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Curium(III) complexation with pyoverdins secreted by a groundwater strain of *Pseudomonas fluorescens*

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Abstract Pyoverdins, bacterial siderophores produced by ubiquitous fluorescent *Pseudomonas* species, have great potential to bind and thus transport actinides in the environment. Therefore, the influence of pyoverdins secreted by microbes on the migration processes of actinides must be taken into account in strategies for the risk assessment of potential nuclear waste disposal sites. The unknown interaction between curium(III) and the pyoverdins released by *Pseudomonas fluorescens* (CCUG 32456) isolated from the granitic rock aquifers at the Äspö Hard Rock Laboratory (Äspö HRL), Sweden, is the subject of this paper. The interaction between soluble species of curium(III) and pyoverdins was studied at trace curium(III) concentrations (3×10^{-7} M) using time-resolved laser-induced fluorescence spectroscopy (TRLFS). Three Cm^{3+}—*P. fluorescens* (CCUG 32456) pyoverdin species, $M_pH_qL_r$, could be identified from the fluorescence emission spectra, CmH_2L^+, CmHL, and CmL^-, having peak maxima at 601, 607, and 611 nm, respectively. The large formation constants, log $\beta_{121} = 32.50 \pm 0.06$, log $\beta_{111} = 27.40 \pm 0.11$, and log $\beta_{101} = 19.30 \pm 0.17$, compared to those of other chelating agents illustrate the unique complexation properties of pyoverdin-type siderophores. An indirect excitation mechanism for the curium(III) fluorescence was observed in the presence of the pyoverdin molecules.

Keywords Curium · Pyoverdin · Fluorescence spectroscopy · TRLFS · Complexation

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Introduction

Actinides have been and will be introduced into shallow and deep groundwater environments via various human activities. These activities include the injection of low- and intermediate-level radioactive waste into deep geological formations (Rumynin et al. 2005), underground atomic bomb testing (Kersting et al. 1999), leaching from mine waste (Kalinowski et al. 2004), and accidental leakage from current and future underground radioactive waste repositories (Pedersen 2005). The process of interaction between microbes and metals may influence the migration behavior of hazardous actinides in the environment once they have been released. Microorganisms can interact with actinides by both direct and

indirect pathways. An example of a direct interaction process is the biosorption of the trivalent actinide element curium by the sulfate-reducing bacterium *Desulfovibrio aespoeensis* (Moll et al. 2004). Indirect processes include the formation of complexes with bioligands secreted by various microorganisms.

Fluorescent *Pseudomonas* species are ubiquitous soil and groundwater bacteria that synthesize bacterial pyoverdin-type siderophores under iron-deficient conditions. For example, Powell et al. (1980) demonstrated the occurrence of hydroxamate siderophores produced by microorganisms in concentrations ranging from 10^{-7} to 10^{-8} M in a variety of soils. Later, pyoverdins excreted by a groundwater strain of *Pseudomonas fluorescens* were found to efficiently mobilize uranium and other trace elements from shale mine tailings (Kalinowski et al. 2004). The strain was isolated from groundwater from borehole KR0013, 70 m underground in the Äspö Hard Rock Laboratory tunnel (Pedersen 1997). The role of the exudates from this strain was further explored in solid–aqueous phase partitioning experiments using pico- to submicromolar amounts of ^{59}Fe, ^{147}Pm, ^{234}Th, and ^{241}Am in the presence of quartz sand (Johnsson et al. 2006). Relative to the control, aerobic solutions containing exudates maintained more than 50% of the added ^{59}Fe, ^{234}Th, and ^{241}Am in solution. The highest amount of metal present in the liquid phase of the anaerobic solutions was found in the case of ^{241}Am, with 40% more ^{241}Am being present in samples than in controls. The observed mobilization effects of the studied pyoverdins of this strain on radionuclides motivated detailed investigation of their complexation characteristics, using a radionuclide suitable for spectroscopic methods.

Therefore, this present focuses on how curium(III) interacts with the pyoverdins secreted by *P. fluorescens*. To understand the great potential of pyoverdins to complex metals, a brief description of their structure is first needed. Pyoverdin molecules (see also Fig. 1) comprise three parts (a) the chromophore (1S)-5-amino-2,3-dihydro-8,9-dihydroxy-1H-pyrimido[1,2-a]quinoline-1-carboxylic acid, (b) a peptide chain consisting of 6–12 mainly hydrophilic amino acids bound via its N-terminus to the carboxyl group of the chromophore, and (c) an acyl chain attached to the NH_2 group of the chromophore consisting of dicarboxylic acid residues, for example succinate or its amide form depending on the growth conditions (Budzikiewicz 2004; Meyer 2000; Schäfer et al. 1991). The functional groups that participate in the metal binding are the catechol group of the chromophore and two ligand sites in the peptide chain, i.e. one or two hydroxamate groups and one or two α-hydroxy acid moieties (see Fig. 1).

To the best of our knowledge, the characteristics of the complexation of pyoverdins with trivalent actinides (e.g., curium) are unknown. To address this lack, we thus present findings regarding the complexation of curium(III) with *P. fluorescens* (CCUG 32456) pyoverdins, obtained using time-resolved laser-induced fluorescence spectroscopy (TRLFS). The speciation experiments were performed at trace concentrations of curium(III) (3×10^{-7} M) by varying the pyoverdin concentration and pH of the test solutions. The different curium(III) pyoverdin complex species could be distinguished on the basis of

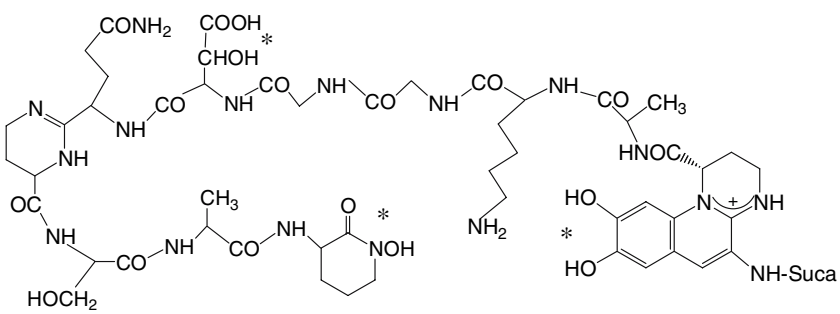

Fig. 1 Structure of the pyoverdin from *P. fluorescens* (CCUG 32456) with a succinamide (Suca) side chain (Suca-Chr-Ala-Lys-Gly-Gly-OHAsp-(Gln-Dab)-Ser-Ala-cOHOrn). Asterisks indicate the complexation sites. The amino acids Ala, Lys, and Gln (underlined) are D-configured

their individual fluorescence emission spectra. The obtained stability constants were compared to those of other chelating agents. The results of this study increase our understanding of the mobilization of actinides by pyoverdins secreted by resident bacteria in a natural environment.

Materials and methods

Bacteria, pyoverdin isolation, and characterization

P. fluorescens (CCUG 32456 A) were grown in batch cultures under aerobic conditions using the standard succinate medium (SSM) as described in (Meyer and Abdallah 1978) at room temperature. After one week, the cultures were pooled and the pooled material was centrifuged at 8000*g* for 10 min in a Sörvall RC-5B superspeed centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). To remove cells remaining after centrifuging, approximately 500 ml of the supernatant was then suction-filtered in a 0.2-μm pore size Filtropur BT 50 bottle top filter (Landskrona, Sweden) into a sterile bottle. The pH of the filtered supernatant was lowered to 6 using 1 M NaOH, and the solution was thereafter frozen at −18°C pending purification. The uncomplexed pyoverdin was purified from the filtered supernatant as outlined in (Meyer and Abdallah 1978). Briefly, the filtered supernatant was loaded onto XAD-4 Amberlite resin (Rohm and Haas, Philadelphia, PA, USA). The resin was then rinsed with Milli-Q water. Elution of the pyoverdin was achieved using 50% methanol; the methanol was evaporated, and the resulting pulverized pyoverdin mixtures were used for the characterization and complexation studies.

Mass spectrometry measurements demonstrated that *P. fluorescens* (CCUG 32456 A) secreted a pyoverdin mixture having the following main components: pyoverdin with a succinamide side chain (see Fig. 1), pyoverdin with a succinic acid side chain, ferribactin with a succinamide side chain, and a ferribactin with a glutamic acid side chain (the identification of a ferribactin is not unusual, because ferribactins are biogenetic precursors of pyoverdins). High-performance liquid chromatography (HPLC) analyses of the secreted bioligand mixture suggest also the presence of four chelators able to complex Fe(III) (Kalinowski et al. 2006). However, the results of an absorption spectroscopy study of the aqueous bioligand mixture demonstrated the dominant influence of the pyoverdins. From the pH-dependent absorption spectra, three pK values could be determined using the general approach that the pyoverdin molecule can liberate four labile protons, LH_4, from the complexing sites most likely responsible for metal binding (Bouby et al. 1998, 1999; Teintze et al. 1981). The following protonation constants were calculated: $\log \beta_{012} = 22.67 \pm 0.15$ ($pK_1 = 4.40$), $\log \beta_{013} = 29.15 \pm 0.05$ ($pK_2 = 6.48$), and $\log \beta_{014} = 33.55 \pm 0.05$ ($pK_3 = 10.47$). The excellent fluorescence properties of the pyoverdin mixture (due to the presence of the chromophore) after excitation with a monochromatic laser beam at 266 nm were investigated using time-resolved laser-induced fluorescence spectroscopy with ultrafast pulses (fs-TRLFS) (Moll et al. 2007). A broad emission spectrum with a maximum at 466 nm was observed between pH 4 and 9. The fluorescence of the pyoverdins did not interfere with the detection of curium(III) fluorescence because (a) the wavelength range was different, and (b) the pyoverdin fluorescence had already abated (fluorescence lifetimes below 6.5 ns) when we started to detect the curium(III) fluorescence.

Preparation of curium(III) pyoverdin solutions

A stock solution of the long-lived curium isotope ^{248}Cm ($t_{1/2} = 3.4 \times 10^5$ years) was used. This solution had the following composition: 97.3% ^{248}Cm, 2.6% ^{246}Cm, 0.04% ^{245}Cm, 0.02% ^{247}Cm, and 0.009% ^{244}Cm in 1.0 M $HClO_4$. The experiments were performed in a glove box under an N_2 atmosphere at 25°C. As a background electrolyte, analytical grade 0.1 M $NaClO_4$ (Merck, Darmstadt, Germany) was used. To prevent the carbonate complexation of curium(III), carbonate-free water and an NaOH solution were used. The curium(III) concentration was fixed at 3×10^{-7} M in all TRLFS measurements. The pH was measured using an InLab 427 combination pH puncture electrode (Mettler-Toledo, Giessen, Germany) calibrated in H^+ concentration units. The pH was changed by adding analytical grade NaOH or $HClO_4$. The pyoverdin concentration in the acidic LH_4 stock solutions was determined using the absorbance at the characteristic

pyoverdin absorption band at 380 nm and an averaged molar absorption coefficient of 20000 L $mol^{-1}cm^{-1}$ (Albrecht-Gary et al. 1994; Meyer and Abdallah 1978; Xiao and Kisaalita 1995).

Three series of experiments were performed to explore the complexation behavior of curium(III) with the isolated pyoverdin fraction. In the first run, we investigated the curium(III) complex formation by varying the pyoverdin concentration between 3×10^{-7} and 1×10^{-5} M at a fixed pH of 4.17; in the second and third runs, the pyoverdin concentrations were kept constant at 3×10^{-6} and 1×10^{-5} M, respectively, while varying the pH between 2.0 and 11.0.

Time-resolved laser-induced fluorescence spectroscopy (TRLFS): experimental setup

The time-resolved laser-induced fluorescence spectra were recorded at 25°C using a pulsed flash lamp pumped Nd:YAG-OPO laser system (Powerlite Precision II 9020 laser equipped with a Green PANTHER EX OPO from Continuum, Santa Clara, CA, USA). The optical parametrical oscillator (OPO) used to tune the wavelength of the emitted laser beam was pumped by the second harmonic oscillation of the Nd:YAG laser (532 nm). The doubled signal output wavelength can be varied between 330 and 500 nm. The laser pulse energy, which was between 1 and 5 mJ depending on the excitation wavelength used, was monitored using a photodiode. The fluorescence emission spectra were detected using an optical multi-channel analyzer-system, consisting of an Oriel MS 257 monochromator and spectrograph with a 300 or 1200 line mm^{-1} grating and an Andor iStar ICCD camera (Lot-Oriel Group, Darmstadt, Germany). The curium(III) emission spectra were recorded in the 500−700 nm (300 line mm^{-1} grating) and 570−650 nm (1200 line mm^{-1} grating) ranges. A constant time window of 1 ms was applied, and two excitation wavelengths of 360 and 395 nm were 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. To measure the excitation spectra of the main components, the excitation wavelength was scanned in the 340−450 nm range. The TRLFS spectra were measured after an equilibration time of 0.5 h.

Results and discussion

TRLFS is an established experimental technique for characterizing various curium(III) complexes with inorganic and organic ligands, based on quantifying the various metal species by deconvoluting the total emission spectra. In aqueous solution, the emission bands of inner-sphere complexes of curium(III) with organic ligands are generally red-shifted compared to the Cm^{3+} aquo ion. An overview of the emission spectra of 3×10^{-7} M curium(III) in 0.1 M $NaClO_4$ measured in the *P. fluorescens* (CCUG 32456) pyoverdin system is presented in Fig. 2. The complexation of curium(III) with these bioligands had started even at pH 4.2 and low pyoverdin concentrations of 3×10^{-7} M. This is depicted in Fig. 2A by the decreased emission band of the Cm^{3+} aquo ion at 593.8 nm and the formation of a shoulder at 602 nm. A pyoverdin concentration of 3×10^{-7} M lies in the

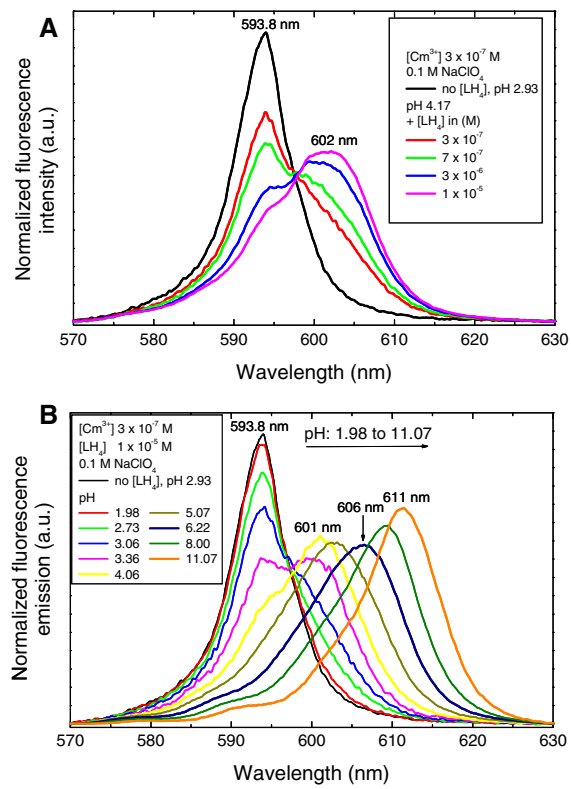

Fig. 2 Fluorescence emission spectra of 3×10^{-7} M curium(III) measured: (**A**) as a function of the pyoverdin concentration, LH_4, at pH 4.17 in 0.1 M $NaClO_4$; and (**B**) at a fixed pyoverdin concentration of $[LH_4]$ 1×10^{-5} M as a function of pH. The spectra are scaled to the same peak area

range of hydroxamate siderophores identified in a variety of different soils (Powell et al. 1980). Figure 2B presents the changes observed in the emission spectra at fixed concentrations of curium(III) and pyoverdin of 3×10^{-7} M and 1×10^{-5} M, respectively, as a function of pH. Three different complex species can be differentiated on the basis of their individual emission bands at 601, 606, and 611 nm.

The spectral changes detected were used in the SPECFIT factor analysis program (Binstead et al. 2004) to describe the complex formation reactions occurring in the Cm^{3+}—*P. fluorescens* (CCUG 32456) pyoverdin (LH_4) system. SPECFIT analyzes TRLFS spectra, for example, by quantitatively decomposing the spectra of mixtures into their various spectral components. Due to the spectroscopic properties of each chemical species, the measured spectra displayed variations depending on the physicochemical parameter (e.g., pH or ligand concentration) varied. These spectral variations are used to determine the spectra and concentration distributions of the individual components, depending on the physicochemical parameter varied. Besides determining the independent components and their relative species distribution curves, SPECFIT also calculates reasonable equilibrium constants. Consequently, the single-component spectra derived by SPECFIT can be attributed to the formed species. In the past, we have demonstrated the successful application of SPECFIT to exploring curium(III) speciation in a variety of organic systems (Moll et al. 2005; Moll and Bernhard 2007a, b). In the present study, all data-sets, i.e. 32 individual spectra, were used for the SPECFIT calculations; the input parameters for the data fitting were the known total concentrations of Cm^{3+} and LH_4, the pH of each sample, and the protonation constants of pyoverdin as determined in our previous study (Moll et al. 2007), i.e. log β_{021} = 22.67 ± 0.15, log β_{031} = 29.15 ± 0.05, and log β_{041} = 33.55 ± 0.05, and in Albrecht-Gary et al. (1994), i.e. log β_{011} = 12.2. As well, the known emission spectrum of the Cm^{3+} aquo ion was used in the SPECFIT calculations. In light of relevant complexation studies of pyoverdin-type bioligands with metals (Albrecht-Gary et al. 1991, 1994; Bouby et al. 1998, 1999), and taking into consideration the deprotonation of the pyoverdin molecule, possible curium(III)—pyoverdin species of the $M_pH_qL_r$ type were introduced into the data analysis procedure. As a result, we were able to develop a chemical model describing the ongoing processes in the Cm^{3+}—*P. fluorescens* (CCUG 32456) pyoverdin system. The variations observed in the emission data (see Fig. 2) could be described by the following equilibria:

$$Cm^{3+} + 2H^{+} + L^{4-} \leftrightarrow CmH_2L^{+} \quad (1)$$

$$Cm^{3+} + H^{+} + L^{4-} \leftrightarrow CmHL \quad (2)$$

$$Cm^{3+} + L^{4-} \leftrightarrow CmL^{-} \quad (3)$$

Formation constants for reactions (1)–(3) were calculated to be log β_{121} = 32.50 ± 0.06, log β_{111} = 27.40 ± 0.11, and log β_{101} = 19.30 ± 0.17, respectively (see Table 1). The corresponding single-component spectra of the individual species are summarized in Fig. 3. These results indicate that

Table 1 Summary of the relevant stability constants of metal bioligand species in comparison to the Cm^{3+}—*P. fluorescens* (CCUG 32456) pyoverdin constants determined in this study

Species/$M_pH_qL_r$	Bacteria/chelating agent	Methods	Ionic strength (M)	log β	Reference
Cm(III)H_2L^{+}/121	*P. fluorescens* (CCUG 32456 A)/ Pyoverdin mixture	Fluorescence spectroscopy	0.1 ($NaClO_4$)	32.50 ± 0.06	This work
Cm(III)HL/111				27.40 ± 0.11	
Cm(III)L^{-}/101				19.30 ± 0.17	
Fe(III)H_2L^{+}/121	*P. aeruginosa* (ATCC 15692)/ Pyoverdin PaA	Spectroscopy & Cyclic voltammetry	0.1 ($NaClO_4$)	47.80 ± 0.20	Albrecht-Gary et al. 1994
Fe(III)HL/111				43.00 ± 0.30	
Fe(III)L^{-}/101				30.80 ± 0.30	
Eu(III)$HDFO^{+}$/ 111	Desferrioxamine B (DFO)	Potentiometry		26.18[a]	Brainard et al. 1992

[a] Recalculated in this study

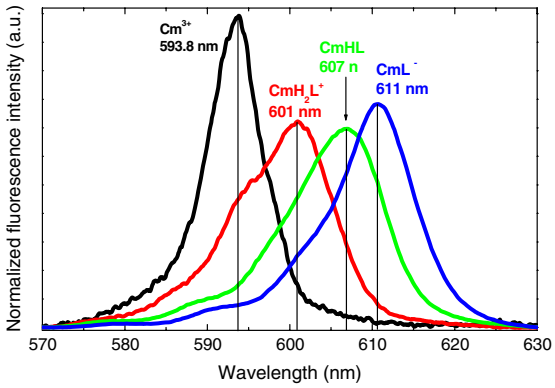

Fig. 3 Fluorescence emission spectra of the single components in the Cm^{3+}—*P. fluorescens* (CCUG 32456) pyoverdin—system, as derived by peak deconvolution using SPECFIT. The spectra are scaled to the same peak area

P. fluorescens (CCUG 32456) pyoverdins form strong 1:1 complexes with curium(III). No published data exist for curium(III), to provide a basis for comparison. Since pyoverdins are chelating agents synthesized by fluorescent *Pseudomonas* spp. to provide the cells with the essential iron(III), it is not surprising that the corresponding formation constants are the largest, as shown in Table 1. Furthermore, Table 1 indicates that pyoverdins are also able to complex elements other than Fe(III) at a considerably high efficiency. Due to the lack of data describing the complexation of trivalent actinides with pyoverdin-type siderophores, the presented results are compared to those obtained for europium(III) with desferrioxamine B; this is a commercially available microbially produced trihydroxamate siderophore with good actinide-binding properties (Jarvis and Hancock 1991; Whisenhunt et al. 1996; Neu et al. 2000). The Cm^{3+}—pyoverdin complexation constant, log β_{111}, is comparable to those of the europium(III) 1:1 complex with desferrioxamine B (Brainard et al. 1992), which emphasizes the good actinide-binding properties of the investigated pyoverdins. The complexation of curium(III) with *P. fluorescens* (CCUG 32456) pyoverdins is stronger than the complexation with EDTA (log β_{101} = 18.41; Choppin et al. 2006), hydroxide (log β_{101} = 6.8 ± 0.5; Edelstein et al. 2006), or carbonate (log β_{101} = 8.1 ± 0.3; Edelstein et al. 2006).

The spectroscopic properties of the curium(III) complexes characterized in this study are summarized in Table 2. The emission peak maximum is shifted from 593.8 nm for the Cm^{3+} aquo ion to

Table 2 Spectroscopic properties of the identified curium(III) species

	Cm^{3+} (aq)	CmH_2L^+ [a]	$CmHL$ [b]	CmL^- [c]
Excitation (nm)		372.6 (5.4)	373[e]	366 (25)
	374.4 (3.6)[d]	377.8 (4.6)	385[f]	377[e]
	379.9 (2.9)	386.0 (7.6)	389[f]	386 (4.8)
	396.0 (2.2)	395.4 (8.4)	395[f]	394[f]
			400[f]	
Emission (nm)	593.8 (8.2)[d]	593.7[e]	602.5[e]	605.3[e]
		602.2 (11.6)	608.4 (7.9)	610.8 (6.9)
				614.6[e]
Lifetime (μs)	68 ± 1	86 ± 2 (80%)	83 ± 2 (64%)	100 ± 5 (71%)
		198 ± 10 (20%)	229 ± 5 (36%)	330 ± 10 (29%)
$I_{rel}(395)$	1	3.36	19.63	8.10
$I_{rel}(360)$	1	197.2	1112.7	575.3

[a] In 1×10^{-5} M LH_4 at pH 4.17 (88% CmH_2L^+ and 12% CmHL)

[b] In 3×10^{-6} M LH_4 at pH 7.00 (98% CmHL and 2% CmH_2L^+)

[c] In 1×10^{-5} M LH_4 at pH 11.07 (98% CmL^- and 2% CmHL)

[d] Values in parentheses are full width at half-maximum

[e] Shoulder

[f] Broad, poorly resolved bands

601, 606, and 611 nm when curium(III) occurs in the three identified 1:1 pyoverdin complexes (see also Fig. 2). Simultaneously, the emission intensity is increased by factors of 3.36, 19.63, and 8.10 for the three pyoverdin complexes, respectively. In agreement with previous findings regarding the absorption spectrum of the Cm^{3+} aquo ion (Carnall and Rajnak 1975), we found that the emission intensity of this species decreased by a factor of 65 when the excitation wavelength was changed from 395 to 360 nm. Under the same conditions, the intensities of the CmH_2L^+ and the CmHL complexes decreased by factors of only 1.12 and 1.16, respectively, whereas the intensity of the CmL^- complex increased by a factor of 1.08. At an excitation wavelength of 360 nm, the intensities of these complexes relative to that of the Cm^{3+} aquo ion are 197, 1113, and 575, respectively. This indicates that fluorescence emission of the Cm^{3+} pyoverdin species can be generated either by direct excitation of the metal ion or by indirect excitation of the ligand followed by energy transfer from the ligand molecule to the metal ion. The excitation spectra of the identified curium(III) complexes are summarized in Fig. 4. The above effects are reflected in the corresponding excitation spectra. Instead of sharp, well-resolved absorption bands like those observed for the Cm^{3+} aquo ion and intense transitions to the H-, G-, and F-states, only broad and poorly resolved absorption bands were measured for the Cm^{3+} pyoverdin species. This behavior indicates the coordination of curium(III) to organic molecules containing aromatic entities (Panak et al. 1995), which could be characterized in the pyoverdins, i.e. pyoverdin-chromophore: (1S)-5-amino-2,3-dihydro-8,9-dihydroxy-1H-pyrimido[1,2-a]quinoline-1-carboxylic acid, as shown in Fig. 1. Flat and structurally poor excitation spectra have previously been reported, for example for Cm^{3+} humate/fulvate complexes (Panak et al. 1995) and Cm^{3+} humates sorbed onto γ-Al_2O_3 (Wang et al. 2004). We tested the idea of selectively exciting the Cm^{3+} aquo ion and the Cm^{3+} pyoverdin species using direct and indirect excitation modes. In the pH 2−4 range, the Cm^{3+} aquo ion coexists with the first 1:1 pyoverdin complex, CmH_2L^+. Irradiation of the test solution with an excitation wavelength of 360 nm induces only a very weak measurable fluorescence of the Cm^{3+} aquo ion (see Fig. 5). When complexation with the pyoverdins starts, energy transfer from excited electronic or vibronic states of the pyoverdin molecule to curium(III) is enabled and fluorescence spectra are obtained. Unlike the results reported for the Cm^{3+}—humic acid—γ-Al_2O_3 system (Wang et al. 2004), we observed emission spectra that are almost congruent and are independent of the excitation mode over the whole pH range investigated

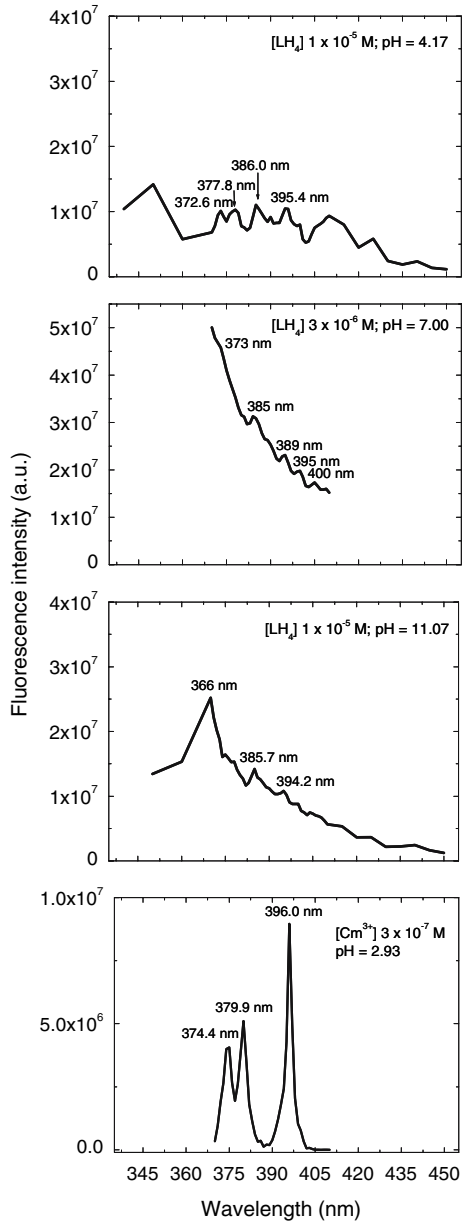

Fig. 4 Excitation spectra of curium(III) measured in the *P. fluorescens* (CCUG 32456) pyoverdin system

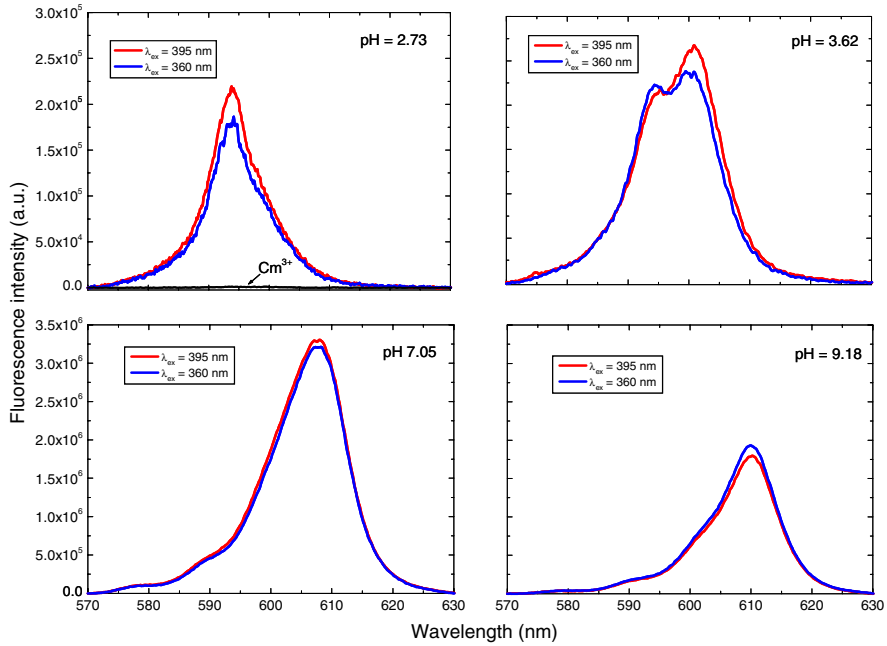

Fig. 5 Comparison of fluorescence emission spectra of curium(III) in aqueous *P. fluorescens* (CCUG 32456) pyoverdin solutions measured at excitation wavelengths of 360 (indirect excitation mode) and 395 nm (direct excitation mode); $[Cm^{3+}]$ 3×10^{-7} M, $[LH_4]$ 1×10^{-5} M, 0.1 M $NaClO_4$

(see Fig. 5). It follows that the unique fluorescence properties of the pyoverdin polyelectrolyte likely inhibit the spectroscopic differentiation of non-pyoverdin-bound and pyoverdin-bound curium(III).

Fluorescence lifetime measurements of curium(III) aqueous species are frequently used to obtain information on the composition of the first coordination sphere and on the kinetics of the complex formation reactions. A linear correlation between the decay rate and the number of H_2O molecules in the first coordination sphere of curium(III) was found by Kimura and Choppin (1994). Our finding of 68 ± 1 μs measured for the Cm^{3+} aquo ion corresponds to nine water molecules, while the value of 1370 μs measured in D_2O and reported in Lindqvist-Reis et al. (2005) corresponds to zero water molecules in the first coordination sphere of curium(III). This linear relation-ship might be inapplicable when separate quench mechanisms, operating via the organic ligand, are interfering. In the pyoverdin mixture under investigation, we cannot exclude such processes in the pyoverdin molecule or between the other constituents of the mixture; therefore, the fluorescence decay behavior is used for comparison (see Fig. 6 and Table 2) and not to draw definite conclusions concerning the structure of the formed species. In all samples in which the Cm^{3+} aquo ion and the first pyoverdin complex, CmH_2L^+, are present, a mono-exponential decay was measured with an average lifetime of 83 μs; this lifetime could correspond to the CmH_2L^+ species. In all samples with pH values above 3.4 and $[LH_4]$ of 3×10^{-6} and 1×10^{-5} M, bi-exponential decay was always detected with average lifetimes of 83 and 210 μs. The latter might correspond to the second pyoverdin species, CmHL. At pH values above 10, the second lifetime increased to 340 μs, indicating the formation of a third complex, CmL^-. This suggests a low ligand exchange rate for the pyoverdin complexes, compared

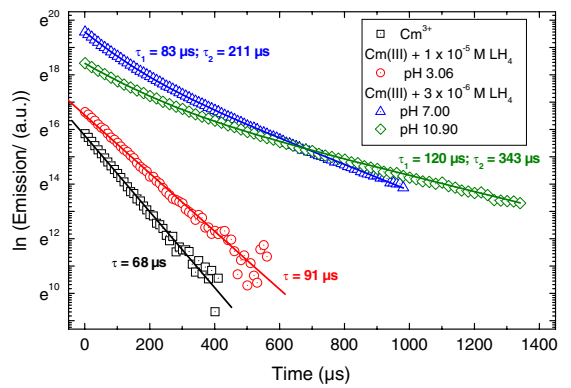

Fig. 6 Fluorescence emission lifetimes of curium(III) in *P. fluorescens* (CCUG 32456) pyoverdin solutions at various pH levels; mono- and bi-exponential decay behavior; $[Cm^{3+}]$ 3×10^{-7} M, 0.1 M $NaClO_4$

to the fluorescence decay rate of the excited Cm^{3+} aquo ion. The increasing lifetimes of the curium(III) species reflect the exclusion of water molecules from the first coordination sphere of curium(III), due to the identified complex formation reactions. The fluorescence lifetimes as measured by direct and indirect excitation match closely.

In conclusion, use of TRLFS in combination with the SPECFIT factor analysis software provides a sensitive method for investigating the speciation of curium(III) in the aqueous *P. fluorescens* (CCUG 32456) pyoverdin system. Strong Cm^{3+} pyoverdin species are formed, indicating the great potential of these unique siderophores to mobilize curium(III) in the biologically relevant pH range. Three Cm^{3+} pyoverdin complexes, CmH_2L^+, CmHL, and CmL^-, could be identified by their individual emission spectra (see Fig. 3). The results of the present work contribute to an improved understanding of the chemistry of curium(III) coordination with natural siderophores of the pyoverdin type in aqueous solution. Such complexation studies of selected bioligands are essential to explain the overall interaction processes of actinides with microbes at a molecular level. The determined stability constants can be used directly in safety calculations to quantify the actinide-mobilizing effect of the bioligands released, for example, in the vicinity of a nuclear waste disposal site.

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