakurai, H.; Inohara, T.; Adachi, Y.; Kawabe, K.; Yasui, H.; Takada, J. *Biorg. Med. Chem. Lett.* **2004**, *14*, 1093.
(27) Ding, M.; Gannett, P. M.; Rojanasakul, Y.; Liu, K.; Shi, X. *J. Inorg. Biochem.* **1994**, *55*, 101.
(28) Thompson, K. H.; Orvig, C. *Coord. Chem. Rev.* **2001**, *219*, 1033.
(29) Amin, S. S.; Cryer, K.; Zhang, B.; Dutta, S. K.; Eaton, S. S.; Anderson, O. P.; Miller, S. M.; Reul, B. A.; Brichard, S. M.; Crans, D. C. *Inorg. Chem.* **2000**, *39*, 406.
(30) Albanese, N. F.; Chasteen, N. D. *J. Phys. Chem.* **1978**, *82*, 910.

Results

Stoichiometry. L-Ascorbic acid reduces aqueous vanadium(V) in both strongly and weakly acidic media according to the following equation:

$$2H^+ + H_2A + 2VO_2^+(aq) \rightarrow A + 2VO^{2+}(aq) + 2H_2O \quad (1)$$

The 1:2 stoichiometry was determined at pH 1 and 4 (the pH range of the kinetic study) using ion-exchange chromatography of the product in the same fashion as that reported by Kustin and Toppen.[14] As other researchers have done, it was assumed that the oxidized L-ascorbate species is dehydroascorbate based on the 1:2 ratio (two-electron oxidation) as well as other reported redox studies.[13] A brown species (λ_{max} = 430 nm) was rapidly formed upon mixing of the two reactants, over the entire pH range investigated. Figure S1 in the Supporting Information shows the spectrum of this intermediate, which subsequently disappeared to give the final products.

Kinetic Studies. Because this intermediate is strongly absorbing, a limited kinetic study of its formation was possible even though the reaction rates are at the limits of stopped-flow capabilities. At 0.40 M HCl under pseudo-first-order conditions with excess reductant, plots of the pseudo-first-order rate constants versus L-ascorbic acid concentrations are linear with a significant *y* intercept (see the Supporting Information).

$$k_{obs}(\text{formation}) = a[H_2A] + b \quad (2)$$

Under pseudo-first-order conditions, with excess L-ascorbic acid, the decrease in the absorbance at 430 nm (the brown intermediate) was first-order with respect to the intermediate. Plots of k_{obs} versus L-ascorbic acid concentrations showed simple saturation kinetics. Below pH 2, the data fit the following general equation, in agreement with the behavior first observed by Kustin and Toppen:[14]

$$k_{obs} = c[H_2A]/(1 + d[H_2A]) \quad (3)$$

Evaluation of *c* and *d* was possible using linear double-reciprocal plots; see Figure 1. However, above pH 2, the double-reciprocal plots showed marked deviation from linearity; see the inset in Figure 2. At these pHs, the data fit an equation with an additional term in L-ascorbic acid.

$$k_{obs} = (c[H_2A] + e[H_2A]^2)/(1 + d[H_2A]) \quad (4)$$

Evaluation of the constants *c*−*e* was possible using nonlinear fitting routines using Origin. Data and a typical fit are shown as a solid line in Figure 2.

EPR and NMR Characterization of Vanadium(IV) Ascorbate and Vanadium(V) Ascorbate Complexes: Low-pH Studies. The reaction of vanadate with L-ascorbic acid and L-ascorbate was investigated using EPR and ^{51}V NMR spectroscopy in the same pH region as the kinetic studies. EPR spectra recorded at ambient temperature of solutions

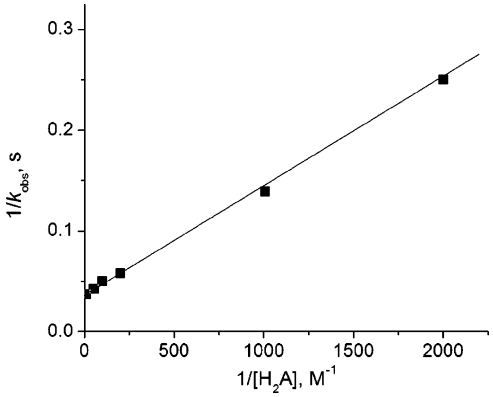

Figure 1. Plot of the inverse rate constant as a function of the inverse substrate concentration. Conditions: pH 0.40, 0.50 mM NH_4VO_3, 0.40 M HCl at 25.0 °C.

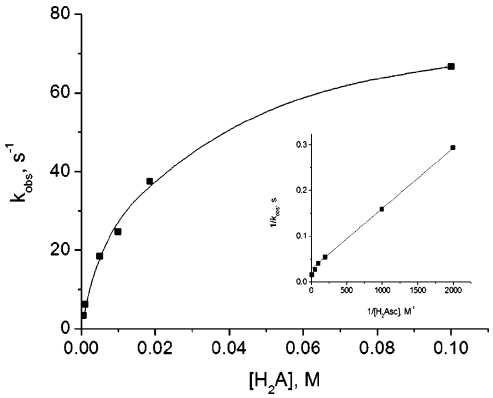

Figure 2. Plot yielding the pseudo-first-order rate constants at pH 2.0. The line shows the fit based on eq 6 in the text. Inset: Double-reciprocal plot at pH 2.0. Conditions: 0.50 mM NH_4VO_3, 10.0 mM HCl, 0.40 M KCl at 25.0 °C.

of vanadium(IV) and L-ascorbate at a 1:1 ratio (5 mM each) and acidic pH show a signal with the characteristic eight-line pattern and with parameters indistinguishable from that of VO^{2+} (Figure 3). This study demonstrates that at a 1:1 ratio L-ascorbate reduces sufficient vanadium(V) for observation. The possible formation of a vanadium(IV) L-ascorbate complex was investigated further using both visible and EPR spectroscopy. Acidic solutions of VO^{2+} in the presence of equimolar L-ascorbic acid and over pH 1−3 gave EPR spectra with parameters characteristic of only aqueous VO^{2+} [vanadium(IV)], thereby indicating no significant amount of complex in these solutions (data not shown). In contrast, EPR spectra (Figure 3) of solutions of VO^{2+} and L-ascorbic acid at pH 3.24 and a 200-fold of L-ascorbic acid indicate the formation of a vanadium(IV) complex (g_0 = 1.984, A_0 = 108 G) similar to species observed for solutions with a 200-fold excess of L-ascorbic acid over vanadate(V) (g_0 = 1.984, A_0 = 108 G). Because these conditions give spectra with identical parameters, it is likely that it is the same vanadium-(IV) species that forms in both solutions.

The EPR spectra of solutions of 5.0 mM vanadate in the presence of 50 mM L-ascorbic acid at various pH values are shown in Figure 4. At pH 1.5 and 3.0, no evidence for complex formation was observed, although the vanadium-(IV) L-ascorbic acid complex is readily observed at a 200-fold excess at these pH values. However, as the pH is

(31) Crans, D. C.; Bunch, R. L.; Theisen, L. A. *J. Am. Chem. Soc.* **1989**, *111*, 7597.

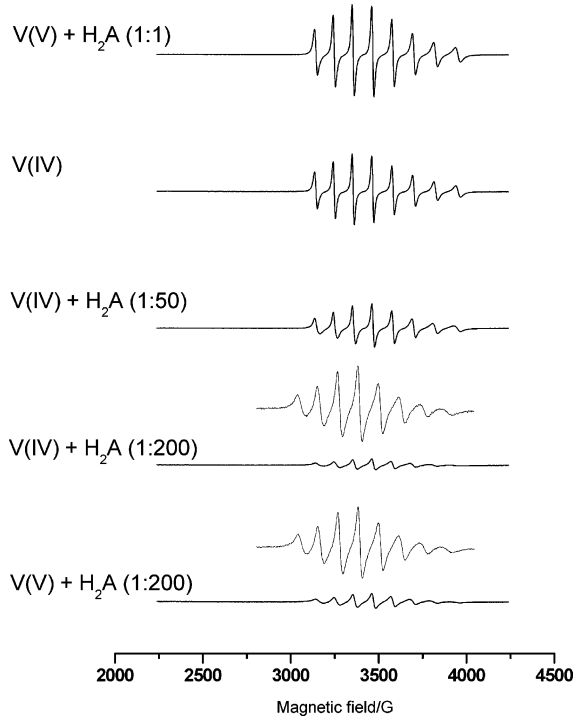

Figure 3. Ambient-temperature EPR spectra of solutions containing 5.0 mM VO_2^+ and 5.0 mM L-ascorbic acid (H_2A) (at pH 2.8) (top), 5.0 mM VO^{2+}, 5.0 mM VO^{2+} reference, and 0.25 M H_2A at pH 3.2, 5.0 mM VO^{2+} and 1.0 M H_2A at pH 3.2, and 5.0 mM VO_2^+ and 1.0 M H_2A at pH 3.2 (bottom). The spectra were recorded using the following parameters: number of scans = 4, center of field = 3238 G, sweep width = 2000 G, resolution = 2048 points, microwave frequency = 9.82 GHz, microwave power = 20 mW, receiver gain = 4.48×10^4, modulation frequency = 100 kHz, modulation amplitude = 5 G, conversion time = 82 ms, time constant = 164 ms, and sweep time = 168 s.

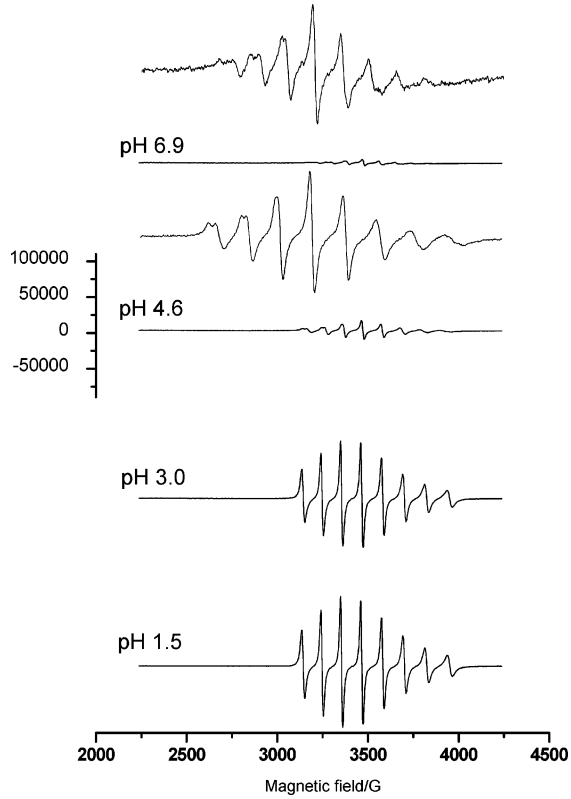

Figure 4. Ambient-temperature EPR spectra of solutions containing 5.0 mM $H_2VO_4^-$ and 50 mM H_2A recorded at pH 1.5, 3.0, 4.6, and 6.9. The spectra were recorded using the parameters listed in the caption for Figure 3.

increased to 4.6, two new species are clearly observed. As the pH is increased further to 6.9, one of the two species in the pH 4.6 solution disappears and a new species with a larger g_0 value appears (Figure 5). The signal-to-noise ratio in the pH 6.9 spectrum has decreased significantly, which indicates a lower quantity of complex compared to the other spectra at lower pH values. However, the fact that two species remain observable at near-neutral pH where a salt solution of $VOSO_4$ would yield an EPR-silent spectrum testifies to the high stability of these complexes.

Spectroscopic Characterization of Vanadium(IV) Ascorbate and Vanadium(V) Ascorbate Complexes: Neutral-pH Studies. In an attempt to resolve the reported ambiguity concerning the L-ascorbate reduction of vanadium(V) at neutral pH, we initiated a series of EPR and ^{51}V NMR studies of the vanadium(V) ascorbate reaction.[24,25,27,28,45] Solutions of vanadium(IV) and L-ascorbate at pH 7.0 led to the formation of a distinct but weak signal with the two eight-line patterns characteristic of two different vanadium(IV) species. The parameters for both species ($g_0 = 1.989$, $A_0 = 96$ G; $g_0 = 1.974$, $A_0 = 106$ G) were different from those of VO^{2+} and thus support the formation of two vanadium(IV) complexes. These are presumably vanadium(IV) complexes with L-ascorbate or an oxidation product of L-ascorbate. These results disagree with those of Ding and co-workers, who reported that no reduction of vanadium(V) took place.[27]

We further investigated this system by varying the concentrations of the vanadate and L-ascorbate ions. Figure 5 shows representative spectra when the vanadium(V) concentration was kept constant at 5.0 mM while the

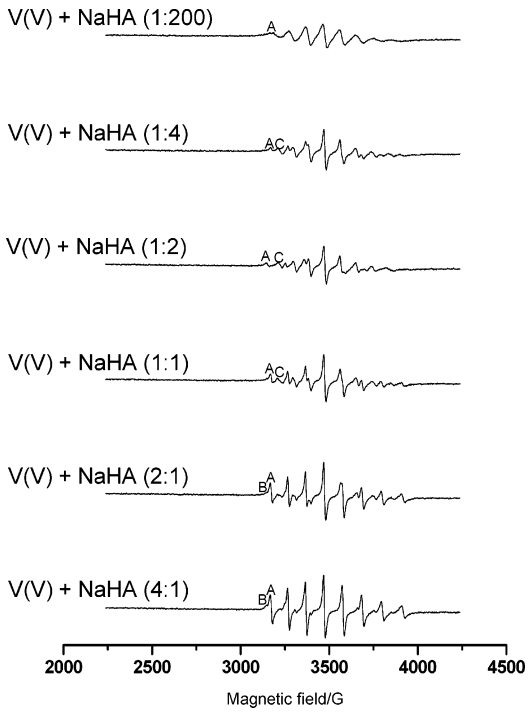

Figure 5. Ambient-temperature EPR spectra of solutions containing 5.0 mM $H_2VO_4^-$ at pH 7.0 in the presence of 1.25−1000 mM L-ascorbate (HA^-). The spectra were recorded using the parameters listed in the caption for Figure 3.

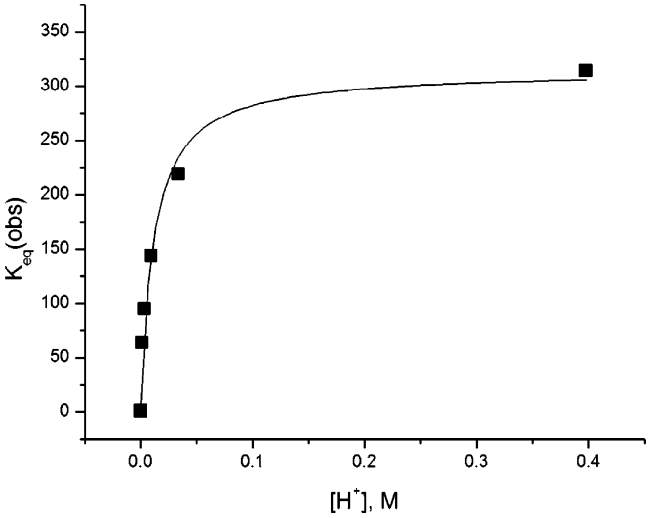

Figure 6. Acid dependence of the observed formation constants determined from kinetic data for solutions of NH_4VO_3/H_2A. Conditions: 0.40 M KCl, 0.50 mM NH_4VO_3 at 25.0 °C.

L-ascorbate concentration was varied from 1 to 1000 mM. The spectra indicate that three different complexes are formed, both of which are different from the third complex shown in Figure 4. The latter forms with a large excess of L-ascorbate ($g_0 = 1.989$, $A_0 = 96$ G). On the basis of these studies and information in the literature,[25,45] we assign the signal at $g_0 = 1.990$ and $A_0 = 97$ G to a 1:1 complex, the signal at $g_0 = 1.979$ and $A_0 = 109$ to a 1:2 complex (species C in Figure 5), and the signal at $g_0 = 1.64$ and $A_0 = 99$ G (species B in Figure 5) to a complex formed with the oxidation product of L-ascorbate, presumably dehydroascorbic acid.[45]

Solutions of vanadate and L-ascorbic acid were also investigated by visible and ^{51}V NMR spectroscopy at pH 3 and 7. Using the reported rate constant at pH 7.4,[33] a solution of 5 mM vanadate in the presence of 5.0 mM L-ascorbate should still contain 20−60% of the vanadium(V) ascorbate complex within 5 min of mixing. Unfortunately, even at 0 °C, no new ^{51}V NMR signals were observed. A minor vanadium(V) signal was observed in some samples but was attributed to vanadate ions.

Discussion

Kinetic Studies. (a) Formation of Intermediate. The reaction between aqueous vanadium(V) and L-ascorbic acid over the entire pH range studied produces a brown intermediate with an absorption maximum at 430 nm. Our results agree with Kustin and Toppen's earlier report with an absorption maximum at 425 nm. The difference in the absorption maxima between this study and Kustin and Toppen's can be explained by the different counterions used in the studies (chloride in this work and perchlorate in Kustin and Toppen's study). In agreement with Kustin and Toppen, we describe the formation of the brown intermediate in terms of a reversible complexation reaction between L-ascorbic acid and aqueous vanadium(V).

Assuming a simple equilibrium for the formation of the vanadium(V) ascorbic acid intermediate, the slope and intercept (a and b) shown in eq 2 represent the forward and reverse rate constants, i.e., $k_f = 5.5 \times 10^4$ M^{-1} s^{-1} and $k_r = 170$ s^{-1} at 0.40 M HCl. The ratio of these rate constants gives a value for K_{eq} ($=k_f/k_r$) equal to 320 M^{-1}, in good agreement with the both results reported by Kustin and Toppen and the reduction data that will be discussed later in the present study.[14]

(b) Reduction of Vanadium(V) at Low pH: Intramolecular Electron Transfer. With the intent to examine the reaction nearer to physiological pH, we expanded Kustin and Toppen's work[14] up to pH 4.0. The loss of the 430-nm intermediate was followed over an acidic range of 10^{-4}–0.40 M HCl, and the reaction was carried out in the absence of a buffer because even inert and "noncomplexing" buffers have been shown to affect vanadium(V) and -(IV) speciation.[31,32] The pH was checked before and after the reaction, which confirmed that no pH change had occurred during the reaction.

The rapid formation of the intermediate was followed by a slower step, intramolecular electron transfer, to produce vanadium(IV) and the L-ascorbate radical; the latter rapidly reduced another vanadium(V) center to account for the reaction stoichiometry. Equations 5−7 show a general (no detailed balancing) reaction scheme where H_2A represents L-ascorbic acid and VO_2^+ the aqueous vanadium(V). A detailed mechanism will be developed later. On the basis of

$$H_2A + VO_2^+ \overset{K_{eq}}{\rightleftharpoons} \text{intermediate} \quad (5)$$

$$\text{intermediate} \xrightarrow{k_{et}} VO^{2+} + HA^\bullet \quad (6)$$

$$VO_2^+ + HA^\bullet \xrightarrow{\text{fast}} VO^{2+} + A \quad (7)$$

this scheme, the saturation kinetics described in eq 4 can now be recast where "c" equals $k_{et}K_{eq}$ and "d" equals K_{eq}; see eq 8. The observed saturation behavior permits evaluation

$$k_{obs} = k_{et}K_{eq}[H_2A]/(1 + K_{eq}[H_2A]) \quad (8)$$

of K_{eq} and k_{et}, both of which remain essentially constant when the acid concentration changes from 1.00 to 0.20 M HCl. The binding constant and the inner-sphere electron-transfer rate constant are 350 M^{-1} and 12 s^{-1}, respectively, which agree well with those reported by Kustin and Toppen.[14]

(c) Reduction of Vanadium(V) by L-Ascorbic Acid at Higher pH: a Second Electron-Transfer Step. At pH 2 and above, a closer examination of the data in Figure 2 shows that the values of k_{obs} do not level off at high reductant concentrations but rather continue to increase gradually. This is confirmed by the deviation from linearity in the double-reciprocal plot at high L-ascorbic acid concentrations (Figure 2, inset). These observations are consistent with the notion that a second reduction process has become competitive with the inner-sphere pathway. An additional term that is second-order in L-ascorbic acid is required to fit the data as shown in eq 4. This is accounted for as shown in eq 9, where k_{et2}

(32) Crans, D. C.; Shin, P. K. *Inorg. Chem.* **1988**, *27*, 1797.

is the rate constant for the new electron-transfer pathway.

$$H_2A + \text{intermediate} \xrightarrow{k_{et2}} V^{IV} + HA^{\bullet} + H_2A \qquad (9)$$

Equation 9 can be incorporated into the overall reaction scheme (eqs 5−7) to yield eq 10, which is equivalent to the observed kinetic dependence in eq 4 with the addition of $K_{eq}k_{et2}$ as "*e*". Figure 2 shows an excellent nonlinear least-

$$k_{obs} = (K_{eq}k_{et}[H_2A] + K_{eq}k_{et2}[H_2A]^2)/(1 + K_{eq}[H_2A]) \quad (10)$$

squares fit (solid line) of the data using eq 10. At pH 2, the k_{et} first-order rate constant is 49 s^{-1} and k_{et2} is 220 M^{-1} s^{-1}. At 0.10 M L-ascorbic acid, the calculated pseudo-first-order second-reduction rate constant is 22 s^{-1}. This rate constant is high enough to compete with the inner-sphere rate constant of 49 s^{-1}.

Several factors point toward the second reduction process being an outer-sphere electron-transfer mechanism: (1) the value for K_{eq} *decreases* dramatically with pH (see the discussion below and Figure 3), which makes a second complexation most unfavorable; (2) no second complexation term appears to be necessary in the denominator of eq 10 (i.e., no $K_{eq2}[H_2A]^2$) to fit the data; (3) no change in the spectrum for the vanadium-containing intermediate (Figure S1 in the Supporting Information) is observed from that at lower L-ascorbic acid concentrations. Unfortunately, however, in the absence of further kinetic data, the assignment cannot be considered as unambiguous and ruling out alternative interpretations.

Orvig et al.[33] have recently reported the L-ascorbic acid reduction rate constants for bis(maltolato)dioxovanadate(V) and during the analysis also generated data for the reaction of "free vanadate(V)", between pH 6 and 7.5. The two rate constants were reported to be 5×10^7 and 10^7 M^{-2} $s^{-1}[H^+]$, respectively. Because these are third-order rate constants, a comparison of the data requires their rate constants to be extrapolated to pH 2. Because the pK_{a1} for L-ascorbic acid is 4.17,[23] the fully protonated L-ascorbic acid is present at pH 2 instead of the monoanionic L-ascorbate ion as in Orvig et al.'s study.[33] Kinetic data on the L-ascorbic acid reduction of bis(hydroxypyridinone)dioxovanadate(V) and *cis*-dioxovanadium(V) at pH 3 give an outer-sphere rate constant of around 300 M^{-1} s^{-1}.[34] If this is true for the present system, a value between 10^1 and 10^2 M^{-1} s^{-1} is expected for Orvig et al.'s "free" and bis(maltolato)oxovanadium(V) complexes.[33] On the basis of the values compiled by Davies[13] for a large number of outer-sphere reductions by L-ascorbic acid and its ions, the ratio of rate constants for the mono- and diprotonated L-ascorbic acid species ranges between 10^3 and 10^4. These values for the proposed outer-sphere reduction reactions are in the same range as the 220 M^{-1} s^{-1} values observed in the present study and lend credence to the proposed outer-sphere step.

(d) Effect of the pH on the Inner-Sphere Pathway. Figures 6 and 7 show the plots of K_{eq} and k_{et} as functions of

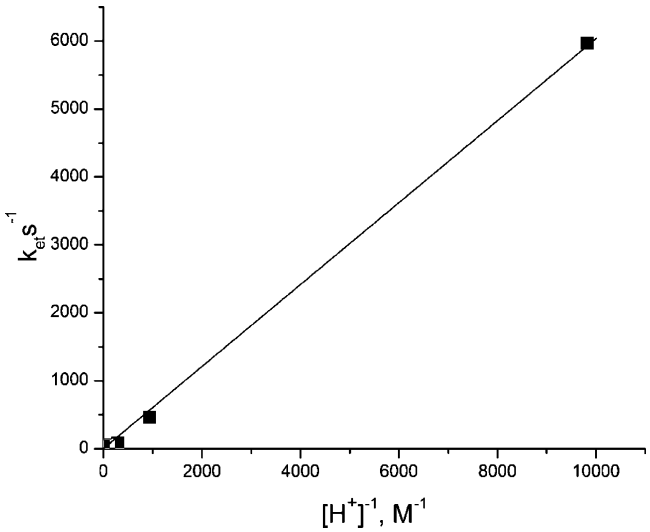

Figure 7. Acid dependence of the observed intramolecular electron-transfer rate constant for the NH_4VO_3/H_2A complex from kinetic data. Conditions: 0.40 M KCl, 0.50 mM NH_4VO_3 at 25.0 °C.

$[H^+]$. It is somewhat surprising that the observed equilibrium constant for the formation of the vanadium(V) ascorbate adduct decreases as $[H^+]$ decreases. Similar behavior was previously noted by Dasgupta et al. in the chromate(VI) oxidation of ascorbic acid.[35] In Dasgupta et al.'s work, the decrease was attributed to a proton from ascorbate forming water from a coordinated hydroxide on chromium(VI), which facilitated the formation of a chromate ascorbate ester.[35] No outer-sphere reduction pathway was observed in these systems. They attributed the lack of reduction to chromate's poor oxidizing potential when fully deprotonated.

In the present kinetic study, there are two possible explanations for the decrease observed in K_{eq} with increasing pH. First, the monoanionic L-ascorbate forms a weak coordination complex with the *cis*-dioxovanadium(V) ion, and as the $[H^+]$ concentration increases, the concentration of monoanionic L-ascorbate decreases. Second, there is a speciation change in the aqueous vanadium(V) system. The binding data for L-ascorbic acid with *cis*-dioxovanadium(V) fit the relationship described in eq 11, as shown by the solid line in Figure 6.

$$K_{eq}(obs) = K_{eq}K_a[H^+]/(1 + K_a[H^+]) \qquad (11)$$

K_a is an acid ionization constant, and K_{eq} is the binding constant for L-ascorbic acid with the *cis*-dioxovanadium(V) ion. The latter is 320 ± 40 M^{-1} at 0.40 M KCl and 25.0 °C. K_a is 68 ± 15 in the present study. The reported pK_{a1} of 4.17 for the first ionization of L-ascorbic acid is significantly smaller than the observed K_a and is therefore more likely to represent a deprotonation on the vanadium(V) oxo unit. Two studies report the ionization constant of the VO(OH) unit to equal 4.5 and 30 M^{-1}.[36,37] The latter is a kinetically derived constant from the reduction of vanadium(V) in strong acid by outer-sphere metal complexes and is in good agreement with our value of 68 M^{-1}. Complexation studies with

(33) Song, B.; Aebischer, N.; Orvig, C. *Inorg. Chem.* **2002**, *41*, 1357.
(34) Crans, D. C.; Johnson, M. D., unpublished work.
(35) Dixon, D. A.; Sadler, N. P.; Dasgupta, T. P. *J. Chem. Soc., Dalton Trans.* **1993**, 3489.

8-hydroxyquinoline also show that protonated oxovanadium oxygens are necessary for reaction.[38] While the Kustin and Toppen study of the complex formation reaction between catechols and vanadium(V) in acidic media did not reveal a similar proton dependence, this can be explained by compensating effects in the forward and reverse rate constants.[37] Therefore, we attribute the decrease in the formation of the vanadium(V) L-ascorbic acid intermediate at higher pH to the deprotonation of an oxohydroxovanadium(V) ion, $VO(OH)^{2+}$ species, formed from the *cis*-dioxovanadium(V) ion, VO_2^+, and which can complex with L-ascorbic acid.

It is interesting to note that vanadium(V) L-ascorbic acid binding constants are substantially smaller than those of structurally similar ligands such as maltol or adenosine with vanadium(V), which have values of $K_{mal1} = 10^{8.8}$ and $K_{adn} = 10^{5.8}$.[39,40] Martell, in his review of metal ion complexation by ascorbic acid,[41] noted that the equilibrium constants for ascorbate are always smaller than expected. As an example, Davies cites the binding constant of monoanionic ascorbate with aluminum(III) as $10^{3.6}$, which is more than 2 orders of magnitude less than anticipated when compared to catechol ($10^{6.3}$).[13] Xu and Jordan[42] have invoked the large bite distance between oxygens in ascorbic acid to account for enhanced substitution rates on iron(III). We also point to the short C=C bond in ascorbic acid as an undesirable structural feature of these complexes that would contribute to destabilization of the complexes. Both these factors could be responsible for ascorbic acid's relatively small equilibrium constants in metal binding.

In contrast to the binding constant, the intramolecular electron-transfer rate constant, k_{et}, increases sharply as the pH is increased (Figure S2 in the Supporting Information). Figure 4 shows a plot of k_{et} versus $1/[H^+]$ to be linear with a significant *y* intercept. This plot corresponds to the two-term rate law shown in eq 12, where the subscripts H and O indicate protonated and deprotonated species, respectively. This kinetic form requires the simplification that $[H^+]$ is much larger than the deprotonation equilibrium constant for the bound L-ascorbic acid. Values for the proton-dependent

$$k_{et} = k_H + k_O[H^+]^{-1} \tag{12}$$

and -independent rate constants, k_H and k_O, are $55 \pm 10\ s^{-1}$ and $0.61 \pm 0.05\ M\ s^{-1}$, respectively, at 0.40 M KCl and 25 °C. Although the kinetic treatment involves a proton equilibrium, it is not possible from these kinetic data to discern where the proton loss in the vanadate L-ascorbate complex occurs. Furthermore, the acid dissociation constant for the vanadium(V) L-ascorbate complex cannot be determined from these studies.

Proton-assisted electron transfers have been proposed for redox reactions between oxometalate centers and reductants with a dissociable proton.[43,44] To test for this in the present study, experiments were carried out by measuring the rate constant in D_2O. At a p*D* equivalent to pH 2, the observed k_H/k_D ratio was 1.0. Because there were no differences in rates measured in H_2O and D_2O, the rate-limiting step does not involve a proton-assisted transfer. This suggests that proton assistance does not occur in this system.

(e) Effects of the pH on the Second Electron-Transfer Step. A detailed treatment of the second electron-transfer rate constant was not possible because relatively precise values could only be obtained over a very limited pH range, pH 2−3. This is because the vanadium(V)−L-ascorbic acid reaction rates at higher pH values are at the limits of the stopped-flow instrument and the second electron-transfer pathway is too slow to be observed at lower pH values.

However, if it is *assumed* that the second pathway proceeds via an outer-sphere pathway, the second pathway likely represents an outer-sphere reduction of the vanadium(V) ascorbate intermediate because the reductions by deprotonated L-ascorbic acid are faster than those by fully protonated L-ascorbic acid. In this case, the rate constant for the observed second electron transfer, k_{et2}, is a composite of K_{a1} for L-ascorbic acid and k_{os}, the rate constant for the reaction between the vanadium(V)−L-ascorbate complex and the L-ascorbate ion. This leads to the expression shown in eq 13. Using the value of k_{os} at pH 2 and K_{a1} equal to $10^{-4.17}$,

$$k_{et2} = k_{os}K_{a1}[H^+]/([H^+] + K_{a1}) \tag{13}$$

a value for k_{os} of approximately $10^6\ M^{-1}\ s^{-1}$ can be calculated.

On the basis of these considerations and the kinetic data and using the abbreviation H_2A (or AH_2) for L-ascorbic acid, a detailed reaction scheme can be constructed as shown in eqs 14−20.

$$H_2A \underset{K_a}{\overset{K_{a1}}{\rightleftharpoons}} H^+ + HA^- \tag{14}$$

$$VO(OH)^{2+} \rightleftharpoons VO_2^+ + H^+ \tag{15}$$

$$H_2A + VO(OH)^{2+}(aq) \overset{K_{eq}}{\rightleftharpoons} HO_2VAH_2^{2+} \tag{16}$$

$$HO_2VAH_2^{2+} \overset{K_a'}{\rightleftharpoons} \{O_2VAH\}H^+ + H^+ \tag{17}$$

$$HO_2VAH_2^{2+} \xrightarrow{k_{etH}} V^{IV} + HA^{\bullet} \tag{18}$$

$$\{O_2VAH\}H^+ \xrightarrow{k_{etO}} V^{IV} + HA^{\bullet} \tag{19}$$

On the basis of this mechanism, the values for k_H and k_O in eq 12 correspond to k_{etH} and $k_{etO}K_a'$ in eqs 17−19. Because

(36) Floriani, C.; Gambarotta, S.; Chiesi-Villa, A.; Guestini, C. *J. Chem. Soc., Dalton Trans.* **1987**, 2099.
(37) Kustin, K.; Liu, S.-T.; Nicolini, C.; Toppen, D. L. *J. Am. Chem. Soc.* **1974**, *96*, 7410.
(38) Giacomelli, A.; Floriani, C.; Ofir de Souza Duarte, A.; Chiesi-Villa, A.; Guastini, C. *Inorg. Chem.* **1982**, *21*, 3310.
(39) Elvingson, K.; Gonzalez Baro, A.; Pettersson, L. *Inorg. Chem.* **1996**, *35*, 3388.
(40) Tracey, A. S.; Jaswal, J. S.; Gresser, M. J.; Rehder, D. *Inorg. Chem.* **1990**, *29*, 4283.
(41) Martell, A. E. *Adv. Chem. Ser.* **1982**, *200*, 153.
(42) Xu, J.; Jordan, R. B. *Inorg. Chem.* **1990**, *29*, 4180.
(43) Bryant, J. R.; Mayer, J. M. *J. Am. Chem. Soc.* **2003**, *125*, 10351.
(44) Binstead, R. A.; McGuire, M. E.; Dovletoglou, A.; Seok, W. K.; Roecker, L. E.; Meyer, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 173.

$$\{O_2VAH\}H^+ \text{ (or } \{O_2VAH_2^{2+}\}) + HA^- \xrightarrow{k_{os}} V^{IV} + HA^{\bullet} + HA^- \quad (20)$$

the value for K_a' is unknown, resolution of k_O into its fundamental kinetic components is not possible. However, because the K_a of a coordinated acid is generally greater than its free (uncoordinated) value, a lower limit of $k_{etO} \sim 10^5$ s^{-1} can be calculated using K_a for free L-ascorbic acid.

EPR and NMR Spectroscopic Investigation of Vanadium−L-Ascorbate Complexes: Acidic-pH Studies. Given the seeming discrepancy in the literature whether vanadate does[26] or does not[27] oxidize L-ascorbate at neutral pH, studies were also carried out at the pH values of the kinetic and neutral studies. Unfortunately, no stopped-flow kinetic studies were conducted above pH 4 because no visible absorbance change was observed when solutions of vanadium(V) and L-ascorbate were mixed at higher pH values. A vanadium(IV) ascorbate adduct was observed in contrast to the previous report that no complex formed.[45] Kriss et al.[45] observed the formation of a complex that they assigned to a 1:1 complex with dehydroascorbic acid having slightly different parameters ($g_0 = 1.966$, $A_0 = 108.0$ G; $g_0 = 1.967$, $A_0 = 108.7$ G). Because the complex in this study was observed with a 200-fold excess of L-ascorbic acid, it is likely that the complexing ligand in our system is L-ascorbate and not dehydroascorbic acid. This assignment is consistent with the report by Ferrer and Baran, who reported the isolation of a 1:1 vanadium(IV) ascorbate complex.[25] Following the reported synthetic procedure using a 1:10 vanadium/L-ascorbic acid ratio, a green powder was isolated. Dissolution of the green powder at pH $\sim$ 6 resulted in a solution that contained two vanadium(IV) complexes ($g_0 = 1.990$, $A_0 =$ 97 G; $g_0 = 1.979$, $A_0 = 109$ G). The minor species ($g_0 =$ 1.979, $A_0 = 109$ G) observed in these studies has parameters indistinguishable from those of the vanadium(IV) complex observed in the present study formed with a large excess of L-ascorbic acid.

The observation of a new vanadium(IV) species in solutions of VO^{2+} and L-ascorbate at low pH values is important because it supports the existence of a vanadium(IV) ascorbate complex. Although the complex only forms in the presence of a large excess of L-ascorbate and readily dissociates, spectroscopic support for such a complex has been provided. The kinetic evidence suggesting an outer-sphere process involving a vanadium(V) ascorbate complex described in this work is therefore supported by the spectroscopic demonstration that a vanadium(IV) ascorbate complex can form.

Spectroscopic Characterization of Vanadium(IV) Ascorbate and Vanadium(V) Ascorbate Complexes: Neutral-pH Studies. In contrast to the reaction in the acidic pH range, ambiguity exists as to whether L-ascorbic acid reduces aqueous vanadium(V) in the absence of potential coordinating agents at neutral pH. Using EPR experiments at ambient temperature, Ding et al.[27] have stated that 4 mM vanadate is not reduced by 1.25 mM L-ascorbate at pH 7. In contrast, Rao et al. reported that the reduction of vanadate by L-ascorbate at pH 7.4−8.0 was a facile process,[24] which is in agreement with Orvig et al.'s work on the L-ascorbate reduction of a complex mixture of free and maltolatovanadium(V) complexes.[33]

On the basis of the literature,[25,45] we assign the signals in Figure 5 to 1:1 and 1:2 complexes formed with L-ascorbic acid and a third complex with the oxidation product of L-ascorbate, possibly dehydroascorbic acid.[25,45] This interpretation differs from that of Kriss and co-workers in that the parameters for our complex with the L-ascorbic acid oxidation product vary.[45] The calculation of the parameters for the latter complex is not precise because the spectra with this complex have poor signal-to-noise ratios and attempts to improve them had limited success. All complexes we observed are different from oxalato(oxo)vanadium(IV) complexes ($g_0 = 1.968$, $A_0 = 105$ G; $g_0 = 1.981$, $A_0 = 89$ G; $g_0 = 1.965$, $A_0 = 102$ G)[46] and related complexes previously isolated from the reaction of vanadate with L-ascorbic acid.[47]

Ding et al.[27] reported a spectrum with the typical eight-line signal for VO^{2+} [aqueous vanadium(IV)] for the EPR spectrum of a solution containing 4.0 mM $NaVO_3$ and 1.25 mM ascorbate in a 50 mM phosphate buffer at pH 7.4 15 min after preparation of the solution. Under these conditions, we were unable to reproduce his results using his and slightly different EPR parameters. In a related spectrum of 4.0 mM $NaVO_3$ and 1.25 mM L-ascorbate in the absence of phosphate, Ding et al.[27] reported that they observed no EPR signal. However, upon examination of their reported figures, a low-intensity signal is apparent in the baseline of the spectrum that corresponds to the 1:1 species we described above. Therefore, it is proposed that both Ding et al.[27] and the present work demonstrate the L-ascorbate reduction of vanadate(V). Because of the speciation chemistry of vanadium(IV) at neutral pH, only a very low intensity signal is visible in the EPR spectrum. Ding et al.[27] observed an enhanced signal in the presence of phosphate, which we were unable to reproduce. We can only speculate that the published spectrum may be that of the vanadyl cation at low pH in the presence or absence of phosphate and may not actually reflect the formation of a ternary vanadium(IV) phosphate ascorbate complex at neutral pH.[48] Indeed, at neutral pH, vanadium(IV) oxoorthophosphate is reported to precipitate. Our results are in agreement with the collective results of Orvig et al.,[33] Sakurai et al.,[26] Rao et al.,[24] and Ferrer and Baran[25] and in contrast to those reported by Ding and co-workers[27] at neutral pH.

Conclusions

L-Ascorbic acid reacts with vanadium(V) over the pH range of 0.40−4.0 to form a brown intermediate that absorbs at 430 nm. The kinetics of the disappearance of this peak,

(45) Kriss, E. E.; Yatsimirskii, K. B.; Kurbatova, G. T.; Grigoreva, A. S. *Russ. J. Inorg. Chem.* **1975**, *20*, 55.
(46) McPhail, D. B.; Goodman, B. A. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 3513.
(47) Baruah, B.; Golub, V. O.; O'Connor, C. J.; Chakravorty, A. *Eur. J. Inorg. Chem.* **2003**, 2299.
(48) Alberico, E.; Micera, G. *Inorg. Chim. Acta* **1994**, *215*, 225.

to form vanadium(IV), require the presence of two different electron-transfer pathways. The effects of the pH on L-ascorbic acid binding to the vanadium(V) center are observed when the major form of the vanadium(V) is HVO_2^{2+}. Unfortunately, the lack of absorbance of vanadium−L-ascorbate complexes above pH 4 prevented kinetic studies at higher pH values. EPR spectroscopic studies were carried out and provided evidence for three different complexes of vanadium(IV) after the L-ascorbic acid reduction of vanadium(V). In contrast, only two different complexes were observed in solutions containing vanadium(IV) and L-ascorbic acid. A 1:2 complex forms only at high ligand-to-metal ratios, whereas a 1:1 complex forms in solutions containing equimolar ligand and metal ions. The 1:2 vanadium(IV) L-ascorbate complex was found in solutions of either vanadate(V) or vanadium(IV) in the presence of a large excess of L-ascorbate. The existence of this complex is consistent with, but does not prove, an outer-sphere electron-transfer process described in this work. Spectroscopic observation of two complexes that form at pH 7 demonstrates that vanadate is reduced by L-ascorbate. These complexes are attributed to 1:1 and 1:2 complexes with L-ascorbate and an oxidation product of L-ascorbic acid, presumably dehydroascorbic acid.

Acknowledgment. We thank the National Institutes of Health for supporting most of this work at both Colorado State University and New Mexico State University. The authors also thank Professor Ken Kustin for his most helpful comments.

Supporting Information Available: Tables of kinetic data, absorption spectrum of the NH_4VO_3−L-ascorbic acid adduct 0.10 s after mixing (Figure S1), and a plot of the inner-sphere electron-transfer rate constants derived from eq 10 (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

IC050749G