A New Approach for Thermodynamic Study on the Binding of Human Serum Albumin with Cerium Chloride

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Thermodynamics of the interaction between Cerium (III) chloride, Ce$^{3+}$, with Human Serum Albumin, HSA, was investigated at pH 7.0 and 27 °C in phosphate buffer by isothermal titration calorimetry. Our recently solvation model was used to reproduce the enthalpies of HSA interaction by Ce$^{3+}$. The solvation parameters recovered from our new model, attributed to the structural change of HSA and its biological activity. The interaction of HSA with Ce$^{3+}$ showed a set of two binding sites with negative cooperativity. Ce$^{3+}$ interacts with multiple sites on HSA affecting its biochemical and biophysical properties.

Key Words: Human Serum Albumin, Isothermal titration calorimetry, Cerium (III) chloride.

Introduction

Human Serum Albumin (HSA), as the most abundant protein constituent of blood plasma, has a high affinity to an extraordinarily diverse range of materials, such as drugs, metabolites, fatty acids and metal ions. HSA can bind and carry through the bloodstream many drugs, which are poorly soluble in water and it is also responsible for the maintenance of blood pH, the drug disposition and efficacy, and the contribution of colloid osmotic blood pressure. The unique feature of albumin is its ability to bind a wide variety of compounds, mainly because of the availability of hydrophobic pockets inside the protein network and the flexibility of the albumins to adapt its shape. The crystallographic analysis of HSA revealed that this protein is a single-chain 66 kDa protein, which is largely α-helical, and consists of three structurally homologous domains that assemble to form a heart-shaped molecule. Each domain is a product of two subdomains, which are predominantly helical and extensively cross-linked by several disulfide bridges.

Erbium is one of the rare chemicals that can be found in houses in equipment such as color televisions, fluorescent lamps, energy-saving lamps and glasses. All rare chemicals have comparable properties. Erbium will gradually accumulate in soils and water soils and this will eventually lead to increasing concentrations in humans, animals and soil particles. The use of erbium is still growing, due to the fact that it is suited to produce catalysers and to polish glass. Erbium is mostly dangerous in the working environment, due to the fact that damp and gasses can be inhaled with air. This can cause lung embolisms, especially during long-term exposure. Erbium can be a threat to the liver when it accumulates in the human body. All erbium compounds should be regarded as highly toxic because the biological properties of the lanthanides, primarily based on their similarity to calcium, have a high affinity for Ca$^{2+}$ sites on biological molecules and hence can act as either Ca$^{2+}$ inhibitors or probes. Although the Lanthanide cannot gain access to intracellular organelles, they have been used as biochemical probes to study calcium transport in mitochondria and other organelles. The biological properties of the lanthanides, primarily based on their similarity to calcium, have been the research basis into the potential therapeutic applications of lanthanides, since the early part of the twentieth century. The lanthanides have similar ionic radii to calcium, but by virtue of possessing a higher charge, they exhibit a high affinity for the Ca$^{2+}$ sites on biological molecules and a stronger binding to water molecules. One of the major physiological effects of the lanthanide (Ln$^{3+}$) ions is to block both the voltage operated and the receptor operated calcium channels. Ln$^{3+}$ can block the Na$^{+}$/Ca$^{2+}$ synaptic plasma membrane exchange and inhibit the skeletal, smooth and cardiac muscle contraction by blocking the Ca$^{2+}$-ATPase in the sarcoplasmic reticulum of the muscle. The Ln$^{3+}$ ions themselves are unable to cross the cell membranes, but they act by blocking the exterior face of the calcium channel. Though Ln$^{3+}$ cannot gain access to the intracellular organelles, they have been used as biochemical probes to study the calcium transport in mitochondria and the other organelles. The lanthanides can substitute calcium in proteins, even though it should be noted that the Ln$^{3+}$ ions can also substitute other metal ions, such as Mg$^{2+}$, Fe$^{3+}$ and Mn$^{2+}$. The calcium dependent enzymes can either be inhibited by lanthanides, or in some cases, be activated to a similar or greater extent by calcium. It has been proposed that the stimulatory or inhibitory effect of the lanthanides may be a function of the role of calcium in the native enzyme. The interest in lanthanides regarding the biochemical reactions arises from the fact that they can be used as probes to unravel the interactions between Ca$^{2+}$ and the biologically important molecules.

This work represents the most comprehensive study on the interactions between Ce$^{3+}$ cations with HSA and provides new evidence for validity of our recently introduced solvation model and more insights into the interactions of Ce$^{3+}$ with HSA for further understanding of the effects of metal ions on the stability and the structural changes of macromolecules.
Materials

HSA was obtained from Sigma and Ce$^{3+}$ was purchased from Merck. Protein concentrations were determined from absorbance measurements at 277 nm in 1 cm quartz cuvettes. All other materials and reagents were of analytical grade, and solutions were made in 50 mM buffer phosphate using double-distilled water.

Method

The isothermal titration calorimetric experiments were carried out on a VP-ITC ultra sensitive titration calorimeter (MicroCal, LLC, Northampton, MA). The microcalorimeter consists of a reference cell and a sample cell of 1.8 mL in volume, with both cells insulated by an adiabatic shield. All solutions were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with HSA solution (40 µM) and the reference cell contained buffer solution. The solution in the cell was stirred at 307 rpm by the syringe (40 µM) and the reference cell contained buffer solution. The samples were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with HSA solution in the cell was stirred at 307 rpm by the syringe with CeCl$_3$ solution involved 30 consecutive injections of the ligand solution, the first injection was 5 µL and the remaining aliquots were 10 µL. In all cases, each injection was done in 6 s at 3 min intervals. To correct the thermal effects due to CeCl$_3$ dilution, control experiments were done in which identical aliquots were injected into the buffer solution with the exception of HSA. In the ITC experiments, the enthalpy changes associated with processes occurring at a constant temperature are measured.$^{17,20}$ The measurements were performed at a constant temperature of 27.0 ± 0.02 °C and the temperature was controlled using a Poly-Science water bath.

Results and Discussion

We have shown previously that the enthalpies of the HSA + Ce$^{3+}$ interactions in the aqueous solvent system can be accounted for quantitatively in terms of three factors: preferential solvation by the components of a mixed solvent, weakening or strengthening of solvent-solvent bonds by the solute and the change in the enthalpy of the solute-solvent interactions.$^{21-32}$ This treatment leads to:

$$\Delta H = \Delta H_{\text{mix}} x_A^s - \delta_{\text{dilut}}^s (x_A^s L_A + x_B^s L_B) - (\delta_{\text{dilut}}^s - \delta_{\text{dilut}}^p)(x_A^s L_A + x_B^s L_B)x_A^s$$  \hspace{1cm} (1)

The parameters $\delta_{\text{dilut}}^s = (a + bN)$ and $\delta_{\text{dilut}}^p = (a + bN)$ are the composite parameters which reflect to the net effect of Ce$^{3+}$ cations on the HSA stability in the low and high Ce$^{3+}$ concentrations respectively, with $a$ and $b$ reflecting the formation of a cavity wherein "m" Ce$^{3+}$ cations become the nearest neighbors of the HSA and $bN$ reflecting the enthalpy change from strengthening or weakening of water + Ce$^{3+}$ bonds of N solvent molecules ($N \geq n$) around the cavity. The positive values for $\delta_{\text{dilut}}^s = (a + bN)$ and $\delta_{\text{dilut}}^p = (a + bN)^p$ indicate that Ce$^{3+}$ cations stabilized the HSA structure and vice versa. The constants $a$ and $b$ reflect the proportion of the total enthalpy of water + Ce$^{3+}$ binding which is associated with the cavity formation and modification of solvent structure (water + Ce$^{3+}$) around the cavity, respectively. Cooperative binding requires that the macromolecule have more than one binding site, since cooperativity results from the interactions between binding sites. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity. Conversely, if the binding of ligand at one site lowers the affinity for ligand at another site, the protein exhibits negative cooperativity. If the ligand binds at each site independently, the binding is non-cooperative. $p < 1$ or $p > 1$ indicate positive or negative cooperativity of macromolecule for binding with ligand respectively; $p = 1$ indicates that the binding is non-cooperative. $x_A^p$ can be expressed as follows:

$$x_A^p = \frac{px_A}{x_A + px_A}$$  \hspace{1cm} (2)

$x_A$ is the fraction of the Ce$^{3+}$ needed for saturation of the binding sites, and $x_B = 1 - x_A$ is the fraction of unbounded Ce$^{3+}$.

The isothermal titration calorimetric experiments were fitted to Eq. 1 over the whole Ce$^{3+}$ compositions. In the procedure the only adjustable parameter ($p$) was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). $\delta_{\text{dilut}}^s$ and $\delta_{\text{dilut}}^p$ parameters have been also optimized to fit the data. The optimized $\delta_{\text{dilut}}^s$ and $\delta_{\text{dilut}}^p$ values are recovered from the coefficients of the second and third terms of Eq. 1. The small relative standard coefficient errors and the high $r^2$ values (0.999999) support the method. The binding parameters for HSA + Ce$^{3+}$ interactions recovered from Eq. 1 were listed in Table 1. The agreement between the calculated
Comparison between the experimental enthalpies, $\Delta H$, for HSA + Ce$^{3+}$ interactions (●) and calculated data (lines) via Eq. 1.

**Table 1.** Enthalpies of HSA + Ce$^{3+}$ interactions, $\Delta H$, in Ce$^{3+}$ solution with water at 300 K in kJ mol$^{-1}$. $\Delta H_{\text{dilut}}$ is the enthalpies of dilution of Ce$^{3+}$ with water. Precision is ± 0.400 µJ or better.

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<th>[Ce$^{3+}$] / µM</th>
<th>$\Delta H$ / µJ</th>
<th>$\Delta H_{\text{dilut}}$ / µJ</th>
<th>[HSA] / µM</th>
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and experimental results (Fig. 1) is striking, and gives considerable support to the use of Eq. 1.

$\Phi$ is the fraction of HSA molecule undergoing complexation with Ce$^{3+}$ which can be expressed as follows:

$\Phi = \frac{\Delta H}{\Delta H_{\text{max}}}$ (5)

$\Delta H_{\text{max}}$ represents the heat value upon saturation of all HSA.

The appearance association equilibrium constant values, $K_a$, as a function of Ce$^{3+}$ free concentration, $[Ce^{3+}]_f$, can be calculated as follow:

$$K_a = \frac{\Phi}{(1-\Phi)[Ce^{3+}]_f} = \frac{\Phi}{(1-\Phi)(1-x_s)[Ce^{3+}]_f}$$ (6)

The association equilibrium constants for successive replacement of water molecules by Ce$^{3+}$ cations are as follows:

$$K_a = x_i^s - \sum_{i=1}^{s} K_a \frac{x_i}{x_i^s}$$ (7)

Figure 1. Comparison between the experimental enthalpies, $\Delta H$, for HSA + Ce$^{3+}$ interactions (●) and calculated data (lines) via Eq. 1.

Figure 2. Comparison between the experimental entropies (●) for HSA + Ce$^{3+}$ interactions and calculated data (lines) via Eq. 8.

Figure 3. Comparison between the experimental free energies (○) for HSA + Ce$^{3+}$ interactions and calculated data (lines).
is negative cooperativity in two binding sites of HSA. Therefore, by the

positive values of

there is negative cooperativity in two binding sites of HSA.

stabilized against unfolding by Ce 3+ ions. Therefore, by the

phyrin bind in the two distinct sites with different affinity on

The Gibbs free energies as a function of Ce3+ concentrations

prediction by Eq. 1 as

This conclusion is in a good agreement with cooperativity

site is inhibited (anticooperativity or negative cooperativity).

Fig. 3.


\( K_a \) values obtained from Eq. 6, have been fitted to Eq. 7 using

a computer program for nonlinear least-square fitting. There-

fore, we can approach to "\( g_p \)" value simply (\( g = 2 \) in this work).

The Gibbs free energies as a function of Ce3+ concentrations

can be obtained as follows:

\[ \Delta G = -R T \ln K_a \]  

(E8)

Eqs. 6 and 7 allow us to have the \( K_a \) values in every

concentrations of Ce3+ with the least standard deviations and

correlation coefficients are so close to one. Gibbs energies, \( \Delta G \), calculated from Eq. 8 have shown graphically in Fig. 2. \( \Delta S \) values were calculated using \( \Delta G \) values and have shown in Fig. 3.

Previous reports revealed that some molecules such as
different species of metal ions of mercury, \( \text{Hg}^{2+} \), platinum and Cu

(II) complex of 5,10,15,20-tetrakis (4-N-benzyl-pyridyl) por-

phyrin bind in the two distinct sites with different affinity on

HSA which is in a good agreement with our results.\(^{34}\)

A nonpolar residue dissolved in water induces a solvation

shell in which water molecules are highly ordered. When two nonpolar groups come together on the folding of a polypeptide

chain, the surface area exposed to the solvent is reduced and

part of the highly ordered water in the solvation shell is

released to bulk solvent which results to an increase in the

entropy. It is possible to introduce a correlation between change in \( \delta_s \) and increase in the stability of HSA+Ce3+ complex. The positive values of \( \delta_s \) and \( \delta_p \) show that HSA is substantially stabilized against unfolding by Ce3+ ions. Therefore, by the

definition given above, HSA can serve as a reasonable model

of specific binding interactions for Ce3+ ions. The positive \( \delta_s \) and \( \delta_p \) values (Table 2) for HSA+Ce3+ interaction, indicate that the HSA structure is stabilized, as a result of its interaction with Ce3+ ions, resulting in an increase in its biological activity. \( \delta_s \) and \( \delta_p \) values are equal, indicating that the most of HSA is in its native form.

\( p \) value is less than one (\( p = 0.28 \)), which indicates that

there is negative cooperativity in two binding sites of HSA.

\( K_s < K_1 \) (Table 2) would indicate that binding of the second

site is inhibited (anticooperativity or negative cooperativity).

This conclusion is in a good agreement with cooperativity

prediction by Eq. 1 as \( p \) value recovered from this equation is

0.28.

HSA is a large globular protein with several physiological

roles ranging from transport of hydrophobic metabolites, such

as fatty acids and bilirubin, to the maintenance of the blood

osmotic pressure and transport of metal ions like Cu\(^{2+}\), Ni\(^{2+}\)

and Zn\(^{2+}\). Human serum albumin contains the N-terminal

sequence N-Asp-Ala-His-Lys, and is able to strongly bind the

metal ions (especially, transition metals such as Co, Cu and

Ni) with a high affinity for nitrogen through the donor set

(NH2, N, N\(_{\text{imido}}\), where NH2 represent the terminal amino,

N\(_{\text{imido}}\) the deprotonated amide groups of residues 2 and 3 and N\(_{\text{imido}}\)

the imidazole nitrogen belonging to His-3.\(^{35,36}\) The characteri-

zation of the metal-transport site of HSA was carried out by

Laussac and Sarkar\(^{36}\) who established the participation of

\( \alpha-NH_2 \), two intervening peptide nitrogen atoms, the imidazole

nitrogen atom of the histidine residue in the third position, and

the side chain carboxyl group of Asp-1 in a penta-coordinate

structure.\(^{36,37}\) Previous studies have shown that that albumin

binds one Vanadium, VO\(^{2+}\), at the N-terminal part (the “strong” site) and several other ions via non-specific interactions with carboxylate side chains of surface amino acids (“weak” sites); the exact number of such non-specific VO\(^{2+}\) binding sites is at least five and possibly as many as twenty.\(^{35}\) Therefore, it might be concluded that one of the binding site of Ce\(^{3+}\) on HSA is located at N-terminal part (the “strong” site, from K value (Table 2)) and the other weaker binding site might be located at carboxylate side chains of surface amino acids. The thermodynamic parameters obtained from the binding studies of the Ce\(^{3+}\) complex to the HSA carrier proteins may be useful in the evaluation of the structural changes induced by the

ligand on the carrier protein structure.

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References


