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The bioinorganic chemistry of actinides in blood

David M. Taylor[a,b,*]

[a]University of Heidelberg and Institute for Toxicology, Kernforschungszentrum Karlsruhe, Karlsruhe, Germany
[b]University of Wales Cardiff, Cardiff, UK

Abstract

The behaviour of actinides (An) in mammalian blood is controlled largely by their strong tendency to hydrolyze and form complexes at physiological pH (~7.0). Except for U(VI) and Th(IV), with 70–90% associated with blood cells, the plasma is the predominant transport medium for An in blood. Numerous studies have demonstrated that, in human and animal blood plasma, Pu(IV), Th(IV), Pa(V), U(VI), and Np(V) appear to bind strongly to the iron-transport protein transferrin (Tf), although the binding characteristics may vary from element to element. The trivalent An, Ac(III), Am(III), Cm(III) and Cf(III), apparently bind to Tf, but also to other proteins, and the association with transferrin appears to be much weaker than that of the other actinides. © 1998 Elsevier Science S.A.

Keywords: Actinides; Blood; Transferrin; Speciation

1. Introduction

Certain long-lived actinide (An) isotopes, ^{238}U, ^{232}Th, ^{244}Pu, are primeval radionuclides and they, and their An decay products, must be considered to have been natural components of the human body ever since the emergence of *Homo sapiens* on this planet [1,2]. Thus, an understanding of An interactions with the proteins and other metal-binding ligands present in human blood and tissues is of fundamental biochemical interest. Since the α-particle emitting An radionuclides, especially ^{228}Th, ^{238,239}Pu, ^{241}Am, ^{242,244}Cm and ^{237}Np are highly radiotoxic [3], such knowledge is also an essential prerequisite for the development of safe and efficacious therapeutic methods for accelerating their slow, natural rate of elimination from the human body, in the event of a serious accidental contamination.

2. Methods for studying actinide speciation in blood

The reactions of An ions in all biological media, especially blood plasma, can be seen as a competition between their very strong tendency to undergo hydrolysis within the physiological pH range, ~5 to ~7.5, and their ability to form stable complexes with the many ligands present in such media [3]. This tendency is so strong that the free An ions cannot exist at physiological pH and the metals exist only in complexed form or as hydrolyzed oxo- or hydroxy-species.

The study of An interactions with proteins and other biological ligands is complicated, both by hydrolytic phenomena and by the risks of denaturing sensitive ligands, especially proteins, in acidic environments, pH ≤5; or of damage caused by intense α-particle irradiation. Further, the strict radiological protection requirements for work with even small quantities of these highly radiotoxic elements now place additional, increasingly severe, limitations on the investigators' choice of methods.

For either in vivo or in vitro studies, the analytical methods must be so selected that they cause minimal disturbance to the natural pattern of chemical speciation within the system. Thus major changes in pH, salt concentration, or temperature must be minimized. The major techniques which have been used during the past five decades to study actinide speciation in blood plasma include: *electrophoresis* (Tiselius moving boundary [4], paper [5], cellulose acetate [6], iso-electric focussing [7], polyacrylamide gel (PAGE) [8]); *electrodialysis* [5]; *chromatography* (size-exclusion, ion-exchange, [9–12], affinity [13], immuno- [14]); *precipitation* [5,15]; *spectroscopy* [16,21].

None of these techniques can be regarded as optimal, since they represent compromises between obtaining a meaningful separation of the different chemical species present, and the avoidance of artifacts due to the dissociation of important complexes and/or the degradation of

*Correspondence address. Department of Chemistry, University of Wales Cardiff, P.O. Box 912, Cardiff, CF1 3TB, Wales, UK.

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sensitive biological ligands. However, they do yield useful, albeit often only semi-quantitative [22], information which provides a broad picture of the pattern of the An–ligand interactions which occur in blood and other biological systems.

3. The distribution of actinides within the blood

The partition of An between the cellular elements and the blood plasma has not been studied systematically. The available data from humans and experimental animals indicate that there is little association, ~2–10%, of Pu(IV), Am(III), Cm(III) or Cf(III) with the cellular elements of the blood, >90% of the element in the blood being found in the plasma. In contrast, the majority of the total blood content of U and Th is associated with the blood cells [23,24]. No data are available for Ac(III), Pa(V) or Np(V).

The large difference in the fractions of Th(IV) and Pu(IV) associated with the cells is as yet unexplained, but it indicates that the bioinorganic chemistry of Th(IV) does not mimic that of Pu(IV) in vivo [17]. The chemical forms in which An are retained within blood cells are unknown but, since red blood cells are quite rich in bicarbonate ions (~11 nmol dm^{-3}), bicarbonate complexes could be involved, especially for U(VI). Pu(IV) has been shown to bind to haemoglobin in vitro and to red cell membranes [25].

4. The physico-chemical state of plutonium in rat serum

Beliayev [5] used electrodialysis to study the 'physico-chemical state' of Pu in rat blood following intravenous injection of ^{239}Pu, either as citrate or as nitrate. Analysis of serum, collected 3 min or 3 h post-injection, revealed <1% of cationic Pu, but ~5–8% was present as negatively charged complexes. Precipitation of the serum proteins with alcohol showed that 90–95% of the serum ^{239}Pu was recovered in the protein precipitate. This appears to be the only direct experimental attempt to measure anionic and cationic Pu species in blood serum or plasma. Although the experimental procedure probably distorted the natural chemical equilibrium, the results agree reasonably well with the predictions of computer simulation of speciation studies [26].

5. Binding to plasma proteins

Fifty years ago Muntz and Barron, using classical Tiselius moving-boundary electrophoresis [4], showed that Pu(IV) appeared to combine with the β-globulins of canine blood serum. Later, paper and cellulose acetate electrophoresis [5,6] confirmed this observation in rat and horse serum, and further demonstrated that 80–90% of the ^{239}Pu migrated at the same rate as the β_2-globulin transferrin, the remainder being bound to α- and γ-globulins and albumin [6].

The transferrins form a homologous family of glycoproteins with a molecular mass of about 80 kDa, with each member containing about 700 amino acid residues. The molecule consists of two homologous *lobes*, each containing two *domains* which enclose a high-affinity iron-binding site within the *interdomainal cleft*. The two binding sites, known as the N- and C-terminal sites, are similar but not identical; each consists of about 330 amino acid residues and binds ferric iron through the imidazole group of a histidine, the phenolic groups of two tyrosines, the carboxyl group of an aspartate, and two oxygens of a bicarbonate, or carbonate anion in a bidentate mode, thus providing a distorted octahedral geometry. The two lobes appear to act independently of each other, the iron-binding properties of one changing only marginally when the other site is occupied. The concentration of Tf in human plasma is ~30 μmol dm^{-3}, about half of which is saturated with Fe(III). The principal function of Tf is to transport Fe from the systemic circulation to the cells and tissues; however, it also binds a number of other metals in vivo (for references see Ref. [27]).

A combination of gel- and ion-exchange chromatography was used to confirm that transferrin was the principal binding agent for ^{239}Pu in rat serum [9,11], and this observation has been extended to rat, rabbit, hamster, dog, horse and human plasma, labelled either in vivo or in vitro [8,13,15,18,23–25]. Over the past four decades, similar methods have also demonstrated that Th(IV) [16,17,24,28], Am(III) [10,13,22,29], Cm(III) [13,29], Np(V) [15,30], Pa(V) [7], U(VI) [23], and probably Ac(III) [31] and Cf(III) [32] bind to the serum Tf of humans and animals in vivo and/or in vitro.

Saturation of the An–Tf with excess Fe(III) generally results in a liberation of the foreign metal, thus suggesting that the association involves the two iron-binding sites on the Tf molecule [6]. However, studies with Pa(V) [7] failed to provide conclusive evidence for the release of this metal upon saturation with Fe(III). These studies have also shown that a synergistic anion, such as bicarbonate, is necessary for the binding of An and lanthanides (Ln) to Tf. Although Tf appears to be an important binding protein for all the An studied, there are differences between the different An metals with regard to the strength and, probably also, the mechanisms of binding. A comparative assessment of the speciation patterns of seven An in blood plasma is presented in Fig. 1. The nature of the low-molecular mass species (LMM) remains to be investigated in detail, but for U(VI) [23] and Pu(IV) [26], carbonate and citrate, respectively, appear to be important ligands.

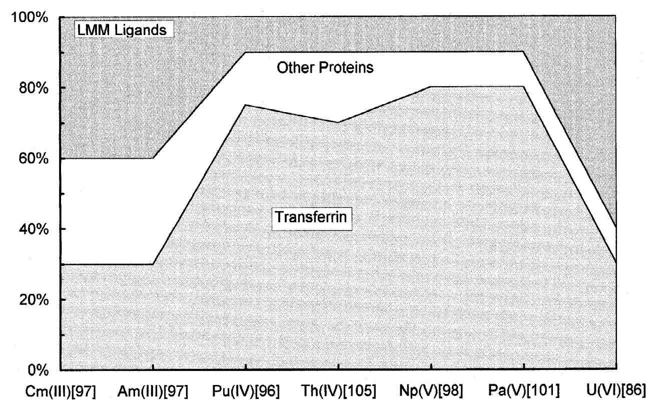

Fig. 1. The partition of actinides between the proteins and other components of blood plasma. The ionic radii, taken from Shannon [42], are shown in square brackets after each element.

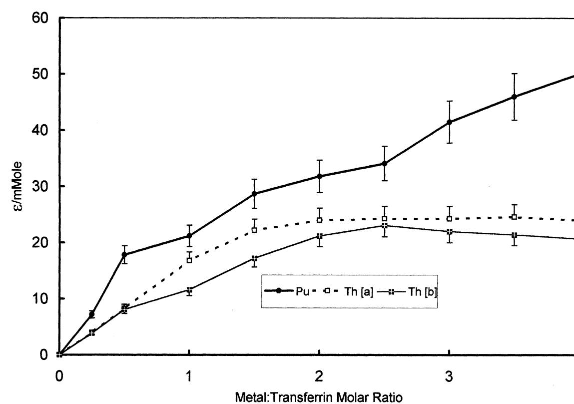

Fig. 2. Spectroscopic data for the titration of plutonium and thorium against transferrin at pH 7.4. Extinction values were measured at a wavelength of 240 nm. The data for Pu shown have been re-plotted from Refs. [16,20]; those for Th from (a) Ref. [16] and (b) Ref. [17].

6. The characteristics of actinide binding to transferrin

The comparative data presented in Fig. 1 indicate that >70% of the total serum Pu(IV), Np(V), Pa(V) and Th(IV) becomes bound to Tf after entry into the blood. In contrast to the apparently tenacious binding of the above metals to Tf, the binding of Am(III), and Cm(III) is much looser, with ~30% of the total plasma An being Tf bound, the remainder being bound to albumin, α- and γ-globulins or to LMM complexes [29].

The apparently strong binding of Np(V) and Pa(V) to Tf was unexpected since the An(V) ions were not expected to form stable protein complexes; and other workers [33] had failed to demonstrate any specific association of Np with rat or canine serum proteins. The formation of stable Pa– and Np–Tf complexes may indicate that, under physiological conditions, the M(V) oxidation state is reduced to the M(IV). This appears theoretically possible, since the redox potentials of Pa(V)→Pa(IV), −0.1 V, and Np(V)→Np(IV), 0.739 V [34], lie within the range of redox potentials expected in aqueous media at pH ~7 [35]. However, the mechanism by which such a reduction might occur in a strongly complexing medium, such as blood plasma, is not clear [27,31].

Spectroscopic, and other, studies of Ln and An binding to Tf in vitro have yielded useful information about both the characteristics and stability of the binding. Spectroscopic data for Th(IV) [16] suggest that the formation of the di-Th–Tf complex involves coordination to three tyrosine residues, two at the C-terminal site, but only one at the weaker N-terminal site. The N-terminal site appears to be slightly smaller than the C-terminal site, and it has been suggested that the Th^{4+}, ionic radius (IR)=105 pm, may be of borderline size to fit into the N-terminal site [16]. This may indicate an important difference between Th^{4+} and the slightly smaller Pu^{4+}, IR=96 pm, since the latter should fit easily into both sites [15]. However, spectroscopic data for Pu(IV) [17,20] provide much less clear-cut evidence for the binding of two Pu(IV) atoms per Tf molecule at pH 7.4. Fig. 2 compares the spectroscopic data for the binding of Th(IV) and Pu(IV) to human Tf and shows clearly that, while the molar extinction coefficient, $\Delta\epsilon$, for Th(IV) reaches a plateau after two atoms are bound per Tf molecule, that for Pu(IV) goes on rising. It was suggested that Th(IV) was bound to Tf as a monohydroxo species [16]. The binding of Th(IV) decreases rapidly with decreasing pH, so that at pH 6 only 0.3 atoms were bound per Tf molecule [16].

No spectroscopic studies with Np(V) have been reported. The high specific activities of Am(III) and Cm(III) preclude spectroscopic studies, but various Ln(III) have been used as surrogates. Spectroscopic and thermodynamic studies have suggested that, at pH 7.4, only one Gd(III) atom was bound per Tf molecule, specifically to the C-terminal metal binding site [36]; binding to the N-terminal site was weak, if it occurred at all. Other spectroscopic data appear to be consistent with the binding of either one or two atoms of Nd(III), Pm(III), Sm(III), Eu(III), Gd(III), Yb(III), Ho(III) or Tb(III) to human Tf [18,19,21], and it appears reasonable to expect that similar conclusions might apply to the An(III) ions.

7. The stability of metal–transferrin complexes

Values for the formation constants for An–Tf complexes are sparse. Yule [20] used UV spectroscopy and ultrafiltration to derive a conditional stability constant (log β) for the Pu–Tf complex of 21.25±0.75(SD). The conditional stability constant derived for the Pu–Tf complex was of the same order as that for Fe(III)–Tf; however, the Pu(IV) complex did not show the same stability with time. The high stability of the Pu(IV)–Tf complex is further illustrated by the fact that a >200-fold molar excess of the powerful chelator DTPA (log β Pu–DTPA=29.4) over Tf

is necessary to release significant amounts of Pu from the Tf complex [37].

Harris [22] measured conditional equilibrium formation constants for Nd(III)– and Sm(III)–Tf complexes and used these, and other data, to derive a linear free energy relationship from which formation constants ($\log K_1$) of 6.3±0.7(SD) and 6.5±0.8(SD) for Am(III) and Cm(III), respectively, were calculated. Zak and Aisen [37] reported a value for the apparent stability constant for the binding of Gd(III) to the C-terminal site of human serum Tf of $\log K_{eff}=6.8$. However, they pointed out that this value was too small to permit any significant role for Tf in the transport of Gd(III) at the physiological concentration of bicarbonate of 27 mmol dm^{-3}, due to competition from the formation of non-binding carbonato complexes. It appears likely that similar considerations might also apply to the binding of Am(III) and Cm(III) in vivo. More work is needed to provide a clear picture of the normal pattern of interaction of trivalent An with blood plasma in vivo.

8. Conformational changes on binding metals to transferrin

High-resolution small-angle X-ray scattering (SAXS) studies can provide valuable information on the size of the metal–Tf complexes, and about inter-domain and inter-lobe movements within the Tf molecule. Comparative studies with [38] SAXS showed that the radius of gyration, R_G, of chicken egg ovo-Tf, a molecule closely similar to human serum-Tf, decreased markedly on binding Fe(III), Cu(II), Al(III), Ga(III), but not on binding Hf(IV) or Th(IV) [39]. These data, which are listed in Table 1, indicate that the Fe(III)–, Cu(II)–, Ga(III)– and In(III)–Tf complexes are smaller than the metal-free *apo*-Tf molecule. The Group IVB element Hf(IV), which shares the same principal oxidation state as Pu(IV), and also binds strongly to Tf in vivo and in vitro, was studied as a surrogate for Pu(IV) [39]. The contraction of the Tf molecule on binding Fe(III), Cu(II), Al(III), Ga(III), etc., is ascribed to closure of the interdomainal clefts; however, in contrast to the other metals, Hf(IV) and Th(IV), and probably also Pu(IV), are unable to trigger this closure [27].

Table 1
The radius of gyration, R_G, for some metal chicken ovotransferrin complexes (from Großman [39])

Metal–protein complex	R_G (pm)	Ionic radius (pm)[a]	
		cn=6	cn=8
Apo-ovo-transferrin (metal-free)	3050±20[b]		
Fe(III)–*apo*-transferrin	2970±20	64	78
Al(III)–ovotransferrin	2970	53	
Ga(III)–ovotransferrin	2980	62	
In(III)–ovotransferrin	2980	80	92
Hf(IV)–ovotransferrin	3040	71	83
Th(IV)–ovotransferrin	3040	94	105

CN, coordination number.
[a] From Ref. [42].
[a] Standard deviation.

9. Biochemical significance of actinide binding to transferrin

The studies discussed above suggest that Tf may play a role in the transport of Pu, Th, Pa and Np in blood plasma in vivo; however, it appears to be questionable if Tf plays a vital role in the transport of the trivalent actinides and of U. Transferrin facilitates the entry of Fe(III) into cells via 'metal–Tf–Tf-receptor' mediated endocytosis, acting through Tf-receptors located on the cell membrane [27]. However, the uptake of Pu(IV) or Am(III) is not facilitated by Tf [40] and, in fact, it has been shown that the Pu–Tf complex does not bind to the Tf-receptor but dissociates at or near the cell surface [41]. The inability of transferrin to facilitate the uptake of Pu^{4+} and Am^{3+} into cells may result from the An–Tf complex not being in the correct conformation to bind to the Tf-receptor on the cell surface, because they do not trigger the closure of the interdomainal cleft. If this is true then Tf could be seen as part of a detoxification mechanism; however, this may be ascribing to teleology what is perhaps more an accident of chemistry. Nevertheless, since U and Th, and even Pu, have always been natural components of the human body [1,2], even though the amounts are very small, it is to be expected that detoxification mechanisms designed to limit any potential chemically induced detriment will have evolved. The observed concentrations of U, and Th, in the plasma of normal non-occupationally exposed humans are about ~50 pmol dm^{-3}, while those of Pu and Np are probably a few amol at most [1,2]; in a normal person the free plasma Tf concentration is ~16 μmol dm^{-3}. Under these circumstances, and bearing in mind the apparent preference for binding to the C-terminal site, it seems probable that, under normal conditions, only one An atom will bind to a Tf molecule. If Tf forms part of a detoxification mechanism, and the binding of an An atom effectively inactivates a Tf molecule, the decrease in the amount of available Tf would be <1/100 000th of the normal plasma transferrin pool. It seems unlikely that this small fraction could cause any detectable direct biochemical effect.

10. Conclusions

During the past half century it has become firmly established that most An can bind to the iron-transport protein, Tf, the binding being apparently stronger for Th, Pu, Np and Pa, than for other An. Other serum proteins and low-molecular mass compounds also appear to be

important An-binding species, especially for the trivalent An. However, the detailed mechanisms of the An–protein interactions and their biological significance remain to be elucidated.

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