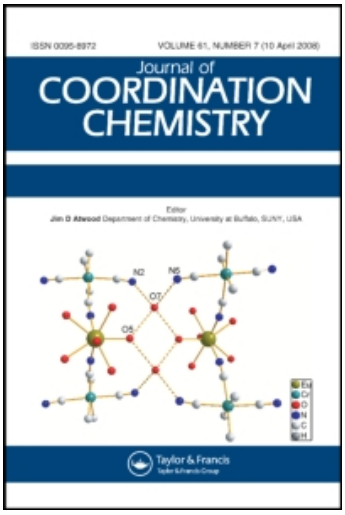

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Complex formation of curium(III) with amino acids of different functionalities: L-threonine and O-phospho-L-threonine

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Complex formation of curium(III) with amino acids of different functionalities: L-threonine and *O*-phospho-L-threonine

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The speciation of curium(III) with L-threonine and *O*-phospho-L-threonine was determined by time-resolved laser-induced fluorescence spectroscopy (TRLFS) at trace Cm(III) concentrations (3×10^{-7} M). Curium species of the type $M_pH_qL_r$ were identified in the L-threonine- and *O*-phospho-L-threonine system. These complexes are characterized by their individual luminescence spectra and luminescence lifetimes. The following formation constants were determined (a) for L-threonine: $\log \beta_{101} = 6.72 \pm 0.07$, $\log \beta_{102} = 10.22 \pm 0.09$, and $\log \beta_{1-22} = -(7.22 \pm 0.19)$ at ionic strength $I = 0.5$ M and (b) for *O*-phospho-L-threonine: $\log \beta_{121} = 18.03 \pm 0.13$ and $\log \beta_{111} = 14.17 \pm 0.09$ at ionic strength $I = 0.154$ M. Possible structures of the identified curium species are discussed on the basis of the luminescence lifetime measurements and the magnitude of the formation constants.

Keywords: Complexation; L-threonine; *O*-phospho-L-threonine; TRLFS; Curium

1. Introduction

For a better understanding of actinide interaction processes in biological systems such as microbes [1–4] and plants [5] on a molecular level, it is essential to explore their coordination chemistry with selected bioligands of relevant functionalities as model compounds. Amino acids are an important group of biomolecules, which are, for instance, basic structural components of proteins. Amino acids can have several functional groups like carboxyl, amino, hydroxyl, sulfuryl and phosphate groups in the fundamental chain and in side chains. All these functionalities are potential binding sites for metals. Recently Merroun *et al*. showed the high affinity of phosporylated amino acids such as phosphothreonine to uranium. Thus phosphothreonine and phosphoserine containing S-layer proteins, which form the outermost cell envelope of many bacteria, are able to bind to uranium with high affinity [6]. Therefore the investigation of the complexation behavior of actinides with amino acids of different functionalities is important.

In the following, a short overview is given about the literature on heavy metal complexation with selected amino acids. Publications describing the interaction of

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amino acids with trivalent actinides are scarce. A critical review of stability constants of metal complexes with amino acids with polar side chains including threonine using mainly potentiometry, polarography, spectrophotometry and NMR was published by Berthon in 1995 [7]. In general 1 : 1 (ML), 1 : 2 (ML_2) and 1 : 3 (ML_3) complexes were found. There are indications for deprotonation of the hydroxyl group in metal chelation even at moderate pH values region. As a consequence species of stoichiometry MLH_{-1}, ML_2H_{-1}, and ML_2H_{-2} having deprotonated hydroxyl groups highlighted by H_{-1} if one –OH group is deprotonated or by H_{-2} in the case of two deprotonated –OH groups are formed in the pH-range above 8. Günther *et al*. identified in a recent study 1 : 1 and 1 : 2 complexes of U(VI) with the zwitterion of threonine (HL) in the acidic pH range from 2 to 4 by means of TRLFS [8]. A key reaction in the activation or deactivation of enzymes in biological systems is the reversible phosphorylation of proteins, which is mainly observed on the hydroxyl group of the essential amino acids serine, threonine or tyrosine. To the knowledge of the authors, no results about the complex formation of phosphothreonine with trivalent actinides have been published. Recent results of a TRLFS study investigating the complexation of UO_2^{2+} with phosphothreonine showed the formation of three different species $UO_2H_3PThr^{2+}$, $UO_2H_2PThr^{+}$ and UO_2HPThr in the pH range from 1 to 4 [8]. The authors conclude a predominant interaction of U(VI) with the phosphate group of phosphothreonine.

To summarize there is very little known about the complexation of amino acids with different functionalities, e.g. threonine and phosphothreonine with trivalent actinides. Therefore, we present results of the curium(III) complexation with those amino acids obtained by TRLFS. The structures of A) L-threonine (threonine) and B) *O*-phospho-L-threonine (phosphothreonine) and the corresponding protolysis species are summarized in figure 1. We want to determine how the substituent on the C3 atom (–OH in the case of threonine and $–OPO_3H_2$ in the case of phosphothreonine) influences the Cm(III) complexation. In contrast to earlier complexation studies described above where the metal ion concentrations are in the millimolar range, we performed our TRLFS experiments with trace amounts of Cm(III) (3×10^{-7} M). The concentration of threonine was varied between 0.001 and 0.25 M in the pH range of 2 to 9.6 using 0.5 M NaCl as background electrolyte. Lower concentrations were applied in the case of phosphothreonine varying from 1×10^{-4} to 0.025 M in the pH range 2.8 to 7.6 using 0.154 M NaCl as background electrolyte.

2. Experimental

2.1. *Solutions and reagents*

The stock solutions of threonine (Carl Roth GmbH + Co. KG, Germany) and phosphothreonine (Sigma-Aldrich Chemie GmbH, Germany) were prepared freshly for each experiment. A stock solution of the long-lived curium isotope Cm-248 ($t_{1/2} = 3.4 \times 10^5$ years) was used. This solution had the following composition 97.3% Cm-248, 2.6% Cm-246, 0.04% Cm-245, 0.02% Cm-247, and 0.009% Cm-244 in 1.0 M $HClO_4$. The experiments were performed in a glove box under N_2 atmosphere at 25°C. As background electrolytes 0.154 M NaCl for phosphothreonine and 0.5 M NaCl for threonine were used. Carbonate free water and NaOH solution were used to avoid

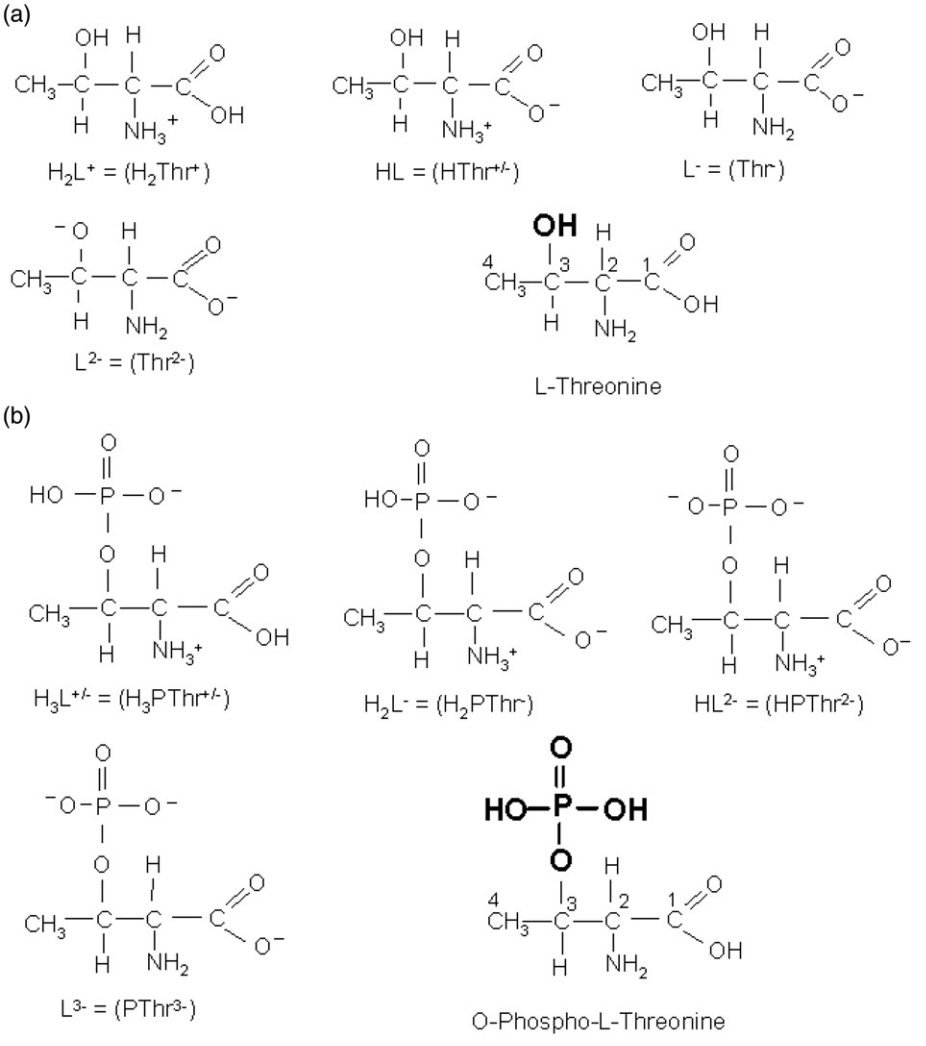

Figure 1. Structures of the amino acids (a) threonine and (b) phosphothreonine investigated. The structures of the corresponding protolysis species are also shown.

carbonate complexation of Cm(III). The Cm(III) concentration was fixed to 3×10^{-7} M in all TRLFS measurements. The ligand concentrations were varied between 1×10^{-4} and 0.25 M. The pH was changed between 2 and 10 by adding analytical grade NaOH (carbonate-free) or $HClO_4$. The pH was measured using a glass electrode (type: InLab427, Mettler-Toledo GmbH, Germany) calibrated in H^+ concentration units.

2.2. *TRLFS measurements*

The time-resolved laser-induced fluorescence spectra were recorded at 25°C using a flash lamp pumped Ti:sapphire laser (Elight, Titania). Details on the experimental

set-up are summarized [9]. For an optimal excitation of the Cm^{3+} luminescence in aqueous solution an excitation wavelength of 395 nm, close to its *F* absorption band, was used with a laser energy of less than 1 mJ controlled by a photodiode. The luminescence spectra were detected by an optical multi-channel analyzer. The system consists of a monochromator and spectrograph (Oriel; MS 257) with a 300 or 1200 lines mm^{-1} grating and an ICCD camera (Andor). The Cm(III) emission spectra were recorded in the 500–700 nm (300 lines mm^{-1} grating) and 570–650 nm (1200 lines mm^{-1} grating) ranges, respectively. A constant time window of 1 ms was used. For time dependent emission decay measurements, the delay time between laser pulse and camera grating was scanned with time intervals between 10 and 20 μs. The TRLFS spectra were measured after an equilibration time of 0.5 h.

In the Cm(III)–threonine system, four series of experiments were performed. In three series, we investigated the complex formation at the pH values of 3.6, 6.5 and 8.7 under variation of [Thr] between 0.001 and 0.25 M. In the fourth series of samples, the pH was changed between 3.1 and 9.4 at a fixed threonine concentration of 0.01 M. Five series of experiments were performed in the Cm(III)–phosphothreonine system. In three series, we investigated the complex formation at the pH values of 2.9, 3.5 and 6.5 under variation of [PThr] between 1×10^{-5} and 0.025 M. In the other two series of samples, the pH was changed between 1.5 and 8.1 at fixed phosphothreonine concentrations of 3×10^{-6} M and 3×10^{-4} M.

2.3. *Evaluation of the luminescence spectra*

The spectra were base-line corrected and normalized using the ORIGIN 6.1G (OriginLab Corporation, USA) code.

Factor analysis of spectroscopic data is a powerful tool for the determination of the number of independent absorbing/emitting species in a series of mixtures. The approach of the factor analysis program SPECFIT [10] to analyze, e.g. TRLFS spectra, is a quantitative decomposition of the spectra of mixtures into different spectral components/constituents. Due to the spectroscopic properties of each individual chemical species, the measured spectra showed variations depending on the physicochemical parameter (e.g. pH and ligand concentration) varied. These spectral variations observed in both systems are used to determine the spectra of the single components and their concentration distribution depending on the physicochemical parameter varied. SPECFIT calculates besides the determination of the independent components and their relative species distribution curves, with the aid of some chemical reasoning, reasonable equilibrium constants. As a consequence, the single component spectra derived from SPECFIT can be attributed to the formed species. We demonstrated the successful application of SPECFIT to describe the complexation of Cm^{3+} with adenosine 5′-triphosphate by using TRLFS data [11]. Input parameters for the data fitting were the known total concentrations of Cm^{3+}, threonine and/or phosphothreonine, the pH of each sample, and the protonation constants (a) for threonine, $\log K_{H_2Thr^+} = 11.17$ and $\log K_{HThr} = 8.89$ determined at an ionic strength of 0.5 M $NaClO_4$ [7] and (b) for phosphothreonine, $\log K_{H_3PThr} = 17.75$, $\log K_{H_2PThr^-} = 15.50$, and $\log K_{HPThr^{2-}} = 9.67$ determined at an ionic strength of 0.2 M KNO_3 [12]. Furthermore, the known luminescence spectrum of the Cm^{3+} aquo ion was used in the SPECFIT calculations.

3. Results and discussion

3.1. *Cm(III) and L-threonine*

TRLFS provides high selectivity to distinguish between various Cm(III) complexes in one sample [1, 9, 11, 15–17, 20, 21, 24]. In aqueous solution the emission bands of inner-sphere complexes of Cm(III) with inorganic and organic ligands are red-shifted compared to the Cm^{3+} ion. An overview of the luminescence spectra of 3×10^{-7} M Cm(III) in 0.5 M NaCl measured in the threonine system showing the spectra at pH 8.7 under variation of the ligand concentration is given in figure 2(a). In the more acidic pH region at pH 3.6, a complex formation between Cm(III) and threonine was detected at

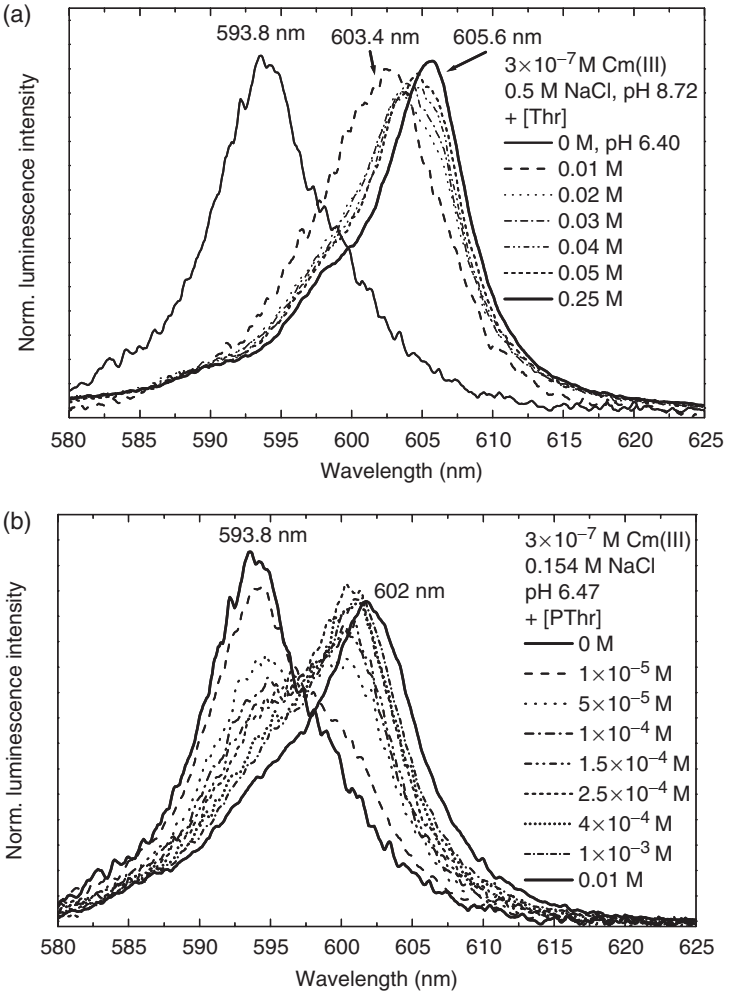

Figure 2. Luminescence spectra of 3×10^{-7} M Cm(III) measured: (a) in the L-threonine system as a function of the threonine concentration at pH 8.7 in 0.5 M NaCl; and (b) in the *O*-phospho-L-threonine system as a function of the phosphothreonine concentration at pH 6.5 in 0.154 M NaCl. The spectra are scaled to the same peak area (not all spectra shown).

ligand concentrations above 0.02 M (see figure S1). The Cm^{3+} aquo ion is characterized by an emission band maximum at 593.8 nm whereas the emission maximum close to 600 nm shown in figure S1 points to the formation of a Cm(III)–threonine species. At threonine concentrations, $\geq$0.1 M, the shoulder at 603.8 nm indicates the occurrence of a further complex. At a large ligand excess and alkaline pH values as depicted in figure 2(a) two species having emission maxima at 603.4 nm and 606 nm were detected. The observed red-shifted emission maxima of 600, 603 and 606 nm compared to Cm^{3+} are the result of changes in the ligand field of the Cm(III) ion due to the formation of different complexes. A speciation calculation using the program SOLGASWATER [13] and taking the protonation constants of threonine [7] showed that in the pH range from 2 to 8 the zwitterion (see figure 1), $HThr^{+/-}$, dominates the species distribution. A very low tendency of Cm to interact with the zwitterion of threonine was detected. Pronounced shifts in the emission bands were detected at larger ligand concentrations and pH values above 6. This correlates with increasing amounts of the anionic form of threonine, Thr^- (see figure 1), implying the formation of Cm–threonine species with the anionic form of the ligand and therefore an influence of Cm^{3+} on the protolysis equilibria of threonine. That the protolysis equilibrium of amino acids can be influenced by metal ions, meaning that deprotonation reactions can occur at lower pH values, was described by Szabó and Grenthe for the uranyl–glycine system [14]. If no threonine is present in the system, speciation calculations using the formation constants for the Cm(III) hydrolysis species [15] showed that 40% of the Cm(III) exists as $Cm(OH)^{2+}$ at pH 8.0. The formation of Cm(III) hydroxo species is connected with a strong decrease in fluorescence intensity. From the the fluorescence emission spectra shown in figure 2(a), we conclude that hydrolysis plays no role in the reaction mechanism.

The spectral changes detected were used in the factor analysis program SPECFIT [10] in order to describe the complex formation reactions occurring in the Cm(III)–threonine system. All data sets (43 individual spectra) were taken for the SPECFIT calculations. In evaluation of relevant complexation studies of threonine with actinides and lanthanides [7] and taking into consideration the deprotonation of the threonine molecule, possible Cm–threonine species of the type $M_pH_qL_r$ were introduced in the data analysis procedure. As a result, we developed a chemical model describing the ongoing processes in the Cm(III)–threonine system. The variations observed in the luminescence data (see figure 2a and S1) could be described by the following equilibria:

$$Cm^{3+} + Thr^- \leftrightarrow CmThr^{2+} \quad (1)$$

$$Cm^{3+} + 2Thr^- \leftrightarrow CmThr_2^+ \quad (2)$$

$$Cm^{3+} + 2Thr^{2-} \leftrightarrow CmH_{-2}Thr_2^- + 2H^+. \quad (3)$$

Formation constants for reactions (1)–(3) were calculated to be $\log ß_{101} = 6.72 \pm 0.07$, $\log ß_{102} = 10.22 \pm 0.09$ and $\log ß_{1-22} = -(7.22 \pm 0.19)$, respectively. The corresponding single component spectra of the individual species are summarized in figure 3(a).

Luminescence lifetime measurements provide information on the composition of the first coordination sphere of Cm(III) [16]. In a recent review [17], summarizing the optical properties of Cm(III) in crystals and solutions, the conclusion was drawn that the luminescence lifetime is sensitive to the number and the nature of the ligands in the first coordination shell in solution and solid complexes. In analogy to Eu and Tb [18],

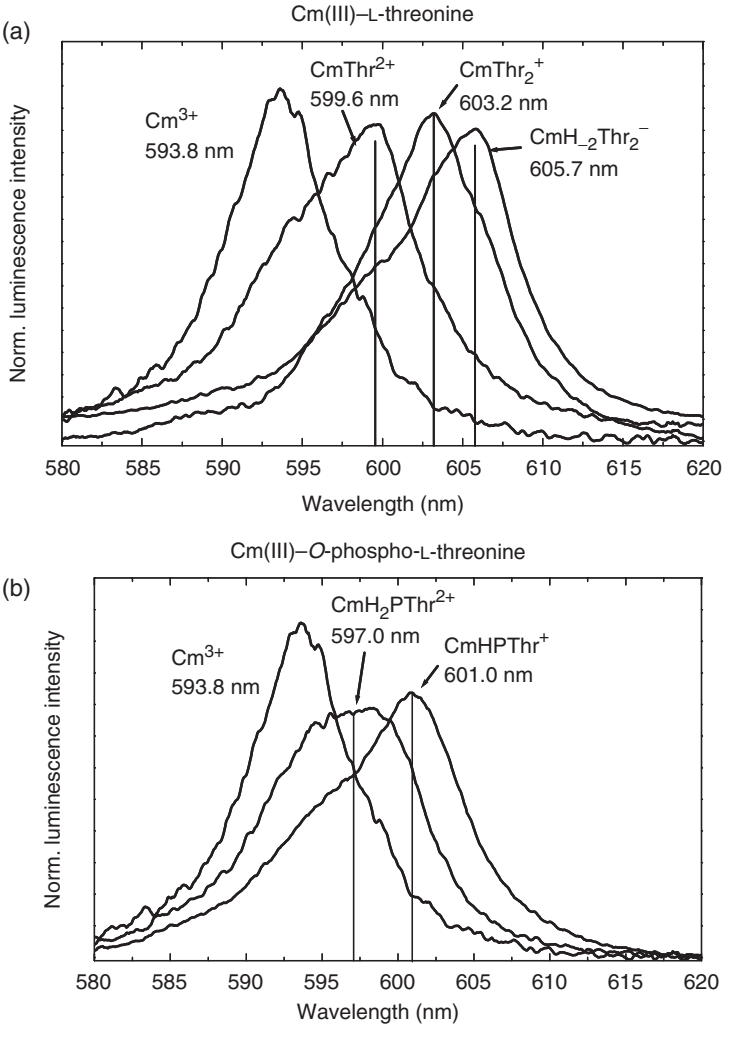

Figure 3. Luminescence spectra of the single components (a) in the Cm–L-threonine-system, and (b) in the Cm–*O*-phospho-L-threonine-system as derived by peak deconvolution using SPECFIT; the spectra are scaled to the same peak area.

a relationship between the lifetime and the hydration number of Cm in liquid and solid phases was established by Kimura and Choppin in 1994 [19]. Luminescence decay measurements of Cm in D_2O–H_2O solutions showed a linear correlation between the decay rate and the number of H_2O molecules in the first coordination sphere of Cm(III). An empirical relation for the hydration number q, the number of H_2O molecules in the first coordination shell, was derived:

$$q = 0.65\, k_{\mathrm{obs}}(\mathrm{Cm(III)}) - 0.88 \tag{4}$$

with a correlation coefficient of 0.9938 and an estimated uncertainty of $q = \pm 0.5$. The quenching behavior of Cm in the D_2O–H_2O system is due mainly to energy transfer from the excited state to OH vibrators of the H_2O molecules bound to the metal [19].

The validity of equation (4) was verified (a) by measurements of Cm doped compounds, and (b) by complexation studies of Cm with polyaminopolycarboxylate ligands (e.g. EDTA) in aqueous solution [19]. The application of equation (4) for selected fluorescent ions in aqueous systems has been discussed [17]. By considering all possible problems, Edelstein *et al*. [17] considered the relation found by Kimura and Choppin [19] as the best available and equation (4) was used [17] as in this study to calculate curium hydration numbers. Increasing lifetimes of Cm(III) species reflect the exclusion of water molecules from the first coordination sphere due to complex formation as found in both systems. $68 \pm 3\,\mu s$ [20–22] measured for the Cm(III) aquo ion, corresponds to nine water molecules, and a value of $1300\,\mu s$ corresponds to zero water molecules in the first coordination sphere of Cm(III) [23]. Furthermore the time variation of the luminescence decay contains information about the kinetics of the complex formation reactions [16, 17]. A mono-exponential luminescence decay with an average lifetime of the species in equilibrium is expected, if the rate of ligand exchange is high compared to the lifetime of the excited Cm(III). If the ligand exchange rate is low in comparison to the fluorescence decay rate of the excited Cm(III), we expect at least a bi-exponential decay. In all samples of the Cm–threonine system, a mono-exponential luminescence decay behavior was measured indicating a high ligand exchange rate of the species in equilibrium compared to the lifetime of the excited Cm(III). In test solutions with dominant amounts of the complexes $CmThr^{2+}$, $CmThr_2^+$ and $CmH_{-2}Thr_2^-$, increasing lifetimes of 76 ± 4, 104 ± 5 and $180 \pm 5\,\mu s$, respectively, were measured (see table 1). By using equation (4), the Kimura and Choppin relation [19], the number of coordinated water molecules were calculated to be: $CmThr^{2+}$, 7.8; $CmThr_2^+$, 5.4; and $CmH_{-2}Thr_2^-$, 2.8. Estimates concerning the structure of the formed species can be made using these values. Eight remaining water molecules in the 1 : 1 complex suggest a monodentate coordination of the anion, Thr^-, via one oxygen atom of the carboxylate group. The larger red shift of the emission maximum of the 1 : 1

Table 1. Luminescence properties of Cm(III) in selected systems determined in this study.

Species/System	Medium	Emission (nm)	Number of coordinated waters[1]	Lifetime (μs)
Cm^{3+}	0.154 M NaCl pH 6.5	593.8	9.0	66
L-Threonine				
88% $CmThr^{2+}$	0.5 M NaCl 0.01 M Thr pH 6.54	599.6	7.8	76
75% $CmThr_2^+$	0.5 M NaCl 0.01 M Thr pH 8.00	603.2	5.4	104
100% $CmH_{-2}Thr_2^-$	0.5 M NaCl 0.25 M Thr pH 9.68	605.7	2.8	180
O-Phospho-L-threonine				
80% CmH_2PThr^{2+}	0.154 M NaCl 0.025 M PThr pH 2.86	597.0	7.6	77
70% $CmHPThr^+$	0.154 M NaCl 0.01 M PThr pH 6.52	601.0	7.0	83

[1]According to the Kimura and Choppin equation [19].

complex, 3.6 nm, compared to the corresponding L2-aminobutyrate complex [24] and also an increased value of the formation constant might result in the assumption that an interaction of the $-NH_2$ group with the Cm center stabilizes the 1 : 1 threonine complex. In the 1 : 2 complex where five remaining water molecules were detected, the two threonine anions are most likely coordinated via one oxygen atom of the carboxylate group and the nitrogen atom of the $-NH_2$ group in a 'glycine-like' chelate ring. A coordination of three threonine molecules in the third complex seems to be unlikely due to steric reasons. The large red shift of the emission band, 11.9 nm, compared to the Cm aquo ion in combination with the increased fluorescence lifetime, 180 μs, points to formation of a 1 : 2 chelate complex with involvement of the deprotonated side-chain hydroxyl group. The structure can be described by formation of a five-membered chelate ring in which the donors are the N (amino) and O (deprotonated hydroxyl group) atoms. The $-COO^-$ groups come near to an axial position and also interact with the Cm center. The resulting number of remaining water molecules would be three, in excellent agreement with the measured value of 2.8. The high values of the complex formation constants are a strong argument for formation of chelate complexes in the Cm(III)–threonine system.

3.2. *Cm(III) and* O-*phospho-*L-*threonine*

In figure 2(b) the luminescence spectra obtained at the fixed pH-value 6.5 under variation of the phosphothreonine concentration are shown. In contrast to the threonine system, we obtained changes in the luminescence spectra due to complex formation reactions already at a pH of 2.9 (figure S2). One dominating species characterized by a luminescence maximum at 599 nm is formed in the phosphothreonine concentration range between 1×10^{-4} and 0.025 M at pH 2.9. By changing the pH to 6.5 an increased influence of a second species could be detected (figure 2b). The main emission band is shifted to 602 nm. The influence of two Cm(III)–phosphothreonine species was also detected in the pH dependence of the luminescence signal measured at a fixed [PThr] of 3×10^{-4} M (data not shown). In contrast to the threonine system, the interaction between Cm(III) and phosphothreonine species were obtained at lower ligand concentrations, e.g. 3×10^{-6} M in the pH range ≥ 5.5 (data not shown). Phosphothreonine occurs as a monoanion, H_2PThr^-, and dianion, $HPThr^{2-}$, in aqueous solution in the pH range 2 to 8 (see figure 1). The outcome of the TRLFS measurements suggests complexation of Cm(III) with these phosphothreonine species.

We conclude from the dependencies observed in the fluorescence emission spectra and from a stronger complexation of Cm(III) with phosphothreonine compared to threonine that hydrolysis plays no role in the reaction mechanism.

The spectral changes measured were again used in the factor analysis program SPECFIT [10] in order to describe the complex formation reactions in the Cm(III)–phosphothreonine system. All data sets, 44 individual spectra, were used for the SPECFIT calculations. By using relevant complexation studies of phosphothreonine with actinides [8] and other metals [7] and taking into consideration the deprotonation of the phosphothreonine molecule, possible Cm–phosphothreonine species of the type $M_pH_qL_r$ were introduced in the data analysis procedure. As a result, a chemical model describing the ongoing processes in the Cm(III)–phosphothreonine system could be developed. In all cases 1 : 1 phosphothreonine species were detected.

The dependencies observed in the luminescence data (see figure 2b and S2) could be summarized by the following equilibria:

$$Cm^{3+} + 2H^+ + PThr^{3-} \leftrightarrow CmH_2PThr^{2+} \quad (5)$$

$$Cm^{3+} + H^+ + PThr^{3-} \leftrightarrow CmHPThr^{+}. \quad (6)$$

Formation constants for reactions (5) and (6) were calculated to be $\log \beta_{121} = 18.03 \pm 0.13$ and $\log \beta_{111} = 14.17 \pm 0.09$, respectively. The corresponding single component spectra of the individual species are summarized in figure 3(b).

In all samples of the Cm–phosphothreonine system, a mono-exponential luminescence decay behavior was measured indicating a high ligand exchange rate of the species in equilibrium compared to the lifetime of the excited Cm(III). In test solutions with dominant amounts of CmH_2PThr^{2+} and $CmHPThr^{+}$, lifetimes of 77 ± 1 and 83 ± 1 μs, respectively, were measured (see table 1). The number of the remaining water molecules in the first coordination sphere of Cm(III) was calculated to be CmH_2PThr^{2+}, 7.6; and $CmHPThr^{+}$, 7.0; by applying equation (4). These low values of displaced water molecules points to predominant 1 : 1 species. Eight remaining water molecules in the 1 : 2 : 1 complex suggest a monodentate coordination of the anion, H_2PThr^{-}, via one oxygen atom of the phosphate or the carboxylate group. In the 1 : 1 : 1 complex where seven remaining water molecules were detected, two possible structures can be postulated. In the first Cm^{3+} is coordinated in a bidentate fashion to the two oxygens of the phosphate group. On the other hand, the species $CmHPThr^{+}$ exhibits the largest red shift of the emission band, 601 nm, for a 1 : 1 complex in this study (see table 1). This red shift of the luminescence could indicate formation of a seven-membered chelate ring. In this structure, Cm(III) binds to one oxygen from the phosphate and one oxygen from the carboxylate group.

4. Summary

This study shows that TRLFS, in combination with the factor analysis program code SPECFIT, is a powerful tool to investigate the coordination chemistry of curium at trace concentrations with amino acids of different functionalities in aqueous solution. The luminescence measurements indicated differences between the luminescence properties of the aqueous species formed in the phosphothreonine and threonine systems. We demonstrated that, depending on the substituent on the C3 atom, the strength of complexation decreases from phosphothreonine to threonine (see figure 4). It can be concluded that the formation of curium species with the two ligands influences the speciation of curium ion at biologically important pH values between 4 and 7. From the luminescence maxima, the luminescence lifetimes, and the magnitude of the stability constants of the individual species (see tables 1 and 2, figure 3), conclusions could be made concerning the structure of the species formed in aqueous solution.

The strongest species were formed in the presence of a phosphate group located at the C3 atom as in phosphothreonine. The complex CmH_2PThr^{2+} is characterized by a luminescence maximum at 597 nm and a luminescence lifetime of 77 μs. These findings and the formation constant $\log \beta^*_{121} = 2.53$ (calculated by involving H_2PThr^{-}) indicate preferred coordination of Cm at the deprotonated oxygen atom of the phosphate moiety. For the species $CmHPThr^{+}$ the largest red shift of the emission maximum for a

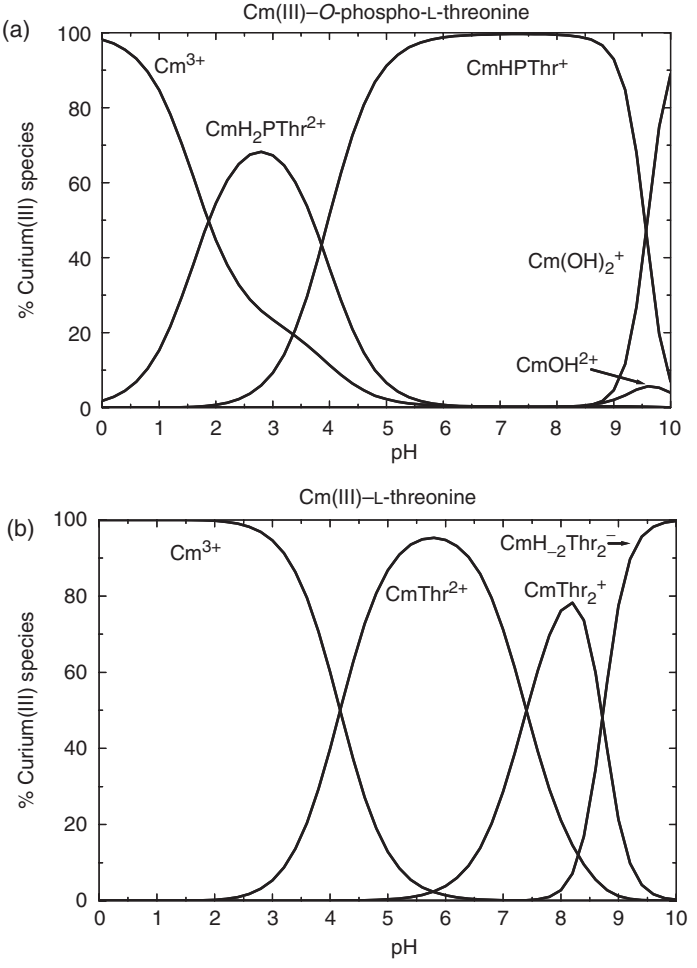

Figure 4. Calculated species distribution in aqueous solution at a curium concentration of 3×10^{-7} M and ligand concentrations of 0.01 M in (a) the phosphothreonine-, and (b) the threonine-system.

1 : 1 complex in this study of 7.2 nm compared to Cm^{3+} aquo ion was measured. This observation, the luminescence lifetime of 83 µs and the higher value of $\log \beta^*_{111} = 4.50$ (calculated by involving $HPThr^{2-}$) compared to the 1 : 2 : 1 complex points to formation of a chelate structure probably a seven-membered ring where the donors are the O from phosphate and the O from carboxylate. A comparison of our results with those of Günther *et al.* [8] shows that uranyl forms slightly stronger complexes with phosphothreonine (see table 2). This can be explained by the larger effective charge of +3.2 for the uranyl ion [25].

Three different Cm–threonine species could be identified by their individual luminescence properties. The 1 : 1 complex, $CmThr^{2+}$, is characterized by a luminescence maximum at 599.6 nm and a luminescence lifetime of 76 µs. Curium most likely coordinates monodentate to the oxygen atom of the carboxylate group. The larger formation constant and red shift of the luminescence compared with the aminobutyrate 1 : 1 complex [24] suggest an incipient interaction with the $–NH_2$ group.

Table 2. Summary of relevant stability constants.

Complex	Method	Log β	T (°C)	I (M)	Reference
H_2Thr^+	Potentiometry	11.17	35	0.5 ($NaClO_4$)	Berthon in [7]
HThr		8.89			
H_3PThr	Potentiometry	17.75	25	0.2 (KNO_3)	Mohan *et al.* [12]
H_2PThr^-		15.50			
$HPThr^{2-}$		9.67			
$LaThr^{2+}$	Potentiometry	3.55	25	0.25 (KCl)	Berthon in [7]
$LaThr_2^+$		6.40			
$GdThr^{2+}$	Potentiometry	3.10	20	0.1 (KCl)	Berthon in [7]
UO_2Thr^+	Potentiometry	6.22	25	0.25 (KCl)	Berthon in [7]
UO_2Thr_2		12.26			
UO_2HThr^{2+}	TRLFS	10.25 ± 0.25	22	0.5 ($NaClO_4$)	Günther *et al.* [8]
$UO_2(HThr)_2^{2+}$		19.94 ± 0.01			
$CmThr^{2+}$	TRLFS	6.72 ± 0.07	25 ± 2	0.5 (NaCl)	This work
$CmThr_2^+$		10.22 ± 0.09			
$CmH_{-2}Thr_2^-$		$-(7.22 \pm 0.19)$			
$UO_2H_3PThr^{2+}$	TRLFS	20.28 ± 0.11	22	0.1 ($NaClO_4$)	Günther *et al.* [8]
$UO_2H_2PThr^+$		18.94 ± 0.29			
UO_2HPThr		15.02 ± 0.23			
CmH_2PThr^{2+}	TRLFS	18.03 ± 0.13	25 ± 2	0.154 (NaCl)	This work
$CmHPThr^+$		14.17 ± 0.09			

The 1 : 2 complex, $CmThr_2^+$, exhibits a luminescence maximum at 603.2 nm with a luminescence lifetime of 104 µs. In combination with the magnitude of the complex formation constant, $\log ß_{102} = 10.22 \pm 0.09$, this shows that the two threonine molecules are chelated where the donors are the O from the carboxylate group and the N from the deprotonated amino group. The large red shift of the third complex, $CmH_{-2}Thr_2^-$, of 605.7 nm, the long luminescence lifetime of 180 µs, and the complex formation constant, $\log ß_{1-22} = -(7.22 \pm 0.19)$, point to formation of a stable chelate structure with involvement of the N from the $-NH_2$ groups, the O from the deprotonated –OH groups and in axial positions the O from the carboxylate groups. In contrast to the results of Günther *et al.* [8] where a complexation of uranyl with the zwitterion of threonine was observed in the acidic pH region, we found only a very weak tendency of Cm to interact with the zwitterionic form of threonine. As observed for ATP [11], Cm(III) forms stronger complexes with threonine compared to those formed with the lanthanide elements La(III) and Gd(III) which serve as homologues for curium (see table 2). One explanation could be the larger charge to radius ratio of Cm(III) [26].

The results of the present work contribute to a better understanding of the curium coordination chemistry with amino acids of different functionalities in aqueous solution. These complexation studies with selected bioligands are essential to explain the interaction processes of actinides in biological systems such as microbes and plants on a molecular level.

Supplementary material

Supplementary data associated with this article: figure S1: luminescence spectra of 3×10^{-7} M Cm(III) in 0.5 M NaCl solution measured in the L-threonine system as a

function of the threonine concentration at pH 3.6; the spectra are scaled to the same peak area (not all spectra shown); figure S2: luminescence spectra of 3×10^{-7} M Cm(III) in 0.154 M NaCl solution measured in the *O*-phospho-L-threonine system as a function of the phosphothreonine concentration at pH 2.9.

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References

[1] H. Moll, Th. Stumpf, M. Merroun, A. Roßberg, S. Selenska-Pobell, G. Bernhard. *Environ. Sci. Technol.*, **38**, 1455 (2004).
[2] M.L. Merroun, C. Hennig, A. Rossberg, T. Reich, S. Selenska-Pobell. *Radiochim. Acta*, **91**, 583 (2003).
[3] P.J. Panak, J. Raff, S. Selenska-Pobell, G. Geipel, G. Bernhard, H. Nitsche. *Radiochim. Acta*, **88**, 71 (2000).
[4] P.J. Panak, H. Nitsche. *Radiochim. Acta*, **89**, 499 (2001).
[5] A. Günther, G. Bernhard, G. Geipel, T. Reich, A. Roßberg, H. Nitsche. *Radiochim. Acta*, **91**, 319 (2003).
[6] M. Merroun, J. Raff, A. Rossberg, C. Hennig, T. Reich, S. Selenska-Pobell. *Environ. Microbiol.*, **71**, 5532 (2005).
[7] G. Berthon. *Pure Appl. Chem.*, **67**, 1117 (1995).
[8] A. Günther, G. Geipel, G. Bernhard, *Radiochim. Acta*. (2006) (Accepted).
[9] Th. Stumpf, Th. Fanghänel, I. Grenthe. *J. Chem. Soc., Dalton Trans.*, 3799 (2002).
[10] R.A. Binstead, A.D. Zuberbühler, B. Jung. *SPECFIT Global Analysis System*, Version 3.0.34 (2003).
[11] H. Moll, G. Geipel, G. Bernhard. *Inorg. Chim. Acta*, **358**, 2275 (2005).
[12] M.S. Mohan, E.H. Abbott. *Inorg. Chem.*, **17**, 2203 (1978).
[13] I. Puigdomenech. *Input, Sed and Predom: Computer Programs Drawing Equilibrium Diagrams*, Trita-OOK-3010, RIT, Stockholm (1983), Version 29 (2002).
[14] Z. Szabó, I. Grenthe. *Inorg. Chem.*, **39**, 5036 (2000).
[15] Th. Fanghänel, J.I. Kim, P. Paviet, R. Klenze, W. Hauser. *Radiochim. Acta*, **66/67**, 81 (1994).
[16] Th. Fanghänel, J.I. Kim. *J. Alloys Comp.*, **271/273**, 728 (1998).
[17] N.M. Edelstein, R. Klenze, Th. Fanghänel, S. Hubert. *Coord. Chem. Rev.*, **250**, 948 (2006).
[18] W.D. Horrocks Jr, D.R. Sudnick. *J. Am. Chem. Soc.*, **101**, 334 (1979).
[19] T. Kimura, G.R. Choppin. *J. Alloys Comp.*, **213/214**, 313 (1994).
[20] H. Wimmer, R. Klenze, J.I. Kim. *Radiochim. Acta*, **56**, 79 (1992).
[21] Th. Fanghänel, J.I. Kim, R. Klenze, J. Kato. *J. Alloys Comp.*, **225**, 308 (1995).
[22] J.V. Beitz, D.L. Bowers, M.M. Doxtader, V.A. Maroni, D.T. Reed. *Radiochim. Acta*, **44/45**, 87 (1988).
[23] W.T. Carnall, H.M. Crosswhite. Report ANL-84-90 (1995).
[24] H. Moll, G. Bernhard, *J. Radioanal. Nucl. Chem.* (2007) (In press).
[25] G.R. Choppin. *Radiochim. Acta*, **32**, 43 (1983).
[26] R.D. Shannon. *Acta Cryst.*, **A32**, 751 (1976).