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Complexation of Cm(III) with blood serum proteins: recombinant human serum albumin (rHSA)

Abstract: The complexation of Cm(III) with the recombi-nant human serum albumin (rHSA) (characterized by single deletion of residue Asp-1), is studied in dependence of pH and rHSA concentration using time-resolved laser fluorescence spectroscopy (TRLFS). A Cm(III) rHSA species is formed between pH 6.4 and 10.0 with the con-ditional stability constant being logK = 6.47 at pH = 7.4. Competition titration experiments with Cu(II) and Zn(II) confirm complexation at the N-terminal binding site (NTS) of rHSA and exclude the involvement of the Multi-Metal Binding Site (MBS). Comparison with a previous study on Cm(III) interaction with native albumin, HSA, points out, that residue Asp-1 is involved in Cm(III) binding to HSA but is not crucial for Cm(III) complexation at the NTS. The results are of major importance for a better understanding of fundamental actinide-protein interaction mechanisms which are highly required for the identification and characterization of relevant distribution pathways of incorporated radionuclides.

Keywords: actinides; binding site; Cm(III); complexation; human serum albumin; spectroscopy.

1 Introduction

Accidentally released radionuclides, in particular actinides, can cause a serious health risk upon incorporation [1]. A detailed understanding of the relevant biochemical reactions of incorporated actinides is strongly required for the development of potential decontamination strategies [2]. One potential reaction is the complexation with human serum albumin (HSA), the most abundant protein in human blood. HSA possesses at least four metal ion binding sites: The amino terminal Cu and Ni binding site (N-terminal site [NTS]) is composed of the first three amino acids Asp-Ala-His of the albumin sequence [3–7]. The Multi-Metal Binding Site (MBS) is the main binding site for Zn(II) [5, 8–11]. Further binding sites, the site around Cys-34 and the so far not located Site B, are less characterized yet [3, 5, 12].

The complexation of HSA with a wide range of metal ions is reported in the literature. Regarding actinides, only the interaction of Th(IV), U(VI) and Cm(III) with HSA has been investigated so far. U(VI) and Th(IV) interact with carbonyl and amide groups of HSA [13]. Two binding sites for U(VI) were identified, with the NTS being the stronger site [14, 15]. In a previous study we identified and characterized Cm(III) HSA complexation in dependence of pH, HSA concentration and temperature [16]. Competition titration experiments showed the repression of Cm(III) HSA complexation with increasing Cu(II) concentration whereas an addition of Zn(II) to Cm(III) HSA has no effect indicating the complexation of Cm(III) at the primary Cu(II) binding site, the NTS. However, since Cu(II) can coordinate to the MBS as well additional experiments are highly required to identify the HSA binding site for Cm(III) and give further insight into the structure of the complex. In the present work we investigated the interaction of Cm(III) with recombinant human serum albumin (rHSA) expressed in *Pichia pastoris* [17, 18]. The variant (Albagen™) is characterized by a single deletion of Asp-1 from the NTS which allows to determine the relevance of residue Asp-1 regarding Cm(III) HSA complexation.

The complexation of Cm(III) with rHSA was studied in dependence of pH and protein concentration as well as in presence of increasing amounts of Cu(II) and Zn(II) using time-resolved laser fluorescence spectroscopy (TRLFS) (setup and measurements described in [16, 19, 20]). This is a very sensitive method for determination of the speciation of lanthanides and actinides, especially Eu(III) and Cm(III) in

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submicromolar concentration ranges. Protein purification and sample preparation were performed according to the protocols described in [16, 21, 22]. The fluorescence spectra of Cm(III) with rHSA in the pH range from 3.5 to 11.0 and the corresponding speciation diagram obtained by peak deconvolution are shown in Figure 1. In dependence of the pH four Cm(III) species are identified: The Cm(III) aquo ion (λ_{max} = 593.8 nm) [23–25], a TRIS-H_2O–OH^- species resulting from Cm(III) interaction with the solvent (λ_{max} = 598.3 nm) [16], the Cm(III) rHSA species (λ_{max} = 601.8 nm) and ternary Cm(III)-OH-rHSA [16] species in the alkaline pH region. In general, the spectra are similar to those of Cm(III) HSA presented in our previous study [16]. However, the emission band of the Cm(III) rHSA complex shows a hypsochromic shift of about 1 nm relative to the emission band of the Cm(III) HSA species (λ_{max} = 602.6 nm). Furthermore, the Cm(III) rHSA species dominates the speciation over a significantly broader pH range (pH 6.4–10.0) and its maximum ratio (100% at pH 8.0) is higher compared to the Cm(III) HSA species (about 60% at pH 8.0) [16]. This discrepancy can be explained by the formation of different Cm(III) complexes with HSA and rHSA. However, the similar shape and shift of the Cm(III) HSA and Cm(III) rHSA emission bands indicate only slight variations in the ligand field introduced by the lack of Asp-1 in rHSA. The results clearly show that the lack of Asp-1 does not prevent Cm(III) binding at the NTS although the coordination environment is slightly different for the Cm(III) HSA and rHSA species.

The fluorescence lifetime of the Cm(III) rHSA species was determined to be τ = 145 ± 10 µs. This is in excellent agreement with the value of τ = 152 ± 10 µs obtained for Cm(III) HSA indicating a similar coordination environment [16]. Both values correspond to 3–4 H_2O molecules [19, 26] and 5–6 coordinating ligands (amino acid residues and/or additional anions like OH^- or CO_3^{2-}) in the first coordination sphere of Cm(III) [27, 28]. These results also confirm that Asp-1 is involved in Cm(III) binding at the NTS but is not crucial for the complexation.

The complexation of Cm(III) with rHSA was studied in dependence of the protein concentration at pH = 7.4 (Figure 2). With increasing rHSA concentration the ratio of the Cm(III) rHSA species increases, resulting in a continuous bathochromic shift of the emission band up to λ_{max} = 601.4 nm. The conditional stability constant at pH 7.4 was determined to be logK = 6.47. This value is compared with that of the Cm(III) HSA species at pH 8.0 (logK = 6.16) [16]. Since both are conditional stability constants obtained at a certain pH a direct comparison is not possible. But taking into account the different pH values and the errors a slightly higher value for Cm(III) rHSA is observed although it

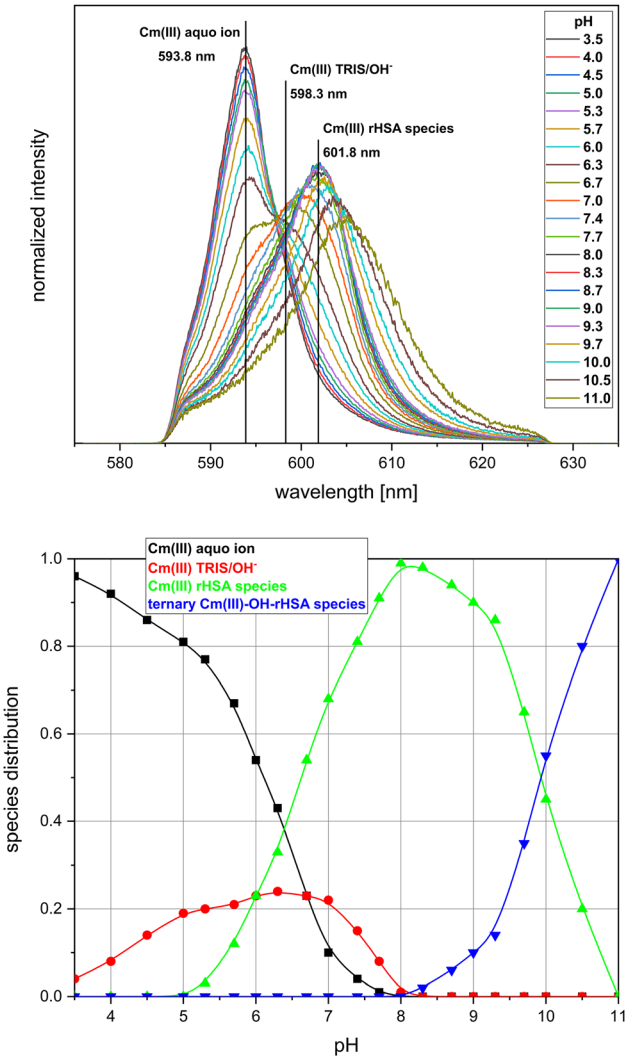
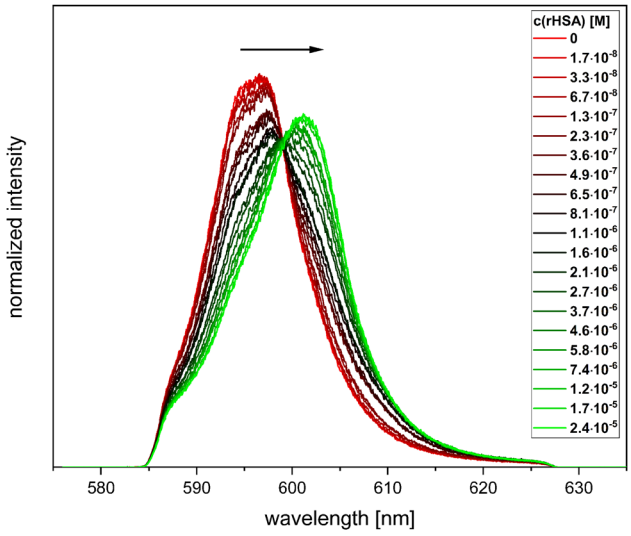


Figure 1: Top: Fluorescence spectra of Cm(III) with rHSA in the pH range between 3.5 and 11.0; Bottom: Speciation of Cm(III) with rHSA as a function of pH; c(Cm) = 1.0·10 ^{7} M, c(rHSA) = 5.0·10 ^{6} M, TRIS 10 mM, NaCl 150 mM, T = 296 K.

was obtained at lower pH. This is also reflected in the speciation diagram with Cm(III) rHSA showing significantly higher ratios in the pH range from 6 to 10 compared to Cm(III) HSA. Consequently, the lack of Asp-1 does not destabilize the Cm(III) complex. On the contrary, the slightly different Cm(III) rHSA complex seems to be more stable than the Cm(III) HSA complex.

For further verification of the Cm(III) HSA binding site competition titration experiments of Cm(III) rHSA with Cu(II) and Zn(II) were performed at pH 8.0. The Cm(III) rHSA complexation is not significantly influenced by increasing the Cu(II) concentration (Figure 3, top). Only a slight broadening and a small hypsochromic shift of the emission band is observed which might be attributed to Cu(II) complexation at other rHSA binding sites. This might



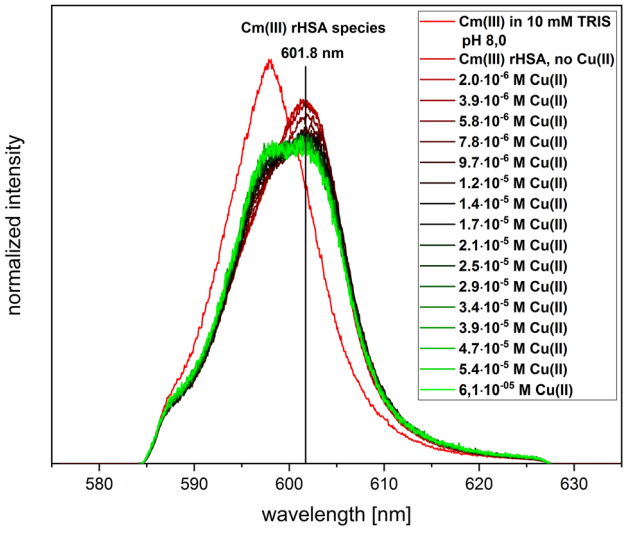


Figure 2: Fluorescence spectra of Cm(III) with increasing rHSA concentration at pH 7.4; c(Cm) = 1.0·10 ^{7} M, c(rHSA) = 0–2.4·10 ^{5} M, TRIS 10 mM, NaCl 150 mM, T = 296 K.

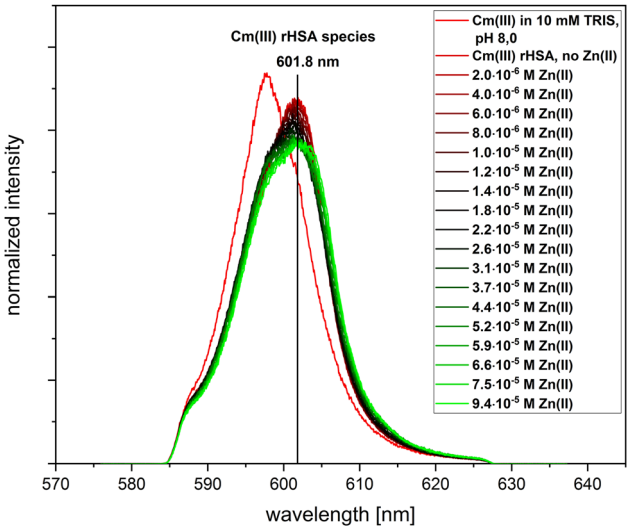


Figure 3: Fluorescence spectra of Cm(III) with rHSA at pH 8.0 in dependence of the Cu(II) concentration (top) and Zn(II) concentration (bottom); c(Cm) = 1.0·10 ^{7} M, c(HSA) = 5.0·10 ^{6} M, c(Cu) = 0–5.4·10 ^{5} M, c(Zn) = 0–9.4·10 ^{5} M, NaCl 150 mM, T = 296 K.

introduce slight changes in the three-dimensional protein structure and influence the coordination environment of the Cm(III) at the NTS. A similar effect was observed for Cm(III) transferrin before [29]. At high pH deprotonation of amino acid residues not directly involved in Cm(III) binding introduce slight changes in the structure of the protein leading to a small hypsochromic shift of the emission band. Cu(II) cannot replace Cm(III) in the rHSA complex which is in contrast to Cm(III) complexation with native HSA. With increasing Cu(II) concentration the Cm(III) HSA complexation is repressed and a hypsochromic shift of the emission band is observed until the Cm(III) solvents spectrum is obtained [16]. These results clearly demonstrate that the lack of Asp-1 prevents Cu(II) from replacing Cm(III) in the rHSA complex.

Additionally to the preferred binding site NTS Cu(II) can also bind to the MBS. To exclude additional complexation of Cm(III) at the MBS a competition titration with Zn(II) was performed (Figure 3, bottom). The results are comparable with those of the Cu(II) experiment: Only a slight broadening of the emission band is observed due to the binding of Zn(II) to other binding sites changing the three-dimensional protein structure. These results are in excellent agreement with those obtained for Cm(III) HSA [16]. For both proteins, Zn(II) does not replace Cm(III) in the protein complex which proves, that Cm(III) does not bind at the MBS neither in presence nor in absence of Asp-1.

In the present study the role of residue Asp-1 for Cm(III) binding to HSA was elucidated. The results confirm that the NTS is the main binding site for Cm(III), although coordination at binding sites with lower affinity cannot be excluded. Residue Asp-1 is involved in Cm(III) complexation with native HSA but is not a crucial ligand for the coordination. Thus, the results contribute to a better understanding of fundamental actinide-protein interaction and the formed complexes which are important for the identification and characterization of relevant distribution pathways of incorporated radionuclides. However, the results obtained so far do not give insight into the composition of the coordination environment. Further experiments including UV/Vis and CD (circular dichroism) spectroscopy, mass

spectrometry and quantum chemical calculations will help to investigate the structure of the Cm(III) rHSA complex.

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