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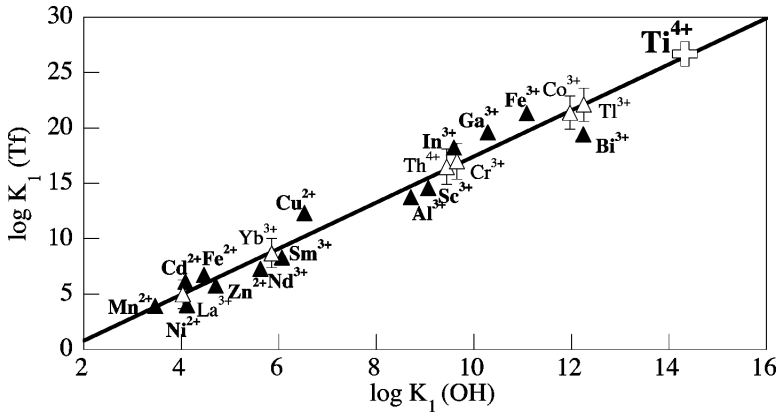


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# Ti(IV) Binds to Human Serum Transferrin More Tightly Than Does Fe(III)

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Human serum transferrin (Tf) holds a central place in the metabolism of Fe(III), but Tf also binds other metal ions. The binding strength correlates with the acidity of the metal.[1−3] Of the few ions predicted to bind more tightly than Fe(III), only Bi(III) has been investigated, and its binding is weaker, probably because of its large size.[4,5] Ti(IV) is nearly the same size as Fe(III) and is a stronger hard Lewis acid (log $K_1$ for $OH^-$ binding is 14.33,[6] compared with 11.21 for Fe(III)[7]). Titanium(IV) binding to transferrin, first demonstrated by Sadler,[8] is implicated in the bioactivity of Ti-containing anticancer drugs[9] and the plasma binding of Ti from imaging reagents[10] and implants.[11−13]

Loading of Ti(IV) into the metal binding sites of Tf was first demonstrated with an in situ-generated Ti(IV) citrate complex with 1.2:1 ligand/metal stoichiometry.[8] The reaction takes 12 h. The transferrin C lobe binds Ti(IV) more tightly than the N lobe, and $CO_3^{2-}$ is the synergistic anion.[9] Delivery of Ti(IV) to Tf from $Cp_2TiCl_2$ is more rapid,[9] but this starting material is very prone to hydrolysis in water,[14] making characterization difficult. Recent Ti(IV) citrate speciation studies reveal the complex species and their stability constants in aqueous solution.[15] At a 1.2:1 citrate/metal ratio, a complicated mix of probable oxo and hydroxo species occurs near neutral pH. In the presence of ≥3 equiv of citrate, however, the complex $[Ti(C_6H_4O_7)_3]^{8-}$ ($[Ti(cit)_3]^{8-}$) predominates between pH 6−9 (Figure S1), and its stability constant has been determined.[15]

This $[Ti(cit)_3]^{8-}$ reacts with Tf in 50 mM Tris (pH 7.4), 10 mM $Na_3$citrate, 20 mM $NaHCO_3$, and the NaCl concentration required to maintain 0.2 M ionic strength. Changes similar to those reported are observed in the UV/vis spectra,[8,9,16] indicating deprotonation of two tyrosines in each binding site and their coordination to Ti(IV). The spectra maximize at 2 equiv of Ti(IV) (Figure S2). The extinction coefficient at 321 nm (10 380 ± 110 $M^{-1}$ $cm^{-1}$ per site) is greater than previously reported (2415 $M^{-1}$ $cm^{-1}$).[9] This difference may reflect more complete loading or a slightly different conformation leading to orbital overlap more conducive to charge-transfer transitions. The $Ti_2$·Tf so prepared can be dialyzed extensively against metal-free buffer without spectral changes. When unbuffered, the very small pH change upon metal binding suggests that three protons are released, as for Fe(III), and these protonate the three citrates released from Ti(IV).

The rate of delivery of Ti(IV) from $[Ti(cit)_3]^{8-}$ to Tf can be monitored by UV/vis spectroscopy. At 10 °C and using dilute solutions under conditions of excess $[Ti(cit)_3]^{8-}$, four kinetic phases are resolved (Figure 1).[17] Similar multiphase binding has also been observed with Fe(III).[18] Bicarbonate is required for this reaction to occur, supporting the finding that $CO_3^{2-}$ is the synergistic anion.[9] Higher temperatures and concentrations lead to faster metal delivery, and at 25 °C and 50−100 μM protein, the reaction is complete in 4 min (Figure S3). A crucial difference between the current conditions and the previously reported ones[8] is the starting Ti(IV) complex. The hydroxo- or oxo-bound species that occur with ≤3 equiv of citrate may result in slower metal delivery. In a result consistent with this idea, Ti(IV) bis(ammoniumlactato)dihydroxide (Figure S4) delivers metal very slowly to the Tf binding site (data not shown).

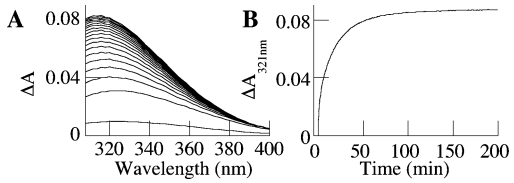


***Figure 1.*** Kinetic data resulting from the reaction of 4.5 μM apo-Tf with 89 μM $[Ti(cit)_3]^{8-}$ at 10 °C. (A) Difference spectra with the apoprotein spectrum subtracted. (B) Absorbance at 321 nm fit by using SpecFit.[17] Four phases were resolved, giving $k_{1obs}$ = 0.7 $min^{-1}$, $k_{2obs}$ = 0.075 $min^{-1}$, $k_{3obs}$ = 0.031 $min^{-1}$, and $k_{4obs}$ = 0.0055 $min^{-1}$.

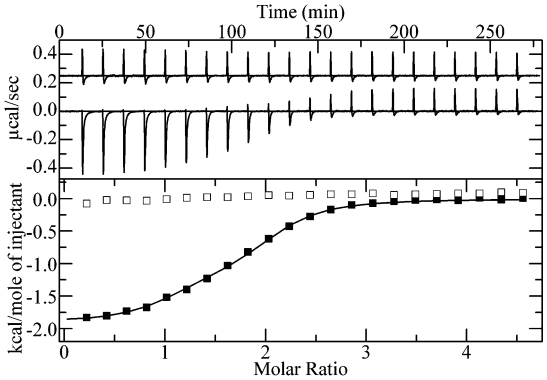


***Figure 2.*** ITC at 25 °C of 2.17 mM $[Ti(cit)_3]^{8-}$ injected into buffer (upper figure, top data offset by 0.25 μcal/s, □) or into 54.4 μM apo-hTF (lower data, ■).

***Table 1.*** Best Fit Parameters for ITC Data in Figure 2

| | $n_{ITC}$ | $\Delta G_{ITC}$[a] | $\Delta H_{ITC}$[a] | $\Delta S_{ITC}$[b] | log $K_{ITC}$ |
|---|---|---|---|---|---|
| C-site | 1.0 | −8.87 | −1.92 | 23.3 | 6.50 |
| N-site | 1.0 | −7.34 | −1.17 | 20.7 | 5.38 |

[a] In kcal/mol. [b] In cal/(mol K).

A stable characterized precursor that delivers Ti(IV) rapidly to Tf makes possible the study of their interaction by isothermal titration calorimetry (ITC). The technique is valuable for the determination of metal−protein binding parameters[19,20] and was used to characterize Fe(III) binding to Tf.[21] Table 1 reports the best thermodynamic parameters for the ITC data in Figure 2, fit to a model of noninteracting sites, each of which gives a stoichiometry of one metal ion.

The binding parameters reflect enthalpy- and entropy-driven binding and a difference in the two binding sites of transferrin. Binding to the tighter site, known to be the C-site,[9] is an order of magnitude greater than that to the N-site. This difference between the two sites is typical of metal−ion binding to transferrin and is consistent with the ITC result for Fe(III).[21] The ITC reveals that the $\Delta\Delta G$ ($\Delta G_C - \Delta G_N = -1.59$ kcal/mol) is due to entropic ($T\Delta S_C$


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***Table 2.*** Thermodynamic Parameters for Ti(IV) Binding to the C- and N-Site of Transferrin

| reactions | log K | $\Delta G$ [a] |
|---|---|---|
| ITC equilibrium | | |
| $Tf(HCO_3)^{2-} + Ti(cit)_3^{8-} \rightleftarrows Ti{-}Tf(CO_3)^{1-} + 3Hcit^{3-}$ | | |
| C lobe | 0.50 | −0.68 |
| N lobe | −0.62 | 0.84 |
| Individual equilibria | | |
| $Ti(cit)_3^{8-} + 3H^+ \rightleftarrows Ti^{4+} + 3\ Hcit^{3-}$ | −4.07[15] | 5.55 |
| $Tf(HCO_3)^{2-} + Ti^{4+} \rightleftarrows Ti{-}Tf(CO_3)^{1-} + 3\ H^+$ | | |
| C lobe | 4.57 | −6.24 |
| N lobe | 3.45 | −4.71 |
| At pH 7.4, $[HCO^{3-}] = 27$ mM | | |
| $Tf + Ti(IV) \rightleftarrows Ti{-}Tf$ | | |
| C lobe | 26.8 | −36.5 |
| N lobe | 25.7 | −35.0 |

[a] In kcal/mol.

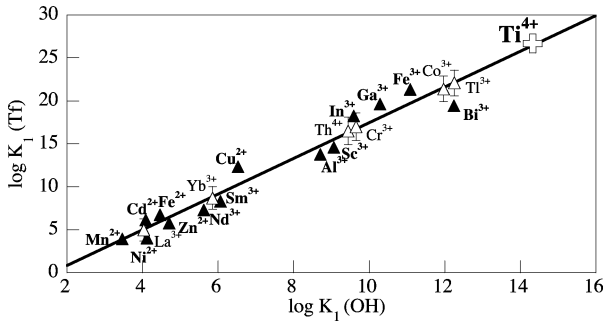


***Figure 3.*** Correlation of binding constant for metal binding to Tf with metal Lewis acidity, as quantified by $K_1$(OH) (adapted from ref 1). Filled triangles represent experimental values, while open triangles represent estimated ones. Data for $Ti^{4+}$ were added.

$-T\Delta S_N = 0.95$ kcal/mol) and enthalpic contributions ($\Delta H_C - \Delta H_N = -0.65$ kcal/mol).

Inclusion of the known free citrate concentration allows a log $K$ for the overall exchange reaction to be calculated from the $K_{ITC}$ values ($K = K_{ITC}[C_6H_5O_7^{3-}]^3$) (Table 2). Accounting for the stability of the starting material[15] and for the blood plasma conditions[1,20] leads to the values in Table 2.

At blood plasma pH and bicarbonate concentrations, Ti(IV) has a stronger affinity (log $K = 26.8$ and 25.7) for each site of transferrin than does Fe(III) (log $K = 22.5$ and 21.4) under the same conditions.[22−24] This result agrees with the predicted correlation between the metal ion affinity for transferrin and for hydroxide (Figure 3). In an apparent contradiction of this result, Fe(III) supplied in previous work as a nitrilotriacetate (NTA) chelate displaced Ti(IV) from the binding site.[9] However, Ti(IV) complexed with NTA does not deliver Ti(IV) to the apoprotein, suggesting that NTA may bind Ti(IV) more strongly than Tf.[16] In that case, the exchange equilibrium may favor binding of Fe(III) to Tf with release of Ti(IV) to NTA. Alternatively, over the extended course of the experiment, Ti(IV) may have precipitated.

The concentration of Fe in normal human blood serum (1180 ± 140 ng/mL) is 10 times that of Ti (117 ± 8 ng/mL).[25] The carrying capacity of Tf is even greater, as only 39% of the Tf isolated from human serum is saturated with iron.[26] Serum exposed to Ti(IV)-containing drugs, implants, or imaging reagents will have even higher Ti concentrations.

In summary, control over the speciation of Ti(IV) in aqueous solution permits the rapid delivery of metal to transferrin from a starting material of known stability. The current work characterizes, by UV/vis kinetics and ITC, the binding to transferrin of Ti(IV). Of the approximately 40 metals known to bind Tf, Ti(IV) is the first shown to bind more tightly than its natural cargo, Fe(III). This very avid binding supports the proposal that the interaction is important for the biological activity of titanium ions released from implants, imaging reagents, and Ti(IV) anticancer medicines.

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**Supporting Information Available:** Experimental details, including five supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Li, H.; Sadler, P. J.; Sun, H. *Eur. J. Biochem.* **1996**, *242*, 387−393.
(2) Harris, W. R. *Struct. Bonding* **1998**, *92*, 121−162.
(3) Sun, H.; Cox, M. C.; Li, H.; Sadler, P. J. *Struct. Bonding* **1997**, *88*, 71−102.
(4) Li, H.; Sadler, P. J.; Sun, H. *J. Biol. Chem.* **1996**, *271*, 9483−9489.
(5) Miquel, G.; Nekaa, T.; Kahn, P. H.; Hemadi, M.; El Hage Chahine, J. M. *Biochemistry* **2004**, *43*, 14722−14731.
(6) Ciavatta, L.; Ferri, D.; Riccio, G. *Polyhedron* **1985**, *4*, 15−21.
(7) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1974−1977.
(8) Sun, H.; Li, H.; Weir, R. A.; Sadler, P. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1577−1579.
(9) Guo, M.; Sun, H.; McArdle, H. J.; Gambling, L.; Sadler, P. J. *Biochemistry* **2000**, *39*, 10023−10033.
(10) (a) Ishiwata, K.; Ido, T.; Monma, M.; Murakami, M.; Fukuda, H.; Kameyama, M.; Yamada, K.; Endo, S.; Yoshioka, S.; Sato, T.; Matsuzawa, T. *Int. J. Rad. Appl. Instrum. A* **1991**, *42*, 707−712. (b) Vavere, A. L.; Welch, M. J. *J. Nucl. Med.* **2005**, *46*, 683−690.
(11) Strietzel, R.; Hosch, A.; Kalbfleisch, H.; Buch, D. *Biomaterials* **1998**, *19*, 1495−1499.
(12) Hallab, N. J.; Mikecz, K.; Vermes, C.; Skipor, A.; Jacobs, J. J. *J. Biomed. Mater. Res.* **2001**, *56*, 427−436.
(13) Hallab, N. J.; Skipor, A.; Jacobs, J. J. *J. Biomed. Mater. Res. A* **2003**, *65*, 311−318.
(14) Toney, J. H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 947−953.
(15) Collins, J. M.; Uppal, R.; Incarvito, C. D.; Valentine, A. M. *Inorg. Chem.* **2005**, *44*, 3431−3440.
(16) Messori, L.; Orioli, P.; Banholzer, V.; Pais, I.; Zatta, P. *FEBS Lett.* **1999**, *442*, 157−161.
(17) *SpecFit*; Binstead, R. A.; Jung, B.; Zuberbühler, A. D.; Spectrum Software Associates: Marlborough, MA, 2001.
(18) Pakdaman, R.; Abdallah, F. B.; El Hage Chahine, J. M. *J. Mol. Biol.* **1999**, *293*, 1273−1284.
(19) Zhang, Y.; Akilesh, S.; Wilcox, D. E. *Inorg. Chem.* **2000**, *39*, 3057−3064.
(20) Zhang, Y.; Wilcox, D. E. *J. Biol. Inorg. Chem.* **2002**, *7*, 327−337.
(21) Lin, L. N.; Mason, A. B.; Woodworth, R. C.; Brandts, J. F. *Biochemistry* **1993**, *32*, 9398−9406.
(22) Aisen, P.; Leibman, A.; Zweier, J. *J. Biol. Chem.* **1978**, *253*, 1930−1937.
(23) Martin, R. B.; Savory, J.; Brown, S.; Bertholf, R. L.; Wills, M. R. *Clin. Chem.* **1987**, *33*, 405−407.
(24) He, Q. Y.; Mason, A. B.; Tam, B. M.; MacGillivray, R. T.; Woodworth, R. C. *Biochemistry* **1999**, *38*, 9704−9711.
(25) Lavi, N.; Alfassi, Z. B. *Analyst* **1990**, *115*, 817−822.
(26) Williams, J.; Moreton, K. *Biochem. J.* **1980**, *185*, 483−488.

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