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Titanium(IV) and Vitamin C: Aqueous Complexes of a Bioactive Form of Ti(IV)

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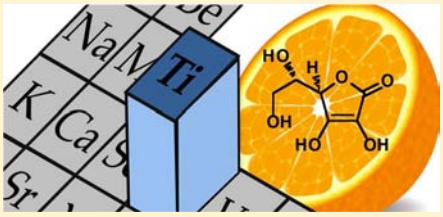
Supporting Information

ABSTRACT: Ascorbic acid is among the biorelevant ligands that render Ti(IV) stable in aqueous solution. A series of pH-dependent titanium(IV) coordination complexes of L-ascorbic acid is described. Directed by spectropotentiometric methods, important aspects of the aqueous interactions in this system are investigated, including ligand binding mode, pH-dependent metal−ligand stoichiometry, and the importance of metal ion-promoted hydrolysis and the binding of hydroxide. Stability constants are determined for all metal ion−ligand−proton complexes by a process of model optimization and nonlinear least-squares fitting of the combined spectropotentiometric titration data to the log β_{MLH} values in the model. A speciation diagram is generated from the set of stability constants described in the model. In the range pH 3−10, the aqueous speciation is characterized by the sequential appearance of the following complexes as a function of added base: $Ti(asc)_2^0 \rightarrow Ti(asc)_3^{2-} \rightarrow Ti(asc)_2(OH)_2^{2-} \rightarrow Ti(asc)(OH)_4^{2-}$. These species dominate the speciation at pH < 3, pH 4−5, pH ∼ 8, and pH > 10, respectively, with minimum log stability constants (β values) of 25.70, 36.91, 16.43, and −6.91. Results from electrospray mass spectrometry, metal−ligand binding experiments, and kinetics measurements support the speciation, which is characterized by bidentate chelation of the ascorbate dianion to the titanium(IV) ion via proton displacement, and a pH-dependent metal−ligand binding motif of ligand addition followed by metal ion-promoted hydrolysis, stepwise ligand dissociation, and the concomitant binding of hydroxide ion. Additionally, the kinetics of ligand exchange of titanium ascorbate with citrate are reported to understand better the possible fate of titanium ascorbate under biologically relevant conditions.



INTRODUCTION

L-Ascorbic acid is an important molecule in both chemistry and biology, and its complexes with metals are of particular interest in both of these areas.[1−3] Their study is complicated because the ligand has multiple protonation and oxidation states (Figure 1), because the metal−ligand complexes are in general not as tight as one might expect for an ene-diol ligand, and because the complexes have mostly defied crystallographic characterization.[1] In most cases, coordination via one of the modes **4**−**6** is invoked, characterized by monodentate or bidentate coordination via the oxygen atoms on C2 or C3, with the ligand singly or doubly deprotonated. Crystal structures are available for metal ascorbate complexes of the soft ions thallium(I)[2] and platinum(II),[3,4] which employ various coordination modes.[5,6] A multinuclear mixed-valent Fe(II)Fe(III)$_2$ complex with the related tetramethyl reductic acid ligand was characterized by X-ray crystallography and in solution.[7] One study reported bidentate binding on titanium dioxide surfaces through binding mode **6**, supported by IR, EXAFS, and XANES data.[8]

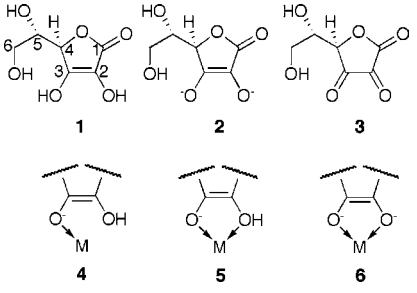


Figure 1. L-Ascorbic acid (H_2asc, **1**), doubly deprotonated ascorbic acid (asc^{2-}, **2**) (pK_a of C3-OH = 4.04 and pK_a of C2-OH = 11.34),[9] dehydroascorbic acid (**3**), and selected metal−ligand binding modes, including monodentate (mono)protonated (**4**), bidentate (mono)protonated (**5**), and bidentate deprotonated (**6**).

Complexes of ascorbic acid with titanium(IV) should be relatively strong,[10] and not prone to the metal-catalyzed ligand oxidation that renders many metal ascorbate complexes so reactive. Accordingly, complexes of ascorbic acid with titanium, first reported in 1936,[11] have been used for detection and quantitation of Ti(IV).[12] Early work was reviewed by Sommer,[13] who combined spectroelectrochemistry and electrophoresis to address the pH-dependent speciation of titanium ascorbate. The data supported the predominant species $Ti(Hasc)^{3+}$ (pH < 1), $TiO(Hasc)^{+}$ (1 < pH < 1.6), $TiO(Hasc)_2$ (1.6 < pH < 2.2), and $Ti(asc)_3^{2-}$ (2.5 < pH < 4.8). The latter species, the one used for metal quantitation,[12] was characterized

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by a broad absorption at λ_{max} = 360 nm, ε = 6800 $M^{-1}cm^{-1}$. The extinction coefficient was determined at a ligand/metal ratio of nearly 2000:1, and the ligand absorption tails into this region; it represents an upper limit.[13] Minority species $TiO(Hasc)_2^{2-}$ and $Ti(asc)_2$ were also detected. Above pH 4.8, the color disappeared, and hydroxo complexes were suspected.[13] This quantitative work agreed well with the original qualitative titrations.[11] Another study invoked $TiO(Hasc)^+$ (λ_{max} = 355 nm, ε = 7800 $M^{-1}cm^{-1}$) and a second, anionic complex with λ_{max} = 375−385 nm that formed at lower ascorbate concentration and higher pH.[14] Complexes formulated as $TiO(Hasc)_2$ (λ_{max} = 364.4 nm, ε = 1202 M^{-1} cm^{-1}) and TiO(OH)(Hasc) (λ_{max} = 344.8 nm, ε = 692 M^{-1} cm^{-1}) were prepared and characterized by elemental analysis and IR and ^1H NMR spectroscopies.[15,16] The former complex is one predicted by Sommer to occur at low pH; the latter, a presumed hydrolysis product prepared by methanol extraction of the former, was not one of the species thought to predominate in aqueous solution.[13] The IR spectra were not reported below 1500 cm^{-1},[16] so the presence or absence of a Ti═O stretch could not be ascertained. A complex formulated as $K_2Ti(asc)_3$ was isolated from EtOH and characterized by elemental analysis, but the ligand dianion was thought to be too basic to form in aqueous solution.[17]

The titanyl ion, $TiO^{2+}(aq)$, is often invoked in the aqueous coordination chemistry of Ti(IV), though the importance of discrete, monomeric TiO^{2+} units in solution has been challenged.[18] One study used ^{17}O NMR spectroscopy to reinvestigate the issue of the aqueous titanyl ion.[19] The "yl" oxygen of $TiO^{2+}(aq)$ exchanged 9 orders of magnitude faster than the "yl" oxygen of $VO^{2+}(aq)$. This finding led to the conclusion that, at low pH and in the absence of any stabilizing ligand, a rapid equilibrium exists among $TiO^{2+}(aq)$, $Ti(OH)_2^{2+}(aq)$, and $Ti^{4+}(aq)$.

The bioactivity of titanium(IV) ascorbate is well established, but in general the complex is prepared *in situ* and not characterized. In one early use of titanium ascorbate in medicine, a solution was used to treat tumors in humans.[20] ^{45}Ti ascorbate has been investigated as a radiopharmaceutical.[21] Metal delivered as the complex by injection into rats bound to albumin in plasma and accumulated in the spleen, liver, and blood. ^{45}Ti concentrated in and was used to image a glioma in the brain of a rat.[21] More recently, ascorbic acid has been shown to increase the uptake of titanium in rat everted gut sacs.[22] István Pais described the use of titanium(IV) ascorbate as a water-soluble and relatively stable compound capable of stimulating the growth of plants.[23] Several reviews have covered the research in this area.[24−27] The generally beneficial effects of titanium solutions on various plants, including peppers,[28−30] wheat,[31,32] oats,[33] apples,[34,35] and plums,[36,37] have been an important focus. Titanium ascorbate was patented as a growth promoter for plants,[38] animals,[39] or both.[40] Other patents cover its preparation as a solid[41] or for aquaculture applications[42] or as a component of a complex fertilizer mixture.[43−46] The formulation applied to organisms is not always described in great detail, but it often comprises titanium(IV) with at least a 20-fold excess of ascorbate, with the pH adjusted to between 5 and 7. The earlier work on titanium ascorbate speciation does not address the complex speciation under these conditions, but instead focuses on lower pH values.

Once administered into a biological system, titanium ascorbate complexes may undergo ligand exchange reactions. Titanium(IV) is a labile metal ion, with exchange rates for terminal water ligands on the order of 3400 s^{-1} at 25 °C.[19] Titanium ascorbate injected into human blood serum at pH 7.4, for example, for an imaging application, where the ascorbate concentration is on the order of 50 μM,[47,48] should undergo ligand exchange reactions with citrate, which is abundant in blood serum at 100 μM[48] and makes quite stable titanium(IV) complexes.[49] Likewise, titanium ascorbate formulated near pH 5 and sprayed onto plants or fed to animals as a growth promoter might encounter citrate or similar α-hydroxy acid competitor ligands. It remains to be evaluated how the rates of these exchanges, involving presumably bidentate ligands, might compare with the benchmark value for water exchange.

In light of the demonstrated bioactivity of titanium ascorbate, the current work revisits these complexes, to further explore this system. This work enumerates the important complexes and equilibria that define the aqueous speciation, particularly in the most bioactive formulations. The ascorbate ligand binding mode is addressed. Finally, ligand exchange reactions are studied to understand the kinetics of such exchange under biorelevant conditions.

EXPERIMENTAL SECTION

Reagents and Chemicals. All aqueous solutions were prepared in Nanopure-quality water (18.2 MΩ cm resistivity; Barnstead), and all chemicals were ACS grade or better. Stock solutions of 1.0 M potassium chloride (KCl, J.T. Baker) and 0.2000 M potassium hydrogen phthalate (KHP, ACS primary standard, Mallinckrodt) were prepared in volumetric flasks by weighing out an appropriate amount of the salt, dissolving in water, and diluting to volume. Solutions of 0.1 M hydrochloric acid (HCl, 1 N volumetric solution, J.T. Baker) and 0.2 M potassium hydroxide (KOH, 1 N carbonate-free Dilut-It Analytical Concentrate, J.T. Baker) were diluted from their respective stock solutions volumetrically, and their concentrations were determined accurately (4 significant figures) by titrating the base against KHP, and the acid against the base. Phenolphthalein (1% in 95% alcohol, Mallinckrodt) was used as an end point indicator, and the analyses were done in triplicate. All solutions were prepared fresh, and were stored under an inert gas (nitrogen or argon) in parafilm-wrapped Nalgene bottles.

For the spectropotentiometric titrations, the ascorbate concentration was constant and the Ti(IV) concentration was varied. An accurately weighed amount (70.45 mg, 0.400 mmol) of L-ascorbic acid crystals (99+ %, Sigma-Aldrich) was added to an argon-purged and foil-wrapped 100 mL volumetric flask containing an aqueous solution of 0.1 M KCl (aq). The titanium(IV) source for all experiments was a neat solution of $TiCl_4$ (l) (99.9%, with SureSeal cap, Sigma-Aldrich) which is highly reactive with water and water vapor and was added to the titration solutions by using a gastight glass syringe (Hamilton Co.). Following addition of metal ion (2−15 μL, 30−170 μmol), a 50.0-mL aliquot of this solution was transferred to the titration vessel by using a volumetric pipet. For other applications, a 0.1000 M stock solution of L-ascorbic acid was prepared volumetrically from the acid crystals and stored under inert gas; additionally, this solution was stored in a foil-wrapped bottle to prevent photo-oxidation. The Ti(IV) concentration was determined from the remaining 50-mL titration solution by flame atomic absorption analysis (SpectrAA-20 flame atomic absorption spectrometer, Varian) by using the method of internal standards.

For kinetic experiments, stock solutions of 10 mM titanium ascorbate were made by dissolving ascorbic acid crystals (0.35 g, 2.0 mmol) in 0.5 M NaCl. To this solution, neat $TiCl_4$ (25 μL, 250 μmol) was added, and allowed to mix for 1 h. The pH of the solution was adjusted to either 7.4 or 4.5 by using NaOH. Individual samples were diluted immediately before kinetics measurements to 125 μM by using 0.5 M NaCl. A stock citric acid solution was made by dissolving citric acid monohydrate (2.6 g, 12 mmol) in 0.5 M NaCl, and the pH was adjusted to either 7.4 or 4.5 using NaOH. Further dilutions were made from this solution to give the range of concentrations used.

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Spectropotentiometric Titrations. All titrations were conducted as described previously,[49] according to the general methods described,[50,51] with minor modifications. The entire assembly was foil-wrapped to prevent any photo-oxidation of the ligand, and kept under positive pressure from argon to prevent oxygen contamination. The total number of equilibrated titration points for which data were collected was between 70 and 90. The fitting software was Specfit/32.[52−56] Full details of the experimental procedure are given in the Supporting Information.

Metal−Ligand Binding Stoichiometry. Ligand-to-metal stoichiometry at pH 4.5 was determined independently by using an aqueous mole ratio method, probed by UV−vis spectroscopy. Repeated volumes of $TiCl_4$ (l) were added to a solution of 4.00 mM H_2asc in 0.1 M KCl under the same conditions discussed above for the titrations. The pH was readjusted to 4.5 for each successive solution by adding acid or base, and total solution volume was monitored. Following each equilibration, a 1.0 mL aliquot was removed, and its absorbance was measured. After data collection, the remaining solution was used for titanium concentration analysis. For each point, an L/M molar ratio value was determined, and the corresponding absorbance at 366 nm, corrected for dilution and reported as an observed molar (Ti(IV)) absorptivity, was calculated.

Mass Spectrometry. Electrospray mass spectra were collected in positive ion mode on a Waters/Micromass ZQ spectrometer. The pertinent voltages were as follows: capillary, 3 kV; cone, 20 V; extractor, 3 V; RF lens, 0.3 V. The aqueous samples were injected directly, and molecular ions were detected in a Waters 2996 Photodiode Array Detector.

Kinetics. Citric acid solutions were prepared in 0.5 M NaCl at concentrations ranging from 3.75 to 250 mM to ensure pseudo-first-order kinetics. The final solutions contained 62.5 μM titanium ascorbate and between 1.88 and 125 mM citrate.

Kinetics measurements were made by using a Cary 50 Bio UV−vis spectrophotometer. The absorbance was measured every 6 s between 300 and 600 nm, or every 0.6 s at 370 nm for single wavelength measurements. Longer time scale experiments were performed over 20 h to confirm complete conversion to the citrate species. These measurements were made every 6 s for the first 2 min, every 30 s for the next 8 min, and then every 10 min for the remainder of the 20 h. The pH was unbuffered except by the solution components, but was constant to within ±0.1 pH unit over the course of the reaction.

Data were fit by using Specfit/32.[53−56] Data at pH 7.4 were best fit to a sequential two step model. Data at pH 4.5 were best fit to a sequential three step model. All data were collected at least in triplicate; error bars represent the standard deviations of all trials.

RESULTS AND DISCUSSION

L-Ascorbic Acid: Protonation States and Oxidation. The spectropotentiometry of ascorbic acid in the absence of metal was performed, in some cases with deliberate ligand oxidation (Figures S1−S3), so that the latter could be recognized and avoided. In the absence of oxidation, the measured pK_a values and species spectroscopic properties agreed with those reported in the literature.[9]

Potentiometric Data Analysis. By examining the unfitted potentiometric data for a series of spectropotentiometric titrations varying only in ligand-to-metal (L/M) molar ratio, several important points relating to the aqueous interactions of Ti(IV) with L-ascorbic acid were established. The raw potentiometric data can be presented as plots of pH as a function of equivalents of base added to a given amount of ligand in the presence of varying amounts of metal ion, or as ligand deprotonation curves showing the pH-dependent removal of acidic ligand protons for the same data (Figure 2). In the former case the most important observations concerned the inflection point for the various titrations. An inflection point in a metal ion−ligand titration is informative on

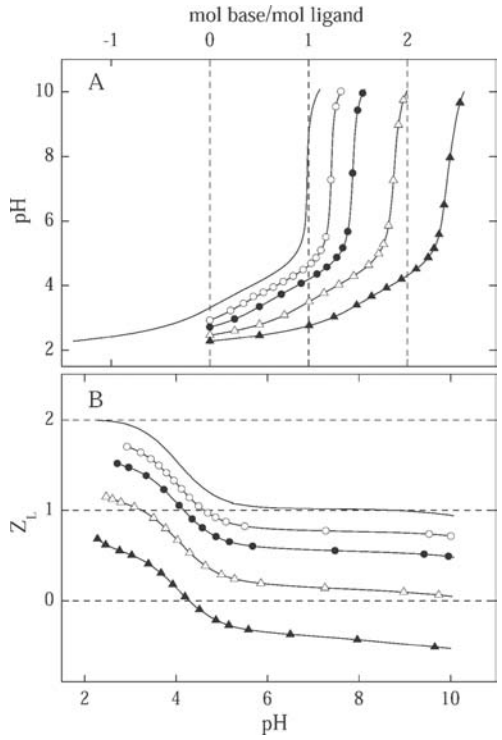

Figure 2. (A) Potentiometric titration curves, as a function of molar equivalents of base added, for 4.00 mM L-ascorbic acid in the presence of various concentrations of Ti(IV). (B) Ligand deprotonation (Z_L) curves, as a function of pH, for 4.00 mM L-ascorbic acid in the presence of various concentrations of Ti(IV). In both panels, data are as follows: no symbol, ascorbic acid alone with no titanium; open circles (○), [Ti(IV)] = 0.297 mM (L/M = 13.5); filled circles (●), [Ti(IV)] = 0.530 mM (L/M = 7.5); open triangles (△), [Ti(IV)] = 1.058 mM (L/M = 3.8); filled triangles (▲), [Ti(IV)] = 1.665 mM (L/M = 2.4). For clarity, in each case, only every fifth data point is symbolized. Dashed lines represent equivalents of acidic ligand protons. All titrations were conducted at 25 °C, in 0.1 M KCl, and under an argon atmosphere.

the basis of both its position relative to the titration of ligand alone and the steepness of the inflection region. For titrations involving a diprotic acid such as L-ascorbic acid, having pK_a values that widely straddle the isopotential pH of water (pH 6.90 in the presence of 0.1 M supporting electrolyte and at 25 °C) are particularly useful, because the region of inflection is isolated from the two ligand deprotonations. Furthermore, in the absence of metal-ion promoted hydrolysis and subsequent hydroxide binding by the metal ion, the inflection region will be as steep as that seen for the titration of ligand alone.

Exactly 1 molar equivalent of base was needed to reach the inflection point in the titration of ligand alone (Figure 1A, no symbols). As metal ion was introduced in subsequent titrations at constant ligand concentration, the position of the inflection was shifted along the *x*-axis, reflecting the extra base needed to consume the additional protons in solution that resulted solely from the presence of metal ion. A comparison was made between experimental and predicted inflection point values, with the former determined from the graph and the latter calculated on the assumption that the metal ion was coordinatively saturated by anionic oxygen donors originating from water or ascorbate. Because the calculated values accounted for the 1 equiv of ligand proton consumed by added base, but did not differentiate between a proton

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(presumably the O(2) hydroxyl proton) of ascorbate versus water as the source of additional protons, the analysis was limited by an inability to distinguish between species related to two limiting cases: $Ti(Hasc)_3(OH)_3^{2-}$ and $Ti(asc)_3^{2-}$. This comparison can be described by the following equations:

$$\text{equiv } H^+ \text{ titrated to midpoint, experimental} = \frac{\text{mol base added to midpoint}}{\text{mol ligand}} \quad (1)$$

$$\text{equiv } H^+ \text{ titrated to midpoint, calculated} = 1 + \frac{3}{\text{L/M molar ratio}} \quad (2)$$

The experimental and calculated midpoint values for the three curves (Figure 1A) describing titrations involving a minimum of a 3-fold excess of ligand were in close agreement (exp/calc for L/M 13.5, 7.5, 3.8 were 1.22/1.22, 1.44/1.40, 1.86/1.90, respectively). While this finding supports the contention (see below) that, in the region before inflection, and in the presence of sufficient ligand for tris-coordination, the prominent metal–ligand complex is the aqueous $Ti(asc)_3^{2-}$ species, it confirms only that, in acidic aqueous solution, Ti(IV) complexation involves octahedral coordination of anionic oxy-donors. The titration at L/M ratio of 2.4, where insufficient ligand precluded the complete tris-coordination of ascorbate by metal ion, gave values not in agreement, with the experimental value significantly higher than the calculated (exp/calc = 2.48/2.25). This finding indicates that, in the presence of insufficient ascorbate for tris-coordination, coordinative saturation is maintained, and metal-ion-promoted hydrolysis and binding of hydroxide has occurred, with the loss of at least one bound ligand.

The argument for metal-ion-promoted hydrolysis and binding of hydroxide in titrations lacking sufficient ligand for tris-coordination is supported by several further observations. In the case of the L/M 2.4 titration, from the fact that the inflection fell outside of the limit of titratable ligand protons (>2); it follows that because the sole source of protons in this region is water, these titratable protons were necessarily produced by hydrolytic binding of hydroxide by the metal ion. Also, as mentioned above, for an aqueous titration in the presence of supporting electrolyte and an acid ligand such as ascorbate the inflection region should be both sharp and steep. The presence of metal ion, however, alters this behavior. The trace for the L/M 2.4 titration, for instance, and to a lesser extent for the L/M 3.8 titration, exhibited some buffering in the inflection region, which can be attributed to the hydrolytic activity of the metal ion.

The Z_L plot, adapted from the work of Öhman,[51] is another convenient tool for visualizing the average number of protons bound per ligand molecule as a function of pH, and, by extension, for identifying regions where mixed ligand–hydroxo complexes are formed, pure Ti(IV) hydroxo species having been shown, in previous work,[49] to be insoluble above pH ~ 3 at the millimolar concentrations used here. The function Z_L was derived from the potentiometric data by using the following equation:

$$Z_L(\text{pH, added base}) = \frac{[H^+]_{\text{bound to L}}}{[H_nL]_{\text{total}}} \quad (3)$$

where

$$[H^+]_{\text{bound to L}} = [H^+]_{\text{total}} - [H^+]_{\text{free}} \quad (4)$$

and

$$[H^+]_{\text{total}} = n[H_nL]_{\text{total}} - [OH^-]_{\text{added}} \quad (5)$$

In cases where the L/M ratio was greater than 3, the curves thus generated (Figure 2B), when compared to the curve for the ligand alone, were uniformly shifted to lower Z_L values as a function of added Ti(IV), and were essentially parallel to the curve for the ligand alone. This finding is an indication primarily that their speciations are similar. This observation is reinforced by comparison with the curve for the L/M 2.4 titration, which has a different overall shape from the others, and which crosses into negative Z_L territory at pH ~ 4. Both of these differences point to a situation where the lack of sufficient ligand for tris-coordination of the metal ion becomes a crucial factor in understanding the speciation of the system.

To address the inability of the above plots to discriminate conclusively between bidentate metal–ligand binding with proton displacement and metal-promoted hydrolysis and binding of hydroxide in mixed metal–ligand–hydroxo complexes in the coordination sphere of Ti(IV), a further graphical tool was employed. This tool, referred to as a Bjerrum plot or *n*-plot, defines a function *n* as the average number of ligands bound per metal ion.[51] The analysis utilizes total ligand and metal concentrations (L_{total} and M_{total}, respectively), base concentration ([base]), initial solution volume ($V_{initial}$), and the conditional pK_w and pK_a values as known quantities, and can be expressed in terms of a diprotic acid ligand:

$$n(\text{pH}, v_{base}, mf_{H_2asc}, mf_{Hasc^-}) = \frac{L_{total} - \frac{2L_{total} - \frac{v_{base}\cdot[base]}{V_{initial}+v_{base}} - 10^{-pH} + 10^{pH-pK_w}}{2\cdot mf_{H_2asc} + mf_{Hasc^-}}}{M_{total}} \quad (6)$$

The experimental quantities determined for each titration point include the pH-dependent mole fractions for the protonation states of the ligand (mf_{H2asc}, mf_{Hasc-}), the pH, and the volume of titrant added (v_{base}). A derivation of the equation used to generate these plots can be found in Supporting Information, Derivation S1. The *n*-curves for a particular metal–ligand system can be shown mathematically to coincide independent of total metal and ligand concentration (and L/M ratio) provided that the system of interest is characterized by a series of $ML–ML_x$ complexes. If, on the other hand, the aqueous speciation is more complicated, including ternary metal–hydroxo–ligand species and protonated metal–ligand species, the assumptions contained in the *n*-plot algorithm are invalidated, and the curves will be unique. Finally, because *n* will likely have an upper limit of 3 for the Ti(IV)–ascorbate coordination system, values exceeding this limit reflect, as noted above, portions of the pH range for which simple $ML–ML_x$ complexes are of minor importance.

For each experimental titration data pair (volume of base added, pH) comprising a given titration, the *n*-value was generated and plotted against $\log[L^{2-}]$, which was independently derived from the speciation of the ligand alone at the particular pH. The results are shown in Figure 3. Three of the curves, all representing titrations with >3-fold ligand excess, were very nearly identical, flattening at $n \sim 3$ before curving upward again above pH 7. The fourth curve, representing the L/M 2.4 titration, was distinct from the other three, with incomplete flattening at $n \sim 2.5$. These observations are

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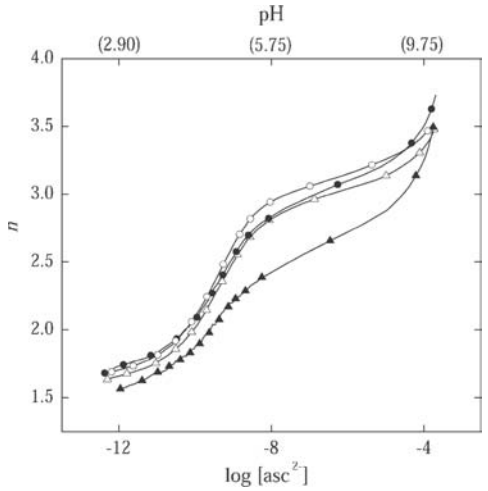

Figure 3. Bjerrum plots, also referred to as *n*-plots, describing equivalents of metal-bound asc^{2-} as a function of $\log[asc^{2-}]$ (bottom axis), with corresponding pH values (top axis), for titrations of 4.00 mM L-ascorbic acid in the presence of varying amounts of Ti(IV). Symbols as follows: open circles (○), [Ti(IV)] = 0.297 mM (L/M = 13.5); filled circles (●), [Ti(IV)] = 0.530 mM (L/M = 7.5); open triangles (△), [Ti(IV)] = 1.058 mM (L/M = 3.8); filled triangles (▲), [Ti(IV)] = 1.665 mM (L/M = 2.4). For clarity, only every fifth data point is symbolized.

evidence that (1) between pH 3 and 6, and in the presence of at least a 3-fold excess of ligand, the aqueous speciation of Ti(IV) ascorbate is characterized by the binding of a third equivalent of asc^{2-}; (2) above pH ∼ 6 ternary metal−ligand−hydroxo species begin to dominate, regardless of L/M ratio; and (3) displacement of the O(2) proton leading to bidentate binding of asc^{2-} is the characteristic aqueous binding mode of the ligand.

Spectral Data Analysis. The UV−vis absorbance changes associated with the titrations were examined independently. For titrations spanning L/M 2−15, the pH-dependent trends were similar, and reflected in several key ways the conclusions of the potentiometric analysis.

The pH-dependent UV−vis behavior for one titration (L/M 13.5) is shown in Figure 4. Overall, the data agree well with the qualitative trends described by Ettori.[11] From pH 3−4 (Figure 4A), a steady increase in the absorbance at 366 nm was observed. This trend supports the conclusions of the potentiometric data, specifically the *n*-plots, which supported for this region the following binding event:

$$Ti(asc)_2^{0} + Hasc^{-} \rightarrow Ti(asc)_3^{2-} + H^{+} \quad (7)$$

The absorbance then remained unchanged from pH 4−5 (Figure 4B), reflecting the stability of the putative tris-ligand complex in this region. In Figure 4C, encompassing the range pH 5−6, the λ = 366 nm absorbance began to decrease slightly, an indication of the onset of metal-promoted hydrolysis and ligand loss. Finally, in the region pH 6−10 (Figure 4D,E), the decrease was more dramatic, and was accompanied by a shift in the LMCT absorbance to 348 nm. This result is significant because it agrees with the λ_{max} proposed for a ternary Ti(IV)−ascorbate−hydroxo species,[16] and it supports the findings of the *n*-plots regarding metal-promoted hydrolysis and replacement of bound ligand by hydroxide. The binding events proposed for this region can be summarized by the following equations:

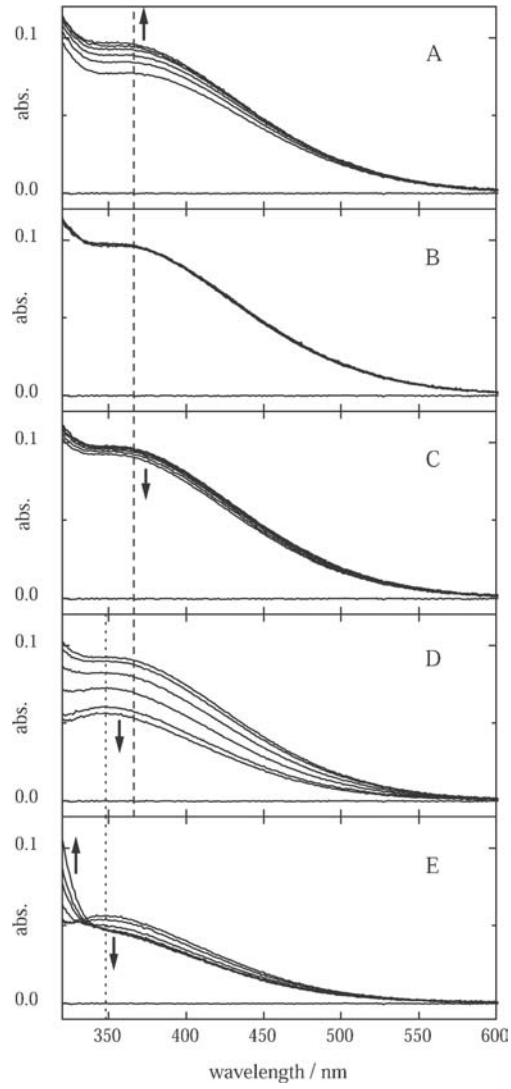

Figure 4. Selected UV−vis absorbance spectra for the spectropotentiometric titration of 4.00 mM L-ascorbic acid in the presence of 0.297 mM Ti(IV) (L/M = 13.5). Ranges as follows: (A) pH 2.9−4.1, (B) pH 4.1−4.8, (C) pH 4.8−5.8, (D) pH 5.8−8.8, (E) pH 8.8−10.0. All traces were normalized against a baseline of 0.1 M KCl (25 °C). For clarity, only six traces are shown per panel out of a total of 76 traces collected. Dashed line is at 366 nm. Dotted line is at 348 nm.

$$Ti(asc)_3^{2-} + 2H_2O \rightarrow Ti(asc)_2(OH)_2^{2-} + Hasc^{-} + H^{+} \quad (8)$$

$$Ti(asc)_2(OH)_2^{2-} + 2H_2O \rightarrow Ti(asc)(OH)_4^{2-} + Hasc^{-} + H^{+} \quad (9)$$

This qualitative description of the aqueous speciation of Ti(IV) with L-ascorbic acid was further investigated by plotting the observed 366 nm molar absorptivity profiles as a function of pH for each of the L/M ratios considered (Figure S4). The most striking observation was that, for the three titrations with L/M > 3, a clear absorbance maximum was seen at pH 4.5. The L/M 2.4 titration peaked slightly before pH 4.5 due to the inability of the metal ion to coordinate fully a third ascorbate ligand. Additionally, the apparent molar absorptivity at a given pH increased as L/M increased. This correlation can be explained by considering the effect of increasing L/M ratio on any aqueous speciation. It can be seen that, as L/M increases,

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both of the following equilibria will be forced to the right in response to the conditions (charges omitted for simplicity):

$$M + \text{large excess } L \rightarrow ML_2 \quad (10)$$

$$M + \text{large excess } L \rightarrow ML_3 \quad (11)$$

However, if the two equilibria are in direct competition over a certain pH range, as they are in the speciation model being constructed, the second equilibrium will be favored. By this reasoning, the Ti(IV) tris-ascorbate complex will dominate the region around pH 4.5 as L/M ratio increases by excluding the neighboring species; this phenomenon is seen in the experimental data as an increase in the observed absorptivity of the species whose wavelength is being monitored. Finally, the pH 4.5 absorbance profiles were investigated as a function of L/M ratio (Figure S5). The results concurred with the above discussion in that the effect of increasing L/M ratio from 1 to 12 changed the speciation, seen in the shift of the λ_{max} from 348 to 366 nm, and in the increasing $\varepsilon_{observed}$, without changing the equilibrium model.

pH-Dependent Speciation of Aqueous Titanium(IV)–Ascorbate Complexes. The data were analyzed first by model-free evolving factor analysis (EFA) (Figures S6 and S7). Specific speciation models were proposed, on the basis of the considerations outlined above (Figure S8) and complemented by modeling of the data. Details of proposed models are outlined in the Supporting Information. The optimized fit returned the following species and stability constants (Table 1).

Table 1. Minimum Stability Constants Determined for Aqueous Ti(IV)–Ascorbate–Hydroxo Species Using L/M = 10 ([H_2asc] = 4.00 mM) in 0.1 M KCl (25 °C, Argon Atmosphere)

equilibrium	log β_{min}
$Ti^{4+} + 2asc^{2-} \leftrightarrows Ti(asc)_2^{0}$	25.70
$Ti^{4+} + 3asc^{2-} \leftrightarrows Ti(asc)_3^{2-}$	36.91
$Ti^{4+} + 2asc^{2-} + 2H_2O \leftrightarrows Ti(asc)_2(OH)_2^{2-} + 2H^+$	16.43
$Ti^{4+} + asc^{2-} + 4H_2O \leftrightarrows Ti(asc)(OH)_4^{2-} + 4H^+$	−6.91

Because free $Ti^{4+}(aq)$ was not detected even at the lowest pH values, these β values are best considered minimum values. Their relative magnitudes are firm, however. An important aspect of a series of stability constants generated for a particular system is the confluence of these constants over a range of L/M values. Assuming that the model is unchanged in the range of conditions employed, the values optimized for each independent data set should produce the same speciation based on the same log β values. This condition depends on the magnitude of the stability constant(s), and the hydrolytic propensity of the metal ion. For a strong complexation with a low propensity for metal ion-promoted hydrolysis, the log β values will be virtually identical regardless of L/M ratio. The opposite is true for values optimized in this system, incorporating relatively weak binding with a strong propensity toward hydrolysis (Table S1 and Figure S9). The reasoning is a reflection of the relative concentrations of metal ion and ligand with respect to pH, wherein solutions with L/M < ~50 will be differentially prone to ligand binding and hydrolysis.[57] This effect was seen in the log β values optimized for the Ti(IV) ascorbate system at different L/M ratios (Table S1), for which slightly different values were determined for each particular species as a function of L/M ratio. Looking at the values generated for L/M > 3, this trend was linear for each particular species (Figure S9), allowing for the reporting of stability constants with reference to a singular L/M ratio. Because a minimum 10-fold ligand excess is a common condition for studies of this type, and because Figure S9 showed that asymptotic linearity began at L/M ~ 10, this ratio was chosen for reporting the log β values characteristic of this system (Table 1). When applied to the spectropotentiometric data, these results produced a speciation that was consistent in all respects with the benchmark findings used to direct the fitting process.

The UV–vis absorbance characteristics were consistent with those predicted from the various analyses of the spectral data, and with the UV–vis characteristics of the species predicted by Jabs et al.,[16] with binary metal–ligand and ternary metal–ligand–hydroxo species exhibiting different λ_{max} values and extinction coefficients based on the numbers of bound ligand and hydroxide (Table 2). Viewed graphically (Figure 5), these

Table 2. Spectral Characteristics of Aqueous Complexes in the Ti(IV) Ascorbate System Predicted for L/M = 10 ([H_2asc] = 4.00 mM) in 0.1 M KCl (25 °C, Argon Atmosphere)

species	λ_{max} (nm)	ε_{max} (M^{-1} cm^{-1})
$Ti(asc)_2^{0}$	370	1260
$Ti(asc)_3^{2-}$	365	1660
$Ti(asc)_2(OH)_2^{2-}$	348	1060
$Ti(asc)(OH)_4^{2-}$	362	700
H_2asc	243	9800
$Hasc^-$	266	15 100
asc^{2-}	299	9700

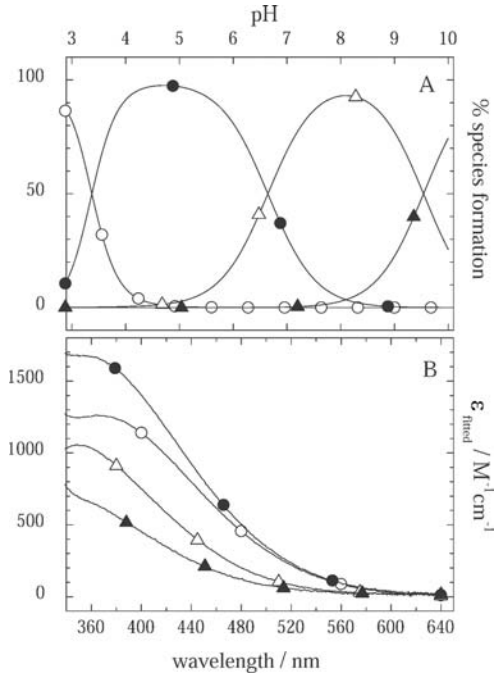

Figure 5. (A) Speciation diagram and (B) corresponding absorbance spectra for spectropotentiometric titration of 4.00 mM L-ascorbic acid in the presence of 0.297 mM Ti(IV) fitted to optimized L/M = 10 log β values. Data as follows: open circles (○), $Ti(asc)_2^{0}$ (β_{120}); filled circles (●), $Ti(asc)_3^{2-}$ (β_{130}); open triangles (Δ), $Ti(asc)_2(OH)_2^{2-}$ (β_{12-2}); filled triangles (▲), $Ti(asc)(OH)_4^{2-}$ (β_{11-4}).

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results, applied to the L/M 13.5 titration data, produced a speciation and absorbance paradigm that could be generalized in form to all L/M ratios. Furthermore, the log β values generated very similar speciation diagrams for all L/M > 3 data sets, changing in a predictable way below this ratio for the reason of insufficient ligand for tris-coordination (Figure S10).

These findings correspond well with the conclusions of the present study, in that, for the pH range 2.5−10, and in the presence of excess ligand (ascorbate), no evidence was found for the titanyl ion. This result can be explained by considering the lability of the "yl" oxygen, and the resulting equilibria between the titanyl ion, the bis-hydroxo species, and the aqueous metal ion. Specifically, as discussed below, the most acidic species described in this investigation, the $Ti(asc)_2^0$ complex, was not anionic according to the potentiometric and fitting results; a putative $Ti(O)(asc)_2^{2-}$ species was therefore ruled out. Furthermore, the binding of a third ascorbate ligand was not found to be accompanied by proton consumption, which would be the case if either $Ti(O)(asc)_2^{2-}$ (aq) or $Ti(OH)_2(asc)_2^{2-}$ (aq) were in significant concentration at this pH. On the contrary, the binding event in this pH region was found to entail proton liberation from the ascorbate monoanion. The combination of these characteristics led to the conclusion that titanyl species are not prevalent in the pH values surveyed.

Metal−Ligand Stoichiometry. Several further analytical procedures were undertaken to substantiate the existence of the tris-coordinated titanium(IV) ascorbate species. In one experiment a solution of ascorbic acid was maintained at pH 4.5 as aliquots of $TiCl_4$ (l) were added. After each addition, the pH of the solution was readjusted to 4.5, and the solution was allowed to re-equilibrate. UV−vis scans were then taken by using a cuvette, and adjusted ligand and metal concentrations were determined. The resulting L/M ratios were plotted against observed molar absorbance at 366 nm, giving the plot shown in Figure 6. The shape of the curve is indicative of a relatively weak binding interaction, and points to the potential for hydroxide to compete with ascorbate for metal binding.

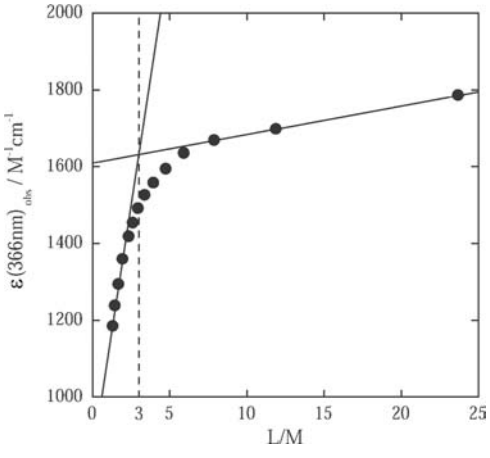

Figure 6. Mole ratio method for ligand binding stoichiometry in the aqueous Ti(IV) ascorbate system at pH 4.5, monitored by the absorbance at 366 nm. Experiments were conducted at 25 °C and under an argon atmosphere by adding aliquots of neat $TiCl_4$ (l) to a solution containing 4.00 mM L-ascorbic acid and 0.1 M KCl. The pH 4.5 ligand-alone absorbance trace was subtracted from each, and all traces were corrected for dilution. The two solid lines intersect at L/M = 3.009 and ε(366 nm) = 1632 M^{-1} cm^{-1}. Dashed line is at L/M = 3.

Extrapolation of the linear portions of the data showed an intersection at almost exactly L/M 3, with an extinction coefficient in close agreement with that predicted from an examination of the raw spectral data (1630 M^{-1} cm^{-1}). Therefore, in addition to predicting the relatively weak binding interaction between metal ion and ligand, this technique supported a Ti(IV) tris-ascorbate species in the aqueous speciation of the system. Additional experiments were conducted at pH = ∼2, ∼6, and ∼9 (data not shown). The asymptote at high L/M was not sufficiently horizontal to allow quantitation, but qualitatively, the L/M ratio for binding in each of these regions was clearly less than 3.

Electrospray mass spectrometry (ESI-MS) offered an additional means of probing the formation of the tris-coordinated species. Several processes, including ligand oxidation, degradation, and protonation, and metal precipitation (as TiO_2), were difficult to avoid in the instrument despite the "soft" ionization that is the hallmark of electrospray; these constraints limited the pH range and L/M ratios under which results could be obtained. At pH = ∼3 and L/M = ∼7.5, with low cone and capillary voltages, however, a spectrum was detected in positive ion mode depicting, based on m/z^+ and isotope distribution, a Ti(IV) tris-ascorbate species (Figure 7).

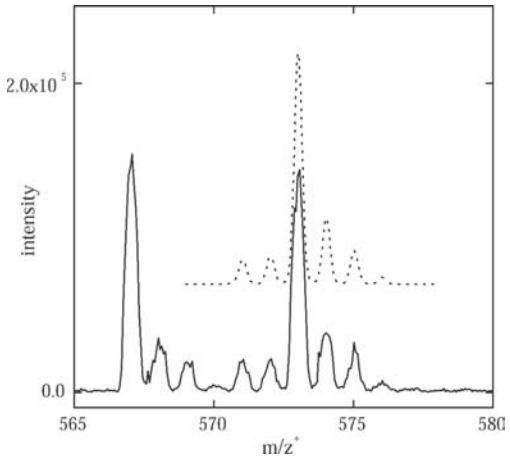

Figure 7. Portion of an electrospray mass spectrum (positive ion mode) for an aqueous solution of Ti(IV) ascorbate (L/M = ∼7.5, pH = ∼3). Peaks centered on m/z = 573 are consistent with the formulation $H_3[Ti(asc)_3]^+$ ($C_{18}H_{21}O_{18}Ti$) in terms of mass and isotope distributions (dotted trace). Peak at m/z = 567 is similarly consistent with the formulation $K(H_2asc)_3^+$ ($C_{18}H_{24}O_{18}K$).

Rates of Ligand Exchange. The binding of Ti(IV) by ascorbate is sufficiently strong to prevent hydrolytic precipitation, but weaker than binding by other common biological ligands such as citrate.[10,49] Speciation models suggest that Ti(IV) ascorbate complexes introduced to blood serum, for example,[58] would undergo ligand exchange with citrate. The rapid exchange rates reported for Ti(IV) using water as a benchmark ligand (>1000 s^{-1})[19] suggest that this exchange among biomolecules might be very fast. But other data, such as the kinetics of delivery of Ti(IV) from citrate to the metal transport protein transferrin,[59] suggest that exchange kinetics might be much slower, on the order of minutes or hours.

The competition for Ti(IV) binding between ascorbate and citrate was studied under pseudo-first-order conditions of excess citrate. Ligand exchange was probed by monitoring the disappearance of absorbance due to the Ti(IV) ascorbate

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complexes at 355 nm after citrate was introduced. Experiments were carried out at the physiological serum pH of 7.4 and also at pH 4.5, near the pH of formulation of the patented growth-promoting complexes, where the tris-ascorbate species is predicted to predominate. The dominant species predicted under these two conditions are[49]

$$Ti(asc)_2(OH)_2^{2-} + 3Hcit^{3-} \rightarrow Ti(cit)_3^{8-} + 3Hasc^- \text{ (at pH 7.4)} \quad (12)$$

$$Ti(asc)_3^{2-} + 3H_2cit^{2-}/H_3cit^- \rightarrow Ti(Hcit)_2(H_2cit)^{4-} + 3Hasc^-/H_2asc \text{ (at pH 4.5)} \quad (13)$$

The data at pH 7.4 fit best to two sequential rate constants (Table S2). The first rate constant was dependent on citrate concentration (Figure 8A) and saturable, yielding a dissociation constant of approximately 11 mM for the first detectable step of the exchange. The second rate constant was not dependent on citrate concentration. For experiments carried out at pH 4.5, three steps were necessary to fit the data. The first two were saturable, with apparent K_d values of 18 mM and 5.4 mM for k_1 and k_2 (Figure 8B). The third did not depend on citrate concentration.

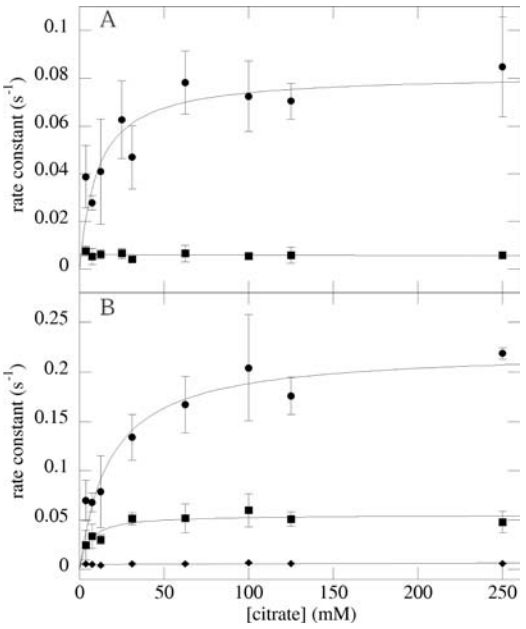
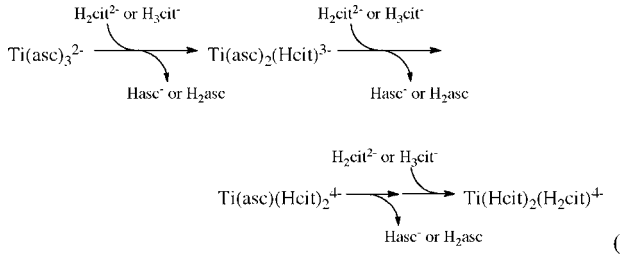


Figure 8. Observed rate constants for the disappearance of the $Ti(Hasc)_2(OH)_2^{2-}$ or $Ti(asc)_3^{2-}$ absorbance as a function of citrate concentration. Error bars represent standard deviations among at least three trials. (A) Data at pH 7.4. (●) k_1: $k_{max} = 0.081 \pm 0.007$ s^{-1}, $K_d = 10 \pm 4$ mM (■) $k_2 = 0.0060 \pm 0.009$ s^{-1}. (B) Data at pH 4.5. (●) k_1: $k_{max} = 0.22 \pm 0.02$ s^{-1}, $K_d = 18 \pm 5$ mM (■) k_2: $k_{max} = 0.055 \pm 0.002$ s^{-1}, $K_d = 5.4 \pm 1.7$ mM (♦) $k_3 = 0.0056 \pm 0.0008$ s^{-1}.

The observation that, at 7.4, two distinct rate constants were detectable by UV–vis, whereas at pH 4.5, three steps were detectable may be related to the titanium ascorbate speciation at those pH values. For the former, a bis-ascorbate species predominates, and the removal of each ascorbate may be detectable in distinct steps. Consistent with this interpretation, the absorbance change for each step is comparable, and the UV–vis spectra of the kinetic species modeled by Specfit/32 match well with titanium species having two, one, and no ascorbate ligands. At pH 4.5, a tris-ascorbate species dominates, and the UV–vis spectra of the kinetic species match well with titanium species having three, two, one, and no ascorbate ligands. The rate constants at the two pH values are remarkably similar: the first ascorbate ligand in the tris-ascorbate species exchanges at ~0.2 s^{-1} ($t_{1/2} \sim 3$ s), the penultimate ascorbate at ~0.06–0.08 s^{-1} ($t_{1/2} \sim 10$ s), and the last ascorbate at ~0.006 s^{-1} ($t_{1/2} \sim 2$ min). The last kinetic step does not exhibit a concentration dependence on citrate, which is consistent with a dissociative mechanism. At pH 4.5, for example, eq 14 is consistent with the observed data.



(14)

At pH 4.5, citrate exists as a mixture of predominantly the H_2cit^{2-} (57%) and H_3cit^- (39%) forms, whereas ascorbate exists as $Hasc^-$ (73%) and H_2asc (27%). These species provide buffering for protonation changes during ligand exhanges. The starting and ending species are those that predominate at pH 4.5 under the solution conditions. The intermediate species are highly speculative, but consistent with the data.

These data serve as a model of processes that may be occurring in blood serum where the ligands studied in the exchange are in relatively high concentrations. The K_d values for the citrate-dependent steps are far higher than the physiological serum citrate concentration of 0.1 mM,[48] and so this model predicts that in serum the conversion of titanium ascorbate to titanium citrate complexes would occur on the order of minutes or longer, and that the rates would depend strongly on the citrate concentration. Physiological citrate concentration varies in certain disease states such as epilepsy and renal failure.[60,61]

The time scale of this exchange is far slower than the rates reported for monodentate ligands, the exchange of which showed no concentration dependence on the incoming ligand, and had forward rate constants ranging from $k_{obs} = 4$ s^{-1} to $k_{obs} > 100$ s^{-1}.[19,62] The faster rates are likely due to the difference in the nature of the ligands binding to the Ti center. In the current case, ascorbate having lost one or both M–L bonds might remain associated with the complex, rebinding rather than exchanging for citrate.

■ CONCLUSIONS

The aqueous speciation model determined for the Ti(IV)–ascorbate complexation system under the reported conditions gave considerable insight into several important facets of the binding. The binding mode was suggested to feature bidentate chelation, with the deprotonated oxy-groups at C2 and C3 as the donors. This finding supports the stipulation that a hard metal ion like Ti(IV) is capable of out-competing protons for the binding of these hard donor atoms. Additionally, direct and indirect evidence for a Ti(IV) tris-ascorbate species at pH = ~4.5 was described. The question of metal-promoted hydrolysis was investigated. The results indicated that, even

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while coordinative saturation was maintained, binding of hydroxide was indicated below physiological pH for this metal ion. Titanium ascorbate complexes convert smoothly to titanium citrate complexes under physiological concentrations of the ligands in multistep processes, with exchange times on the order of minutes. Taken together, the results reported here delineate many thermodynamic and kinetic features of one of the longest-known and most bioactive forms of Ti(IV).

■ ASSOCIATED CONTENT

Supporting Information

UV−vis spectra of ascorbic acid protonation states; potentiometric plots and UV−vis spectra depicting oxidation of ascorbic acid; mathematical derivation of *n*-plot; graphical displays showing effect of L/M ratio on λ(366 nm) absorbance as a function of pH, and on pH 4.5 absorbance as a function of wavelength; graphical representation of EFA smoothing; comparison of EFA profiles at different L/M ratios; speciation diagram comparison of 3- vs 4-species model; table and graph comparing fitted stability constants as a function of L/M ratio; comparison of speciation diagrams for different L/M ratios using singular log β values. Full spectra kinetics and single wavelength cuts across citrate concentrations for 4 min exchange reactions. UV−vis absorbance of the full spectra for overnight kinetics of exchange at pH 4.5. Specfit/32 determined species and speciation for both pH 7.4 and 4.5 kinetics data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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