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Research paper

The interaction of human serum albumin with selected lanthanide and actinide ions: Binding affinities, protein unfolding and conformational changes

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Human serum albumin (HSA), the most abundant soluble protein in blood plays critical roles in transportation of biomolecules and maintenance of osmotic pressure. In view of increasing applications of lanthanides- and actinides-based materials in nuclear energy, space, industries and medical applications, the risk of exposure with these metal ions is a growing concern for human health. In present study, binding interaction of actinides/lanthanides [thorium: Th(IV), uranium: U(VI), lanthanum: La(III), cerium: Ce(III) and (IV)] with HSA and its structural consequences have been investigated. Ultraviolet–visible, Fourier transform-infrared, Raman, Fluorescence and Circular dichroism spectroscopic techniques were applied to study the site of metal ions interaction, binding affinity determination and the effect of metal ions on protein unfolding and HSA conformation. Results showed that these metal ions interacted with carbonyl ($C{=}\ddot{O}:$)/amide ($\ddot{N}-H$) groups and induced exposure of aromatic residues of HSA. The fluorescence analysis indicated that the actinide binding altered the microenvironment around Trp214 in the subdomain IIA. Binding affinity of U(VI) to HSA was slightly higher than that of Th(IV). Actinides and Ce(IV) altered the secondary conformation of HSA with a significant decrease of α-helix and an increase of β-sheet, turn and random coil structures, indicating a partial unfolding of HSA. A correlation was observed between metal ion's ability to alter HSA conformation and protein unfolding. Both cationic effects and coordination ability of metal ions seemed to determine the consequences of their interaction with HSA. Present study improves our understanding about the protein interaction of these heavy ions and their impact on its secondary structure. In addition, binding characteristics may have important implications for the development of rational antidote for the medical management of health effects of actinides and lanthanides.

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1. Introduction

Increasing applications of actinides (An) and lanthanides (Ln) in nuclear power, defense industry, space exploration and civil applications generated a significant numbers of sites for nuclear material processing, inventories and disposal [1]. Among the actinides, uranium (U) and thorium (Th) have prime positions in nuclear energy either due to the natural presence in fissile form (i.e. U-235) or their potential to be transformed into fissile fuel (Pu-239/U-233). In recent years, the Th-232-based nuclear energy has gained significant attention owing to potential advantages such as i) large Th reserves, ii) proliferation resistance, iii) significantly lower level of fission products (cesium and strontium) as well as iv) low level of long-lived alpha-emitters in the nuclear wastes [2–4]. Therefore, in-future, large-scale handling of Th-containing materials such as monazite, thorianite etc. [which mainly contain ThO_2 (~2.5–10%), UO_2 (<1%), cerium dioxide (CeO_2, ~30–40%) and other lanthanides (Ln)] may increase the risks of occupational and accidental exposures of Th, U, La and Ce to nuclear workers, and environmental exposure to human population [5]. Studies on occupationally-exposed subjects (workers in mines, purification and processing plants) of several countries have reported significant levels of Th and U in biological samples [6–12]. High levels of Ln in human

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tissues and body fluids of industrial workers are suggested to cause pulmonary and other pathologies [13,14]. Pneumoconiosis in human subjects showed association with higher level of Ce-containing particles in their bronchoalveolar lavage (~10^5/ml) and lung tissue (~10^7/g) [15]. Moreover, administration of Ln (La, Ce) as contrast agents in humans was found to elicit health effects such as thrombophlebitis, thrombosis, hemoglobinemia etc [16].

Human intake of An and Ln occurs through inhalation as well as oral, dermal, or wound routes. After absorption, these metal ions are most likely to interact with cellular and acellular blood components before accumulation in the target organs (liver, bone and kidney) [17–20]. Biokinetic data of Th and U, which have been reviewed by ICRP suggested ~6 and 70% of urinary excretion, respectively, within 5–6 days of administration [17,18]. Previously, we observed that Th ions interact with negatively-charged sialic acid rich-extracellular domain of glycophorin protein in human erythrocytes and caused concentration-dependent cell lysis or aggregation [21]. In blood, most of the An ions tend to associate with plasma proteins [albumin (HSA), globulin and transferrin (Tf)]. However, in all An cases, a fraction of complexes with ligands of low molecular weight ions (e.g. carbonate, phosphate and citrate) is also identified, which depending on their charge-to-ionic-radius-ratio (z/r), varies from small (<10%) for trivalent, tetravalent and pentavalent An to large (≥50%) for U-dioxocations (UO_2^{2+}) [22]. Among plasma proteins, HSA (due to its abundance in human blood, 6.5×10^{-4} M) and Tf (due to the presence of iron-binding sites) serve as major metal transport carriers [23]. However, fetuin-A, a minor blood protein in-terms of its concentration has recently been shown to bind with U(VI) ions [24]. HSA has been discussed to carry a significant fraction of divalent metal ions like Ca^{2+} and Mg^{2+} [22]. Thus, plasma proteins including HSA would have higher affinity of multivalent Ln/An to occupied/unoccupied metal-binding sites. Evidently, in serum condition, ~95% of incubated Ln was found in albumin fraction [25]. Trivalent lanthanides (Ln^{3+}) have been shown to substitute for metal ions such as Ca^{2+}, and to a lesser extent, Mg^{2+}, Fe^{3+} and Mn^{2+} [26]. Ln^{3+} therefore interact with many proteins, which either have an absolute dependence on Ca^{2+} or whose activity is stimulated by Ca^{2+} [26]. Hence, studies on the interaction of An/Ln with HSA is important in understanding: i) the mechanisms of their transport/distribution, ii) molecular effects at protein level and iii) uptake by the target organs [27].

Human serum albumin (HSA) is the most abundant protein (~55% of total protein, 3.5–5 g/dl) in blood plasma, followed by globulins (38%), fibrinogen (7%) and regulatory proteins (>1%) [28]. HSA (66 kDa) is a monomer of three homologous domains (DI, DII and DIII), each containing two sub-domains with mainly helical conformations, connected by flexible loops. HSA has a pivotal role in the transport of a range of water-insoluble molecules such as fatty acids, hormones, bilirubin, heme, drugs and metal ions [29]. Previously, binding of HSA with metal ions [30,31], drugs [32,33], and bioactive compounds [34] have been studied. Previously, interaction of U with bovine and human albumin has been reported [35–37]. However, there are limited studies on binding mechanism of An/Ln with HSA and the consequent protein conformational changes.

Actinide exhibits a range of oxidation states in aqueous media from +II to +VII. The most stable oxidation states are +IV for Th/Pu and +VI for U [38]. Due to large positive charge, U(VI) exists as dioxo-cation UO_2^{2+} (also known as uranyl ion) in aqueous solution. The coordination number for Th/Pu(IV) ions ranges from 6 to 12 and for uranyl ions from 2 to 8 [39]. The stable oxidation state of Ln in aqueous condition is +III and they show a wide range of coordination number (generally 8–10). Ce mainly exhibits +IV state having ability to extract an electron due to its favorable redox potential [26]. Thus, the interaction of Ln and An with biological ligands is complex and sensitive to the presence of cation/anion(s), ionic strength, redox potential, temperature, gas–liquid–solid phase equilibria and biological microenvironment [27,40].

Since, U has been the mainstay of global nuclear power and its significant level in human has been reported in public/occupational scenarios. It was selected to study its effect on HSA. Due to the realization of Th as a nuclear fuel [4] and its exposure to nuclear workers, Th was choosen as another relevant An in the present study. Monazite, an important Th ore contains significant fraction of Ce and La, which may get internalized in human during extraction/purification of Th and interact with HSA. Hence, in the present study, the interactions of An [Th(IV) and U(VI)] and Ln [La(III), Ce(III) and Ce(IV)] with HSA under the physiological conditions were investigated, using various spectroscopic techniques. We also addressed the structural alteration of HSA due to the above interactions that provided a better understanding of the biological effects of these heavy ions at molecular level.

2. Material and methods

2.1. Materials

HSA (>99% by agarose gel electrophoresis, essentially fatty acid and globulin free, Cat. No. A3782), La(III) nitrate (Cat. No. 203548), Ce(III) nitrate (Cat. No. 429406) and CeO_2 (Cat. No. 202975) were purchased from Sigma, MO, USA. Analytical grade ^{232}Th- and ^{238}U-nitrate salts were obtained from the Radiochemistry Division, BARC, Mumbai, India.

2.2. Preparation of the experimental solutions

The HSA solution (4 μM) was freshly prepared in Hepes-buffered saline (153 mM, pH ~7.4), considering the molecular weight of HSA as 66,500 Da. The concentration of HSA was determined using molar extinction coefficient of 33,434 M^{-1} cm^{-1} at 278 nm [41] in spectrophotometer (V-550, Jasco), and this concentration (4 μM) was used previously for sensitive optical spectroscopic investigation [42]. Stock solutions (10–100 mM) of the metal nitrates were prepared by dissolving the respective salts in 0.01 N nitric acid. For preparing Ce(IV)-nitrate solution, CeO_2 was dissolved in concentrated sulfuric acid, the acid evaporated by heating, and the residual slurry dissolved in 0.01 N HNO_3 to obtain the stock solution (50 mM) [43]. The stock solutions of Ce(IV) were used to obtain the required concentrations of metal ions for the experiments. The presence of Ce(III) in Ce(IV) stock solution was verified by using difference in fluorescence emission maxima [44]. A solution of $CeCl_3$ was taken as a standard. It was 0.04%. The stability of Ce(IV)/Ce(III) ions in buffered-saline was confirmed by cyclic voltammetry.

2.3. Treatment of HSA with metal nitrates

The HSA solutions were treated with 1 μl of metal nitrate and incubated for 1 h at 37 °C (physiological temperature). Control experiments were carried out as above wherein nitric acid (1 μl, 0.01 N) was used instead of the metal nitrates. Only nitric acid treatment to HSA under experimental conditions did not show any noticeable changes in the spectroscopic measurements. The spectrum of saline was taken as a blank for protein solution (control), and the spectrum (UV–VIS, FT-IR, CD and fluorescence) of buffered saline-treated with metal ions were substracted from that of the respective metal ion-treated HSA solutions to nullify the effect of free metal ions if any, on the spectra. Protein precipitation was not observed at studied metal ion concentration.

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