[15] H. Stoll, J. Flad, E. Golka, T. A. Krüger, *Surf. Sci.* **1981**, *106*, 251 – 257.

[16] M. J. Frisch, G. W. Trucks, H. B. Schlegel, P. M. W. Gill, B. G. Johnson, M. A. Robb, J. R. Cheeseman, T. Keith, G. A. Petersson, J. A. Montgomery, K. Raghavachari, M. A. Al-Laham, V. G. Zakrzewski, J. V. Ortiz, J. B. Foresman, J. Cioslowski, B. B. Stefanov, A. Nanayakkara, M. Challacombe, C. Y. Peng, P. Y. Ayala, W. Chen, M. W. Wong, J. L. Andres, E. S. Replogle, R. Gomperts, R. L. Martin, D. J. Fox, J. S. Binkley, D. J. Defrees, J. Baker, J. P. Stewart, M. Head-Gordon, C. Gonzalez, J. A. Pople, GAUSSIAN 94 (Revision A.1), Gaussian, Pittsburgh (PA), USA, **1995**.

The First Specific Ti^{IV} – Protein Complex: Potential Relevance to Anticancer Activity of Titanocenes**

Hongzhe Sun, Hongyan Li, Richard A. Weir, and Peter J. Sadler*

Biological interest in titanium complexes arises from current clinical trials of two anticancer agents $[Ti^{IV}(bzac)_2(OEt)_2]$ (Hbzac = 1,3-diphenyl-1,3-propanedione) and $[Ti^{IV}Cp_2Cl_2]$,[1, 2] and the potential radiopharmaceutical use of compounds containing ^{45}Ti isotopes.[3] Moreover, since enormous amounts of titanium occur in a wide variety of materials, especially TiO_2, there are abundant opportunities for Ti ions to enter into biochemical pathways in living systems. However, very little is known about the biological chemistry of Ti compounds. There appear to be no reports of specific binding of Ti ions to proteins, and the mode of action of Ti anticancer complexes is poorly understood. We chose to study the binding of Ti^{IV} ions to the blood plasma protein transferrin since this protein has been implicated in the transport and delivery of metal ions such as Ga^{III} (as ^{67}Ga) and Ru^{III} to cancer cells.[4, 5] We show here that Ti^{IV} forms a strong complex with human serum transferrin (hTF) by binding to the specific Fe^{III} binding sites in this protein. This appears to be the first characterization of a specific Ti – protein complex, one which may play a role in the mechanism of action of the titanium anticancer drugs.

Our initial experiments focussed on the reaction of titanium(IV) citrate[6] with human serum apotransferrin.[7] When titanium(IV) citrate was added to an aqueous solution of apo-transferrin (apo-hTF), three new bands gradually appeared in the UV difference spectrum and increased in intensity over a period of 12 h, Figure 1. The wavelengths of the two sharp bands at 241 and 295 nm are typical of

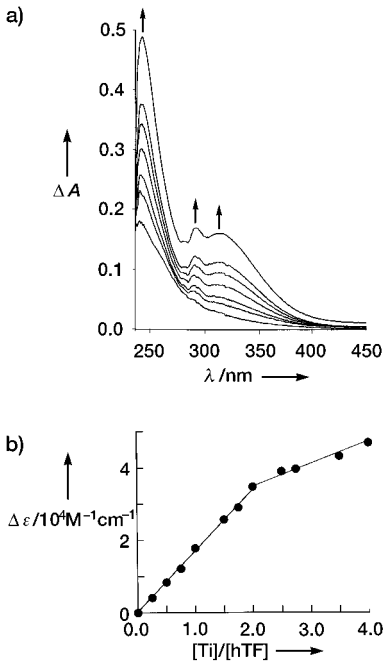


Figure 1. a) UV difference spectra of apo-hTF (13 μM in 10 mM Hepes, 5 mM HCO_3^-, pH 7.4, 310 K) after addition of 2 mol equiv titanium(IV) citrate. Reaction times (from bottom to top): 1, 5, 10, 20, 40, 150, and 720 min. b) Titration curve for addition of titanium(IV) citrate to apo-hTF (conditions as above). Each solution was left > 12 h to reach equilibrium. $\Delta\varepsilon$ is the absorbance at 241 nm divided by the transferrin concentration.

phenolate groups generated by the binding of metal ions to Tyr residues (Tyr 95 and Tyr 188 in the N-lobe, Tyr 426 and Tyr 517 in the C-lobe) in the specific iron binding sites.[8] The third, broader band at 315 nm ($\Delta\varepsilon = 4700 \pm 300\ M^{-1}cm^{-1}$) is likely to be a ligand-to-metal charge-transfer (LMCT) band. However, we could find no reports of such LMCT bands for titanium(IV) phenolate complexes in the literature. To confirm this assignment, titanium(IV) citrate was titrated into a solution of the model ligand ethylenebis(*o*-hydroxyphenylglycine) (EHPG, pH 7.4, 10 mM Hepes buffer), a ligand used previously for mimicking Fe^{III} binding.[9] The UV difference spectra were almost identical to those from reaction of titanium(IV) citrate with transferrin and ^1H NMR shifts of the aromatic protons of EHPG induced by titanium(IV) citrate are consistent with the binding of Ti^{IV} ions to phenolate groups on the ligand.

Analysis of the titration curve for reaction of apo-hTF with titanium(IV) citrate (Figure 1b) suggests that two Ti^{IV} ions bind strongly to transferrin (i.e. one in each lobe). A similar stoichiometry of Ti:hTF = 1.9(±0.1):1 was obtained from the measurement of the titanium content of purified titanium transferrin by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The value of the extinction coefficient for the first step of Ti^{IV} binding to transferrin (occupation of one lobe), $\Delta\varepsilon_1$, is $17000 \pm 400\ M^{-1}cm^{-1}$ at 241 nm, which is the same as the value determined for Ti^{IV} binding to ethylenebis(*o*-hydroxyphenylglycine) ($\Delta\varepsilon \approx 17000\ M^{-1}cm^{-1}$), suggesting that two tyrosines are involved in binding Ti^{IV} in both the N- and C-lobes (linear increase in $\Delta\varepsilon$ with occupation of sites, see Figure 1b). The resulting yellow Ti_2 – hTF solution was stable at ambient temperature.

[*] Prof. Dr. P. J. Sadler, Dr. H. Sun, H. Li, R. Weir
Department of Chemistry, University of Edinburgh
West Mains Road, Edinburgh EH9 3JJ (UK)
Fax: (+44) 131-650-6452
E-mail: p.j.sadler@ed.ac.uk

[**] This research was supported by the Biotechnology and Biological Sciences Research Council, Engineering and Physical Sciences Research Council, and Scottish Higher Education Funding Council. We are grateful to the GlaxoWellcome for a fellowship (H.S.) and the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom for an Overseas Research Student Award (H.L.).

  1433-7851/98/3711-1577 $ 17.50+.50/0

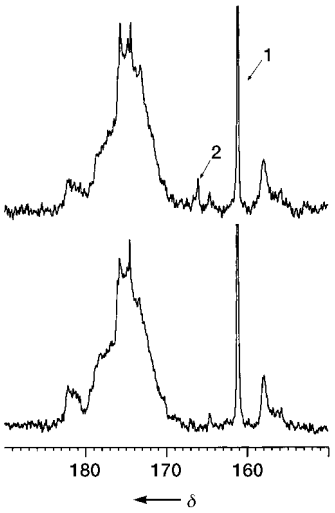


Figure 2. ^{13}C NMR spectra (125 MHz) of apo-hTF. Top: 1.6 mM, 10 mM $H^{13}CO_3^-$, 100 mM KCl, 70% H_2O/30% D_2O, pH 7.5. Bottom: Same solution treated with 2 mol equiv Ti^{IV} (10 mM aqueous solution of $[TiCp_2Cl_2]$ solubilized by addition of 1.2 mol equiv sodium citrate). A similar signal for bound $^{13}CO_3^{2-}$ was also seen on reaction of hTF with titanium(IV) citrate. This peak is weaker than that of, for example Bi_2–hTF,[14] suggesting a difference in the extent of lobe closure and anion exchange rate. Labels 1 and 2 refer to signals of free HCO_3^- and bound CO_3^{2-}, respectively.

Attempts to determine by UV spectroscopy whether the anticancer drug $[TiCp_2Cl_2]$ can transfer Ti ions to hTF were hampered by the absorption of the drug itself. Therefore ^1H and ^{13}C NMR spectroscopy were used to establish this interaction. We reasoned that in blood plasma $[TiCp_2Cl_2]$ may be present as a citrate adduct in view of the high concentration of plasma citrate (ca. 100 μM). Therefore reactions were carried out with the drug solubilized by approximately 1 mol equiv citrate. The exact nature of this complex was not established and we refer to it as $\{TiCp_2\}^{2+}$. It is clear from ^1H NMR spectra that the Cp ligands remain bound and citrate probably displaces the Cl^- ligands by analogy to recently reported amino acid complexes.[10] In the ^{13}C NMR spectrum a new signal assigned to bound $^{13}CO_3^{2-}$ appeared at $\delta = 166.1$ (Figure 2), almost identical to signals observed on reaction of hTF with titanium(IV) citrate in the presence of $H^{13}CO_3^-$. The chemical shift of this bound anion is close to that observed previously for other metallotransferrins ($\delta = 165.4$ for Al^{III}, $\delta = 166.5$ for Ga^{III}, $\delta = 166/166.2$ for Tl^{III},[11, 12] $\delta = 166.8/167.2$ for Sc^{III},[13] and $\delta = 165.8$ for Bi^{III}).[14]

Even though ^1H NMR spectra of hTF are complicated by the overlap of a large number of resonances, it was clear that Ti^{IV} binding caused characteristic changes in certain regions, for example new peaks appeared in the *N*-acetyl region ($\delta =$ 2.066 and 2.055 due to glycan chains), and in the high-field region ($\delta = 0.284, 0.050, -0.073$, Figure 3). Resonances due to bound Cp ($\delta \approx 6.2-7.0$) disappeared and new peaks at approximately $\delta = 6.0$ can be assigned to displaced Cp ligands.

The apparently strong binding of Ti^{IV} ions to transferrin is consistent with predictions based on metal ion acidity: the most acidic metal ions bind the most strongly.[15] In view of the pK_a values of Ti^{IV} and Fe^{III} ions (<1 and 2.7, respectively),[16] Ti^{IV} might be expected to bind to transferrin even more strongly than Fe^{III} (although in practice this may be influenced by other hydrolytic equilibria). We found that Fe^{III} ions can displace Ti^{IV} ions from transferrin but only slowly. Upon addition of 2 mol equiv $[Fe^{III}(nta)_2]$ (nta = nitrilotriacetate) to Ti_2–hTF (pH 7.4) a band appeared at approximately 465 nm, which is characteristic of iron transferrin. This reaction seems

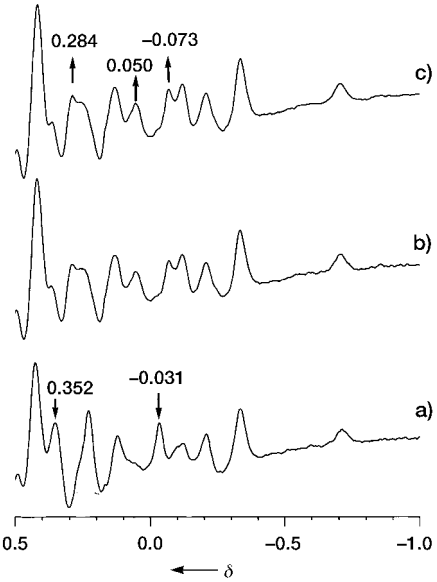


Figure 3. a) High-field region of the ^1H NMR spectrum (500 MHz) of apo-hTF and changes induced by added b) titanium(IV) citrate, and c) $\{TiCp_2\}^{2+}$ (2 mol equiv, pH* 7.4, 0.1 M KCl, 10 mM bicarbonate). The signals in this region are largely from methyl groups situated over aromatic rings in the protein. The spectra are complicated by the broadness of the signals from this 80 kDa protein and by peak overlap, but it is clear that Ti^{IV} binds similarly in both cases with displacement of the citrate and Cp ligands.

to proceed in two steps, the first lasting about 20 min and the second lasting over 5 h.

Since hTF in blood plasma is only about 30% saturated with Fe^{III} ions,[17] Ti^{IV} ions entering the blood, for example from anticancer complexes, could readily be taken up by this protein. Transferrin could serve to deliver Ti^{IV} to cancer cells and release it inside cells (at low pH), as is well-documented for Ga^{III} and Ru^{III} ions.[4, 5] Biological investigations of titanium transferrin would therefore appear to be warranted.

Experimental Section

Apo-hTF was purchased from Sigma (Catalog No. T0519) and was washed three times with 0.1 M KCl using Centricon 30 ultrafilters (Amicon) to remove low molecular mass impurities. Protein concentrations were determined by means of the extinction coefficent $\varepsilon_{280} = 93\,000\ M^{-1}cm^{-1}$.[18] Ethylenebis(*o*-hydroxyphenylglycine) (Sigma), $Ti^{III}Cl_3$ (Sigma), sodium citrate (Sigma), titanocene dichloride (Aldrich), $NaHCO_3$ (Sigma), and $NaH^{13}CO_3$ (MSD isotopes, >99% enriched) were used without further purification.

Measurements of pH were made using a Corning 145 pH meter equipped with an Aldrich microcombination electrode calibrated with Aldrich buffer solutions of pH 4, 7, and 10. Meter readings for D_2O solutions are designated as pH*.

A 200 mM fresh purple solution of $[Ti^{III}(cit)_{1.25}]$ (cit = citrate) was prepared under N_2 by adding sodium citrate (1 mL of a 250 mM solution) to a known amount of $Ti^{III}Cl_3$, and the pH was adjusted to approximately 6.0 by addition of solid $NaHCO_3$.[19] After bubbling with O_2, a colorless solution of titanium(IV) citrate was obtained, which was then diluted to roughly 3.3 mM for UV experiments. UV spectra were recorded on a computer-controlled Perkin Elmer lambda 16 spectrometer; the temperature of the cell (1 cm) was maintained at 310 K.

A 10 mM yellow titanocene solution was prepared by dissolving a known amount of $[TiCp_2Cl_2]$ in a sodium citrate solution (Ti:cit = 1:1.2), and the pH was adjusted to 4.5 (1 M NaOH).

  1433-7851/98/3711-1578 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **1998**, *37*, No. 11

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DMX 500 NMR spectrometer at 500 and 125 MHz, respectively. The experimental conditions were similar to those reported previously.[14] The ^{1}H NMR reference was endogenous formate ($\delta = 8.465$ at pH* > 7.0 relative to trimethylsilyl propionate (TSP)), which was always present in transferrin samples as a minor impurity. The ^{13}C NMR reference was added dioxane ($\delta = 67.4$ relative to TSP).

The titanium content of transferrin was measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Thermo Jarrell Ash IRIS, at 323.40 nm). The Ti_2–hTF samples for ICP-AES were prepared as follows: titanium(IV) citrate (2.2 mol equiv) was added to apo-hTF (in 10 mM Hepes buffer, pH 7.4, 10 mM bicarbonate) and left to equilibrate for over 24 h at 310 K. The protein was then purified by ultrafiltration (Centricon 30, Amicon), washing four times with 0.1 M KCl and twice with water. An aliquot of the final solution was diluted with concentrated HNO_3 (ca. 6 M) and the titanium content was measured without any digestion of the sample.[20] The transferrin concentration in this sample was determined by titrating with Fe^{III} ions (added as $[Fe(NTA)_2]$) by measuring the absorbance at 465 nm ($\Delta\varepsilon = 4950\ M^{-1}\,cm^{-1}$ for diferric transferrin).[21]

Received: December 17, 1997 [Z 11273 IE]
German version: *Angew. Chem.* **1998**, *110*, 1622 – 1625

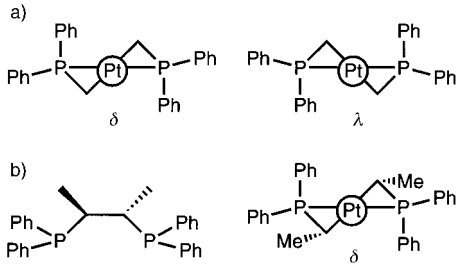
Keywords: antitumor agents · bioinorganic chemistry · titanium · transferrin

[1] B. K. Keppler, C. Friesen, H. Vongerichten, E. Vogel in *Metal Complexes in Cancer Chemotherapy* (Ed.: B. K. Keppler), VCH, Weinheim, **1993**, pp. 297 – 323.
[2] a) P. Köpf-Maier, H. Köpf in *Metal Compounds in Cancer Therapy* (Ed.: S. P. Fricker), Chapman & Hall, London, **1994**, pp. 109 – 146; b) C. Christodoulou, D. Ferry, D. Fyfe, A. Young, J. Doran, G. Sass, A. Eliopoulos, T. Sheehan, D. J. Kerr, *Proc. 88th Annu. Meeting Am. Assoc. Cancer Res.* **1997**, *38*, 222.
[3] K. Ishiwata, T. Ido, M. Monma, M. Murakami, H. Fukuda, M. Kameyama, K. Yamada, S. Endo, S. Yoshioka, T. Sato, T. Matsuzawa, *Appl. Radiat. Isot.* **1991**, *42*, 707 – 712.
[4] S. G. Ward, R. C. Taylor in *Metal-based Antitumor Drugs* (Ed.: M. F. Gielen), Freund, London, **1988**, pp. 1 – 54.
[5] F. Kratz, M. Hartmann, B. K. Keppler, L. Messori, *J. Biol. Chem.* **1994**, *269*, 2581 – 2588.
[6] Titanium(IV) citrate was prepared by oxidation of titanium(III) citrate as described in the Experimental Section. Titanium(III) citrate has been previously used as a strong reductant for proteins: M. Schreiner, R. K. Thauer, *Eur. J. Biochem.* **1997**, *243*, 110 – 114. Reaction of titanium(III) citrate with apo-transferrin leads to the incorporation of Ti^{IV} ions into the protein.
[7] Serum transferrin (hTF) is an 80 kDa glycoprotein of 679 amino acids with two similar specific octahedral Fe^{III} binding sites in flexible interdomain clefts in the N- (and C-) lobes, which consist of the carboxylate of Asp 63 (Asp 392), phenolate of Tyr 95 (Tyr 426) and Tyr 188 (Tyr 517), imidazole N of His 249 (His 585), and synergistic bidentate carbonate anion. X-ray crystal structures of serum transferrins: a) S. Bailey, R. W. Evans, R. C. Garratt, B. Gorinsky, S. Hasnain, C. Horsburgh, H. Jhoti, P. F. Lindley, A. Mydin, R. Sarra, J. L. Watson, *Biochemistry* **1988**, *27*, 5804 – 5812. b) H. J. Zuccola, Ph.D Thesis, Georgia Institute of Technology, **1993**.
[8] D. C. Harris, P. Aisen in *Iron Carriers and Iron Proteins* (Ed.: T. M. Loehr), VCH, Weinheim, **1989**, pp. 69 – 82.
[9] W. R. Harris, C. J. Carrano, V. L. Pecoraro, K. N. Raymond, *J. Am. Chem. Soc.* **1981**, *103*, 2231 – 2237.
[10] a) T. M. Klapötke, H. Köpf, I. C. Tornieporth-Oetting, P. S. White, *Angew. Chem.* **1994**, *106*, 1587 – 1589; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1518 – 1519; b) J. H. Murray, M. M. Harding, *J. Med. Chem.* **1994**, *37*, 1936 – 1941.
[11] I. Bertini, C. Luchinat, L. Messori, A. Scozzafava, G. Pellacani, M. Sola, *Inorg. Chem.* **1986**, *25*, 1782 – 1786.
[12] J. M. Aramini, P. H. Krygsman, H. J. Vogel, *Biochemistry* **1994**, *33*, 3304 – 3311.
[13] J. M. Aramini, H. J. Vogel, *J. Am. Chem. Soc.* **1994**, *116*, 1988 – 1993.
[14] H. Li, P. J. Sadler, H. Sun, *J. Biol. Chem.* **1996**, *271*, 9483 – 9489.
[15] a) H. Li, P. J. Sadler, H. Sun, *Eur. J. Biochem.* **1996**, *242*, 387 – 393; b) H. Sun, M. C. Cox, H. Li, P. J. Sadler, *Struct. Bonding* **1997**, *88*, 71 – 102.
[16] P. Pettit, L. D. Pettit, *IUPAC Stability Constants Database*, IUPAC/Academic Software, Otley, UK, **1993**.
[17] A. Leibman, P. Aisen, *Blood* **1979**, *53*, 1058 – 1065.
[18] C. K. Luk, *Biochemistry* **1971**, *10*, 2838 – 2843.
[19] M. Goubeaud, G. Schreiner, R. K. Thauer, *Eur. J. Biochem.* **1997**, *243*, 110 – 114.
[20] N. Cols, N. Romero-Isart, M. Capdevila, B. Oliva, P. Gonzàlez-Duarte, R. Gonzàlez-Duarte, S. Atrian, *J. Inorg. Biochem.* **1997**, *61*, 157 – 166.
[21] P. K. Bali, W. R. Harris, *Arch. Biochem. Biophys.* **1990**, *281*, 251 – 256.

Platinum(II) Complexes with Constructive and Destructive Interaction of Diphosphane and Binaphthol Ligands**

Nicole M. Brunkan, Peter S. White, and Michel R. Gagné*

(*S*,*S*)-2,3-Bis(diphenylphosphanyl)butane ((*S*,*S*)-Chiraphos) and its achiral analogue 1,2-bis(diphenylphosphanyl)ethane (dppe) belong to a class of chelating diphosphanes that form five-membered rings with late transition metals. These diphosphanes usually adopt a δ- or λ-*skew* conformation (Scheme 1a).[1] With the achiral dppe, the two conformers are



Scheme 1. a) *skew* conformations of dppe in platinum complexes. b) (*S*,*S*)-Chiraphos and its preferred δ conformation in the complex.

equally favorable; however, backbone modification of chiral diphosphanes makes one conformer more stable than the other.[2] For example, the *trans*-methyl substituents of (*S*,*S*)-Chiraphos favor the δ conformer (pseudoequatorial versus

[*] Prof. M. R. Gagné, N. M. Brunkan, P. S. White
Department of Chemistry, CB#3290
University of North Carolina
Chapel Hill, NC 27599-3290 (USA)
Fax: (+1) 919-962-6341
E-mail: mgagne@unc.edu

[**] This work was supported in part by the National Science Foundation under the auspices of a CAREER development award to MRG (CHE-9624852) and an equipment grant to the University of North Carolina for the purchase of a CCD diffractometer (CHE-9709977). We also acknowledge the Petroleum Research Fund, administered by the ACS, for partial support. NMB is a UNC Board of Governors Graduate Fellow. We thank Prof. H. Holden Thorp (UNC-Chapel Hill) for generous access to his UV/Vis spectrometer.