Influence of carbonate on the complexation of Cm(III) with human serum transferrin studied by time-resolved laser fluorescence spectroscopy (TRLFS)

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The complexation of Cm(III) with transferrin is investigated in the pH range from 3.5 to 11.0 in the absence of carbonate and at c(carbonate)tot = 25 mM. In the absence of carbonate two Cm(III) transferrin species I and II are formed depending on pH. An increase of the total carbonate concentration favors the formation of the Cm(III) transferrin species II with Cm(III) bound at the Fe(III) binding site of transferrin at significantly lower pH values. The spectroscopic results directly prove that carbonate acts as a synergistic anion for Cm(III) complexation at the binding site of transferrin. At c(carbonate)tot = 25 mM the formation of the nonspecific Cm(III) transferrin species I is suppressed completely. Instead, three Cm(III) carbonate species Cm(CO3)1+, Cm(CO3)2− and Cm(CO3)3− are formed successively with increasing pH. The formation of Cm(III) carbonate species results in a decreased fraction of the Cm(III) transferrin species II at pH ≥ 7.4 which indicates that carbonate complexation is an important competition reaction for Cm(III) transferrin complexation at physiological carbonate concentration.

Introduction

If radionuclides are accidentally released into the environment, in particular actinides can cause a serious health risk upon incorporation. Since they have no essential function in the human body there is only deficient knowledge about the biochemistry of actinides in man. With regard to the development of potential decontamination therapies, a detailed understanding of the mechanisms of relevant biochemical reactions is required.1

One potential reaction that incorporated actinides might undergo is the coordination to human serum transferrin. Transferrin is an iron carrier protein in blood with a molecular mass of 79 570 Da and consists of 679 amino acids.2–4 Each lobe consists of α/β-subunits and is divided into two domains separated by a cleft housing the metal binding site for Fe(III). The coordination of Fe(III) and the synergistic anion leads to a conformational change of transferrin from an open to a closed form. This structural change stabilizes the metal ion transferrin complex and is important for its recognition by the receptor.2,6

In both lobes Fe(III) is coordinated by two tyrosines, one aspartate, one histidine and the synergistic anion in a distorted octahedral geometry.7,8 Under physiological conditions the synergistic anion is carbonate. It is required for the complexation of metal ions to transferrin since neither the anion nor the metal ion are bound strongly in the absence of the other (except for VO2+).9 Carbonate coordinates to Fe(III) in a bidentate mode and is linked to the protein by hydrogen bonding which leads to the formation of a very stable metal Fe(III)–carbonate–transferrin ternary complex.7,8 Furthermore, complexation of the synergistic anion reduces the positive charge of the protein which originates from an arginine sidechain and a helix N-terminus directed towards the binding site. This facilitates the coordination of a positively charged metal ion.2 Additionally, protonation of the carbonate anion may also play a role in iron release during endocytosis.2,10

Besides carbonate, other anions like oxalate, malonate, glycolate or lactate can also act as synergistic anions and form Fe(III)–anion–transferrin ternary complexes.11,12 These are typically much weaker than the respective complexes with carbonate and most of them are observed only under carbonate free conditions. The key structural feature of these anions is the presence of a carboxylate group and a proximal functional group within 6.3 Å in a “carbonate-like” conformation.11

In blood serum transferrin is about 30% saturated with iron. Consequently, non-saturated transferrin is available for the
complexation of other metal ions. Besides ferric iron about 30 other metal ions were identified to bind to transferrin, among them are actinides like Th(IV), Pa(V), U(VI), Np(IV), Pu(IV), Am(III) and Cm(III). The complexation of Cm(III) with transferrin was studied in the pH range from 3.5 to 11.0 at ambient carbonate concentration. Two different Cm(III) transferrin species were identified and characterized depending on pH. At pH ≥ 7.4 Cm(III) is bound at the Fe(III) binding site of transferrin whereas at lower pH a nonspecific Cm(III) transferrin species is formed. Stability constants for the N- and C-lobe of ferrin was studied in the pH range from 3.5 to 11.0 at ambient carbonate concentration.

In the present work we investigate the influence of the carbonate concentration on the Cm(III) transferrin complexation using time-resolved laser fluorescence spectroscopy (TRLFS). To date all measurements with Cm(III) and transferrin are performed at ambient carbonate concentration. Since carbonate acts as a synergistic anion for Fe(III) complexation at the binding sites it is also of particular interest to study the impact of carbonate on the formation of the Cm(III) transferrin species I and II. Therefore, fluorescence spectra of Cm(III) transferrin in the pH range from 3.5 to 11.0 in the absence of carbonate and at physiological carbonate concentration are measured at room temperature as well as at physiological temperature to get information on the influence of carbonate on the Cm(III) transferrin interaction.

TRLFS is a very sensitive method to study complexation reactions of lanthanides and actinides in the submicromolar concentration range. Cm(III) is used as a representative for trivalent actinides because of its excellent fluorescence properties. Spectroscopic parameters like shape and position of the emission bands provide information on the coordination environment of the metal ion and the complex geometry.

Experimental section

Chemicals and sample preparation

The protein samples were prepared in TRIS (Amresco) buffered solutions (10 mM, pH 7.4) with a physiological sodium chloride medium of 150 mM NaCl (Merck Millipore) using ultrapure water (Millipore, Billerica, MA, USA; 18.2 MΩ cm). The ionic strength of the samples is determined by the concentration of NaCl and fixed at I = 0.16 m. Human serum apo-transferrin of high reagent grade (apo-transferrin human, 98%) was purchased from Sigma. It was purified using size exclusion chromatography (Sephadex G-25 medium, GE Healthcare) followed by filtration (Amicon Centrifugal Filter Units, 30 kDa) before use according to the protocol of Harris et al. The protein concentration of the transferrin stock solution was determined by UV/Vis spectroscopy at λ = 280 nm using an extinction coefficient of ε = 93 000 M⁻¹ cm⁻¹.

The Cm(III) stock solution used for the TRLFS studies (c(Cm) = 3.33 × 10⁻⁷ M in 15.7 mM HClO₄) had an isotopic mass distribution of 89.7% Cm-248, 9.4% Cm-246, ≤ 1% Cm-243, Cm-244, Cm-245 and Cm-247. The Cm(III) concentration of the TRLFS samples was fixed at 1.00 × 10⁻⁷ M by adding 30 μl of the Cm(III) stock solution to 970 μl of a buffered transferrin solution with a concentration of 4.85 × 10⁻⁶ M resulting in a final transferrin concentration of 5.00 × 10⁻⁶ M. Complexation studies were carried out at varying pH values between 3.5 and 11.0 using NaOH and HCl solutions (Merck Millipore) of different concentrations (1.0 M, 0.5 M, 0.1 M, and 0.01 M) for adjustment. Unless stated otherwise the pH was adjusted starting from pH 11 downwards to pH 3.5. TRLFS measurements were performed at room temperature (296 K) and physiological temperature (310 K).

For measurements in the absence of carbonate the purified transferrin stock solution was degassed using seven freeze–pump–thaw cycles. The sample preparation and the adjustment of the pH was performed in an argon glove box using degassed solvents and chemicals, exclusively. For the measurements at physiological carbonate concentration the samples were prepared with TRIS buffer containing 150 mM NaCl and 25 mM NaHCO₃ (Sigma-Aldrich).

Time-resolved laser fluorescence spectroscopy

TRLFS was performed using a Nd-YAG (Continuum Surelute Laser) pumped dye laser system (NARROWscan D-R Dye Laser) with a repetition rate of 10 Hz. For excitation of Cm(III) a wavelength of 396.6 nm was used. Emission spectra were recorded in the range of 580 to 635 nm after a delay time of 1 μs to discriminate short-lived fluorescence of organic compounds. After spectral decomposition by a spectograph (Shamrock 303i) with a 1199 lines mm⁻¹ grating the spectra were recorded using an ICCD camera (iStar Gen III, ANDOR) containing an integrated delay controller. For better comparison all spectra are normalized to the same peak area.

Results and discussion

Under physiological conditions [pH 7.4, T = 310 K, c(carbonate)ₗₒₑₜ = 25 mM, c(NaCl) = 150 mM] Fe(III) is complexed by four amino acids and a bidentate coordinating carbonate at the binding site of transferrin. Although we suppose the coordination environment of the Cm(III) transferrin species II to be similar to the Fe(III) transferrin complex the TRLFS data provide no direct information on the nature of the synergistic anion. In principle, carbonate or bicarbonate could act as a synergistic anion for Cm(III) transferrin complexation. In a closed system with a total carbonate concentration of c(carbonate)ₗₒₑₜ = 25 mM the concentrations of CO₃²⁻, HCO₃⁻ and CO₂(aq) depending on pH (Fig. 1) are calculated from the log K values of the carbonate equilibria which have been taken from the Thermodynamical Database of the Nuclear Energy Agency (NEA – TDB) and extrapolated to I = 0.16. CO₂(aq) dominates in the acidic pH range up to pH 6.0. With increasing pH the bicarbonate concentration increases. HCO₃⁻ is the dominating species between pH 6.2 and 9.8. Carbonate is formed at pH ≥ 7.4 and dominates the speciation above pH 9.8. In the present study the influence of carbonate on the complexation of Cm(III) with transferrin is investigated with dependence on pH. The total carbonate concentration, which means the total concentration of the species CO₃²⁻, HCO₃⁻ and CO₂(aq), was varied from 0 to 25 mM.
For reasons of simplicity, total carbonate concentrations of \( c(\text{carbonate})_{\text{tot}} = 0.23 \text{ mM} \) and \( c(\text{carbonate})_{\text{hot}} = 25.00 \text{ mM} \) are referred to as “ambient carbonate concentration” and “physiological carbonate concentration” with the speciation of \( \text{CO}_3^{2-}, \text{HCO}_3^- \) and \( \text{CO}_2(aq) \) depending on the pH.

### Complexation of Cm(III) with transferrin in the absence of carbonate

Complexation of Cm(III) with transferrin as a function of pH was studied under carbonate free conditions at room temperature (296 K). At each pH step a fluorescence spectrum was recorded. The normalized fluorescence spectra in the pH range from 4.0 to 10.5 are shown in Fig. 2. The spectra display a strong pH dependency of the complexation reaction. Up to pH 6.0 the system is dominated by the Cm(III) aquo ion (\( \lambda_{\text{max}} = 593.9 \text{ nm} \)). A further increase of pH leads to the successive formation of two Cm(III) transferrin species with emission maxima at \( \lambda_{\text{max}} = 599.8 \text{ nm} \) and \( \lambda_{\text{max}} = 619.9 \text{ nm} \), respectively. The spectrum of the second Cm(III) transferrin species is characterized by a hot band at \( \lambda_{\text{max}} = 602.9 \text{ nm} \), which results from the fluorescence emission from thermally populated higher ligand field levels of the excited state.

The emission bands of both Cm(III) transferrin species formed under carbonate free conditions are identical to those observed at ambient carbonate concentration (\( c(\text{carbonate})_{\text{hot}} = 0.23 \text{ mM} \)) in our previous study. The emission band with \( \lambda_{\text{max}} = 599.8 \text{ nm} \) corresponds to the Cm(III) transferrin species I. The shift of this emission band relative to the emission band of the Cm(III) aquo ion is 5.9 nm which is in the usual range of Cm(III) complexes with various organic ligands. Furthermore, the emission band of the Cm(III) transferrin species I is rather broad which is typical for the formation of Cm(III) sorption species. Shape and shift of the emission band as well as the fluorescence lifetime indicate that this is a nonspecific Cm(III) transferrin species with five water molecules and four additional ligands in the first coordination sphere.

The emission spectrum of the second Cm(III) transferrin species is characterized by a very sharp emission band with maxima at \( \lambda_{\text{max}} = 619.9 \text{ nm} \) and \( \lambda_{\text{max}} = 602.9 \text{ nm} \) (hot band). This Cm(III) transferrin species II has been observed also at ambient carbonate concentration in our previous study. The bathochromic shift of 26 nm relative to the emission band of the Cm(III) aquo ion is extraordinary and indicates strong complexation of Cm(III) at the Fe(III) transferrin binding site. The fluorescence lifetime of the emission band of the Cm(III) transferrin species II was determined to be \( \tau = 229 \pm 10 \mu s \) under carbonate free conditions which is in very good agreement with the value measured at ambient carbonate concentration (\( \tau = 221 \pm 5 \mu s \)). This proves the formation of identical species both at ambient carbonate concentration and in the absence of carbonate. The fluorescence lifetime corresponds to two quenching water molecules in the first coordination sphere. Assuming an overall coordination number of nine for Cm(III), the TRLFS results show that Cm(III) is coordinated by four amino acids of apo-transferrin, two \( \text{H}_2\text{O} \) molecules and three additional ligands (e.g. synergistic anions like carbonate).

The fluorescence spectra of the pure components (Cm(III) aquo ion and Cm(III) transferrin species I and II) were determined from the pH dependent fluorescence spectra. They were used for determining the fractions of the three species at various pH values by peak deconvolution of the emission spectra. Fig. 3 shows the species concentrations as a function of pH (species distribution). The Cm(III) aquo ion dominates the speciation up to pH 4.5, with the concentration decreasing with increasing pH. The Cm(III) transferrin species I is formed in the pH range from 4.0 to 9.7 and becomes the dominating species between pH 4.5 and 8.5. Above pH 7.7 the concentration of the Cm(III) transferrin species II increases continuously until it is solely present in solution above pH 8.7.

The species distribution obtained from the measurements in the absence of carbonate is compared to the data for Cm(III) transferrin complexation at ambient carbonate concentration published in our previous study. Besides the carbonate
concentration all experimental conditions are identical for both studies which allows a detailed comparison. The absence of carbonate has no significant impact on the formation of the Cm(III) transferrin species I which confirms that carbonate is not required for the formation of this species. In contrast, carbonate has a distinct influence on the complexation of Cm(III) at the Fe(III) binding site (Cm(III) transferrin species II).

At ambient carbonate concentration the complex formation starts at pH 7.4 whereas under carbonate free conditions the Cm(III) transferrin species II is formed at pH ≥ 7.7. This indicates that carbonate acts in fact as a synergistic anion for Cm(III) complexation at the binding site of transferrin. Hence, in the absence of carbonate higher pH values are required for the complexation of Cm(III) at the Fe(III) binding site of transferrin.

Although the Cm(III) transferrin samples have been prepared under carbonate free conditions in a glove box small traces of carbonate might still be available. Since the Cm(III) concentration used for TRLFS measurements is very low \((c(Cm) = 1 \times 10^{-7} \text{ M})\) even trace concentrations of carbonate are sufficient for the formation of a ternary Cm(III)-transferrin-carbonate complex. This might be the reason why the Cm(III) transferrin species identified under carbonate free conditions matches the Cm(III) transferrin species II which has been observed at ambient carbonate concentration. Nevertheless, our results clearly demonstrate the positive influence of carbonate on the complexation of Cm(III) at the Fe(III) binding site of transferrin.

Complexation of Cm(III) with transferrin at physiological carbonate concentration

Complexation of Cm(III) with transferrin as a function of pH was studied at physiological carbonate concentration (25 mM) and room temperature (296 K). The normalized fluorescence spectra in the pH range from 3.5 to 11.0 are shown in Fig. 4. At low pH the spectra are dominated by the emission band of the Cm(III) aquo ion \((\lambda_{\text{max}} = 593.9 \text{ nm})\). In contrast to the measurements in the absence of carbonate no emission band of the Cm(III) transferrin species I is observed at physiological carbonate concentration. Instead, three Cm(III) carbonate species \(\text{Cm(CO}_3^+)\), \(\text{Cm(CO}_3^2^-\) and \(\text{Cm(CO}_3^3^-\) with emission maxima at \(\lambda_{\text{max}} = 599.1 \text{ nm}, 603.3 \text{ nm and 605.9 nm}\) are formed.\(^{41}\)

Comparison with blank solutions (without transferrin) in the same pH range proves that these emission bands result from Cm(III) carbonate interaction and do not represent additional transferrin species. Above pH 7.0 the spectra display the increasing emission band of the Cm(III) transferrin species II at \(\lambda_{\text{max}} = 620.0 \text{ nm}\).

Fig. 5 shows the fluorescence spectra of the pure components the Cm(III) aquo ion, \(\text{Cm(CO}_3^+)\), \(\text{Cm(CO}_3^2^-\), \(\text{Cm(CO}_3^3^-\) and the Cm(III) transferrin species II. They were determined from the pH dependent fluorescence spectra of Cm(III) with and without transferrin at physiological carbonate concentration. The fractions of the different Cm(III) species at various pH values (species distribution, Fig. 6) were determined by peak
deconvolution of the emission spectra using the spectra of the pure components. The fraction of the Cm(III) aqua ion decreases with increasing pH. In contrast to the measurements at ambient carbonate concentration and in the absence of carbonate, the Cm(III) transferrin species I is not formed at physiological carbonate concentration. This again proves that it is a nonspecific and only weakly bound Cm(III) transferrin species.

In the presence of a competing ligand like carbonate the formation of this species is completely suppressed. Instead, the Cm(III) mono-, di- and tri-carbonate complexes are formed successively with increasing pH. The Cm(CO$_3$)$^{2-}$ and Cm(CO$_3$)$_2^{4-}$ species dominate the speciation in the pH range from 5.3 to 7.2 and from 7.2 to 8.7, respectively. Additionally, up to 30% of the Cm(CO$_3$)$_3^{6-}$ complex are formed in the pH range from 7.7 to 11.0. The Cm(III) transferrin species II is formed at pH $\geq$ 7.0. In comparison, the complex formation in the absence of carbonate starts at a significantly higher pH value (pH 7.7). Hence, an increase of carbonate concentration favors the formation of the Cm(III) transferrin species at lower pH values. This confirms again that carbonate has the function of a synergistic anion for the Cm(III) complexation at the Fe(III) binding site of transferrin.

As shown in Fig. 6, on the one hand carbonate favors the complexation of Cm(III) at the Fe(III) binding site of transferrin in its function as a synergistic anion, on the other hand it is a competitive ligand. Hence, the formation of the Cm(III) transferrin species I is completely suppressed at physiological carbonate concentration. Furthermore, carbonate complexation is also a competitive reaction to the complexation of Cm(III) at the Fe(III) binding site of transferrin. In the absence of carbonate the increase of the fraction of the Cm(III) transferrin species II from 0% to 100% occurs in the pH range from 7.7 to 9.7. At physiological carbonate concentration a significantly wider pH range (pH 7.0–11.0) is required to form the Cm(III) transferrin species II solely. This is attributed to the competition between Cm(III) complexation with carbonate on the one hand and transferrin on the other hand and has to be considered when complexation studies with transferrin are performed under physiological conditions. The competitive effect of carbonate on the complexation of metal ions to transferrin has also been observed in previous studies for some lanthanide and actinide ions. Based on complexation studies of Gd(III) with transferrin in vitro, Zak and Aisen suggest that transferrin may not be an important carrier protein for Gd(III) in vivo because of the formation of Gd(III) carbonate complexes. Taylor et al. reported that only about 20% of Eu(III), Yb(III), Am(III) and Cm(III) are bound to transferrin in vivo which is also attributed to the competition with carbonate.

Besides quantitative information on the speciation of Cm(III) with transferrin at physiological carbonate concentration the nature of the synergistic anion is of particular interest. Fig. 1 and 6 show the speciation of CO$_3^{2-}$, HCO$_3^-$ and CO$_2$(aq) in a closed system ([c(carbonate)]$_{tot}$ = 2.5 x 10$^{-2}$ M) and the speciation of Cm(III) with transferrin at a total carbonate concentration of [c(carbonate)$_{tot}$] = 2.5 x 10$^{-2}$ M (physiological carbonate concentration). Comparison depicts that the increase of the carbonate concentration with increasing pH (black line in Fig. 1) corresponds to the formation of the Cm(III) transferrin species II depending on pH (blue line in Fig. 6). The formation of CO$_3^{2-}$ as well as the Cm(III) transferrin species II starts at pH $\geq$ 7.0. The analogy between carbonate speciation and Cm(III) complexation at the Fe(III) binding site indicates that carbonate acts as a synergistic anion. Furthermore, this corresponds with the fact that bicarbonate is a very weak ligand for trivalent actinides and lanthanides. For example the stability constants of various lanthanide bicarbonate complexes in seawater (0.7 m ionic strength) are in the order of log $K$ = 1.5–1.9. These values are comparable to the stability constant of CmHCO$_3^-$ which was estimated to be log $K$(CmHCO$_3^-$) = 1.9 $\pm$ 0.2$^{47,48}$ whereas the stability constants of the Cm(III) carbonate complexes are significantly higher (log $K$(CmCO$_3^2$) = 8.30, log $K$(Cm(CO$_3$)$_2$) = 13.52 and log $K$(Cm(CO$_3$)$_3$) = 15.52.$^{41}$ The Cm(III) bicarbonate complex has been characterized at an elevated CO$_2$ partial pressure of 11 bar in a 1 M NaCl solution (pH 4.4) at room temperature. Under these conditions the formation of carbonate complexes can be neglected. Reduction of the CO$_2$ partial pressure and an increase of the pH both favor the complexation of Cm(III) with carbonate. Thus, under physiological conditions (pH 7.4 and atmospheric pressure) the complexation of Cm(III) with bicarbonate is insignificant. Consequently, the synergistic anion for the complexation of Cm(III) at the binding site of transferrin is carbonate as well as it is for Fe(III).

**Complexation of Cm(III) with transferrin at physiological carbonate concentration and temperature**

In addition to the measurements at room temperature the complexation of Cm(III) with transferrin as a function of pH was studied at physiological carbonate concentration (25 mM) and physiological temperature (310 K). Fig. 7 shows the normalized fluorescence spectra in the pH range from 3.5 to 11.0. The spectra are rather similar to those observed at physiological carbonate concentration and room temperature.
formation of the Cm(III) transferrin species II does not start present above pH 10.5. In comparison, at room temperature the transferrin species II increases continuously until it is solely Cm(CO₃)⁺, Cm(CO₃)₂⁻ dominates the speciation up to pH 4.5. With increasing pH Cm(CO₃)₃⁻ three Cm(III) carbonate species Cm(CO₃)⁺, Cm(CO₃)₂⁻ and Cm(CO₃)₃⁻ are formed successively. Compared to room temperature conditions the formation of the Cm(III) carbonate species starts at slightly lower pH values. Above pH 6.3 the concentration of the Cm(III) aquo ion, Cm(CO₃)⁺, Cm(CO₃)₂⁻ and Cm(CO₃)₃⁻ species are formed successively with increasing pH.

The species distribution (Fig. 8) was obtained from the pH dependent emission spectra by peak deconvolution using the spectra of the pure components (Fig. 5). The Cm(III) aquo ion dominates the speciation up to pH 4.5. With increasing pH Cm(CO₃)⁺, Cm(CO₃)₂⁻ and Cm(CO₃)₃⁻ species are formed successively. Compared to room temperature conditions the formation of the Cm(III) carbonate species starts at slightly lower pH values. Above pH 6.3 the concentration of the Cm(III) transferrin species II increases continuously until it is solely present above pH 10.5. In comparison, at room temperature the formation of the Cm(III) transferrin species II does not start before pH 7.0. Hence, an increase of the temperature favors the formation of the Cm(III) transferrin species II at lower pH values. This is comparable to the results on the temperature dependency of Cm(III) transferrin interaction at ambient carbonate concentration. In this case, the Cm(III) transferrin species II is formed at pH values of about 0.5 units lower at physiological temperature compared to room temperature.

Conclusions

In the present study, the influence of carbonate on the complexation of Cm(III) with transferrin was investigated using Time-Resolved Laser Fluorescence Spectroscopy (TRLFS). Our results show that an increase of the total carbonate concentration favors the formation of the Cm(III) transferrin species with Cm(III) bound at the Fe(III) binding site of transferrin at significantly lower pH values, which distinctively proves that carbonate acts as a synergistic anion for Cm(III) complexation with transferrin.

Furthermore, carbonate complexation is an important competition reaction which suppresses Cm(III) transferrin complexation significantly. At physiological carbonate concentration the formation of the nonspecific Cm(III) transferrin species I is suppressed completely but an increase of carbonate also has a significant impact on the fraction of the Cm(III) transferrin species II. Hence, the competitive effect of carbonate has to be considered when complexation studies with transferrin are performed under physiological conditions.

Under physiological conditions (pH 7.4, T = 310 K, c(carbonate)tot = 2.5 × 10⁻² M, c(NaCl) = 150 mM) 29% Cm(CO₃)⁺, 38% Cm(CO₃)₂⁻, 18% Cm(CO₃)₃⁻ and 15% of the Cm(III) transferrin species II are present in solution. Results from our previous study show the formation of about 70% of the Cm(III) transferrin species I and 30% of the Cm(III) transferrin species II at pH 7.4, ambient carbonate concentration and physiological temperature (310 K). This again proves that carbonate complexation is an important competition reaction which suppresses Cm(III) transferrin complexation significantly. Nevertheless, 15% of the Cm(III) transferrin species II are formed under physiological conditions. As a consequence, the Cm(III) transferrin complex might be recognized by the receptor followed by endocytosis which could be a possible pathway for the distribution of Cm(III) in the human body.

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