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A HIGH PERFORMANCE METHOD FOR THERMODYNAMIC STUDY ON THE BINDING OF HUMAN SERUM ALBUMIN WITH ERBIUM CHLORIDE

G. Rezaei Behbehani[1*], *A. Divsalar*[2,3], *A. A. Saboury*[2], *F. Faridbod*[4] *and M. R. Ganjali*[4]

[1]Chemistry Department, Imam Khomeini International University, Qazvin, Iran
[2]Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[3]Department of Biological Sciences, Tarbiat Moallem University, Tehran, Iran
[4]Departments of Chemistry, University of Tehran, Tehran, Iran

Thermodynamics of the interaction between erbium(III) chloride, Er^{3+}, with human serum albumin (HSA), was investigated at pH 7.0 and in phosphate buffer by isothermal titration calorimetry. Our recently, solvation model was used to reproduce the enthalpies of HSA interaction by Er^{3+} over a broad range of metal ion concentration. The solvation parameters recovered from our new model, attributed to the structural change of HSA and its biological activity. The binding parameters for the interaction of Er^{3+} and HSA indicate that the concentrations of Er^{3+} have no significant effects on the structure of HSA.

Keywords: *erbium(III) chloride, human serum albumin, isothermal titration calorimetry*

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 [1–3]. 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 [4, 5]. 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 [6–8]. 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 [8–10].

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 damps 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 [11]. 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 [12]. 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 [12–14]. 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 exte-

* Author for correspondence: grb402003@yahoo.com

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

rior 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 [15, 16]. 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 [17–19].

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

Experimental

Materials

HSA was obtained from Sigma and Er^{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 (equipped with micro propeller) filled with $ErCl_3$ solution (500 μM) to ensure rapid mixing. Injections were started after baseline stability had been achieved. The titration of HSA with $ErCl_3$ solution involved 30 consecutive injections of the ligand solution, the first injection was 5 μL and the remaining ones were 10 μL. In all cases, each injection was done in 6 s at 3-min intervals. To correct the thermal effects due to $ErCl_3$ 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. 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. The measurement accuracy of VP-ITC ultra sensitive titration calorimeter was 0.4 μcal.

Results and discussion

We have shown previously that the enthalpies of the HSA+Er^{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 [20–25]. This treatment leads to:

$$\Delta H = \Delta H_{max} x'_B - \delta^{\theta}_A (x'_A L_A + x'_B L_B) - \\ -(\delta^{\theta}_B - \delta^{\theta}_A)(x'_A L_A + x'_B L_B) x'_B \quad (1)$$

The parameters $\delta^{\theta}_A = (\alpha n + \beta N)^{\theta}_A$ and $\delta^{\theta}_B = (\alpha n + \beta N)^{\theta}_B$ are the composite parameters which reflect to the net effect of Er^{3+} cations on the HSA stability in the low and high Er^{3+} concentrations respectively, with αn resulting from the formation of a cavity wherein nEr^{3+} cations become the nearest neighbors of the HSA and βN reflecting the enthalpy change from strengthening or weakening of water+Er^{3+} bonds of N solvent molecules ($N \geq n$) around the cavity. The positive values for $\delta^{\theta}_A = (\alpha n + \beta N)^{\theta}_A$ or $\delta^{\theta}_B = (\alpha n + \beta N)^{\theta}_B$ indicate that Er^{3+} cations stabilized the HSA structure and vice versa. The constants α and β reflect the proportion of the total enthalpy of water+Er^{3+} binding which is associated with the cavity formation and modification of solvent structure (water+Er^{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'_B can be expressed as follow:

$$x'_B = \frac{px_B}{x_A + px_B} = \frac{\nu}{g} \quad (2)$$

where x_B is the fraction of the Er^{3+} needed for saturation of the binding sites, and x_A=1–x_B is the fraction of unbounded Er^{3+}. Now the model is a simple mass action treatment, with metal ions replacing water molecules, at the binding sites in the present case. We can express x_B fractions, as the total Er^{3+} concentrations divided by the maximum concentration of the Er^{3+} upon saturation of all HSA as follow:

$$x_B = \frac{[Er^{3+}]_T}{[Er^{3+}]_{max}} \quad x_A=1-x_B \quad (3)$$

where $[Er^{3+}]_T$ is the total concentration of Er^{3+} and $[Er^{3+}]_{max}$ is the maximum concentration of the Er^{3+} upon saturation of all HSA. In general, there will be 'g' sites for binding of Er^{3+} per HSA molecule and ν is defined as the average moles of bound Er^{3+} per mole of total HSA. L_A and L_B are the relative contributions of unbounded and bounded Er^{3+} to the enthalpies of dilution with the exception of HSA and can be calculated from the enthalpies of dilution of Er^{3+} in buffer, ΔH_{dilut}, as follow:

$$L_A = \Delta H_{dilut} + x_B \frac{\partial \Delta H_{dilut}}{\partial x_B},$$
$$L_B = \Delta H_{dilut} - x_A \frac{\partial \Delta H_{dilut}}{\partial x_B}, \quad (4)$$

The enthalpies of HSA+Er^{3+} interactions, ΔH, were fitted to Eq. (1) over the whole Er^{3+} compositions. In the procedure the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached

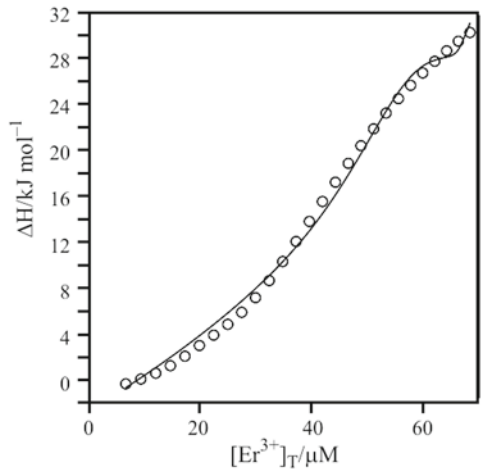

Fig. 1 Comparison between the ○ – experimental enthalpies, ΔH, for HSA+Er^{3+} interactions and — – calculated data via Eq. (1). $[Er^{3+}]_T$ is total concentrations of Er^{3+} solutions

Table 1 Enthalpies of HSA+Er^{3+} interactions, ΔH, in Er^{3+} solution with water at 300 K in kJ mol^{-1}. ΔH_{dilut} is the enthalpies of dilution of Er^{3+} with water.

$[Er^{3+}]$/μM	ΔH/kJ mol^{-1}	ΔH_{dilut}/kJ mol^{-1}
1.385	–0.273	10.180
4.132	–0.736	11.286
6.849	–0.406	7.785
9.537	0.134	5.856
12.195	0.805	4.682
14.825	1.667	3.878
17.426	2.774	3.303
20.000	4.042	2.873
22.546	5.280	2.556
25.066	6.566	2.285
27.559	8.022	2.082
30.026	9.805	1.893
32.467	11.899	1.742
34.884	14.246	1.605
37.275	16.730	1.491
39.642	19.252	1.365
41.985	21.777	1.265
44.304	24.257	1.166
46.599	26.700	1.054
48.872	29.020	0.958
51.122	31.251	0.868
53.350	33.375	0.788
55.556	35.351	0.728
57.739	37.223	0.664
59.902	38.974	0.605
62.044	40.639	0.542
64.165	42.192	0.485
66.265	43.620	0.435
68.345	44.959	0.389

*Precision is ±0.002 kJ mol^{-1} or better

(Fig. 1). δ^{θ}_A and δ^{θ}_B parameters have been also optimized to fit the data. The optimized δ^{θ}_A and δ^{θ}_B 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 (0.99999) support the method. The binding parameters for HSA+Er^{3+} interactions recovered from Eq. (1) were listed in Table 1. The agreement between the calculated and experimental results (Fig. 1) is striking, and gives considerable support to the use of Eq. (1).

Φ is the fraction of HSA molecule undergoing complexation with Er^{3+} which can be expressed as follows:

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

where ΔH_{max} represents the heat value upon saturation of all HSA. The association equilibrium constant values, K_a, as a function of Er^{3+} free concentration, $[Er^{3+}]_F$, can be calculated as follow:

$$K_a = \frac{\Phi}{(1-\Phi)[Er^{3+}]_F} = \\ = \frac{\Phi}{(1-\Phi)(1-x_B)[Er^{3+}]_T} \quad (6)$$

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

$$K_a = x_A^g - \sum_{i=1}^{g} K_i \frac{x_B^i}{x_A^{i-g}} \quad (7)$$

where K_is are the equilibrium products for the equilibria:

$$HSA(H_2O)_g + i(Er^{3+}) \Leftrightarrow HSA(H_2O)_{g-i}(Er^{3+})_i \quad (E1)$$

K_a values obtained from Eq. (6), have been fitted to Eq. (7) using a computer program for non-linear least-square fitting. Therefore, we can approach to g value simply (g=2 in this work). ν values can be calculated at any concentration of Er^{3+} via Eq. (3). The Gibbs free energies as a function of Er^{3+} concentrations can be obtained as follow:

$$\Delta G = -RT\ln K_a \quad (8)$$

Gibbs energies, ΔG, calculated from Eq. (8) have shown graphically in Fig. 2. ΔS values were calculated using ΔG values and have shown in Fig. 3. Therefore for the first time, we managed to calculate ΔG and ΔS values with using one set of experimental data in one temperature. Binding parameters for HSA+Er^{3+} interactions using the new model are as follow:

K_1=1.950±0.009 μM^{-1} K_2=1.134±0.008 μM^{-1} g=2

Equations (6) and (7) allow us to have the K_a values in every concentrations of Er^{3+} with the least standard deviations and correlation coefficients are so close to one. The abrupt breaks in Figs 2 and 3 are a good evidence for having two different binding sites on HSA. It is possible to see these changes in Fig. 1 too. The low K_a values in the low Er^{3+} concentrations reflect to lower affinity of HSA for Er^{3+} in this domain (Tables 2 and 3). The less negative Gibbs free energies in the low Er^{3+} concentrations (Fig. 2) also indicate the lower affinity in this region. Previous reports revealed that some molecules such as different species of metal ions of mercury [26], paclitaxel and Cu(II) complex of 5,10,15, 20-tetrakis (4-N-benzylpyridyl) porphyrin bind in the two distinct sites with different affinity on HSA which is in a good agreement with our results [27].

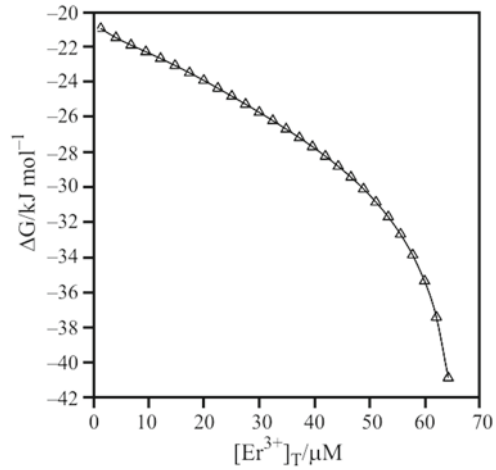

Fig. 2 Comparison between the △ – experimental Gibbs energies values for HSA+Er^{3+} interactions and — – calculated data via Eq. (8). $[Er^{3+}]_T$ is total concentrations of Er^{3+} solutions

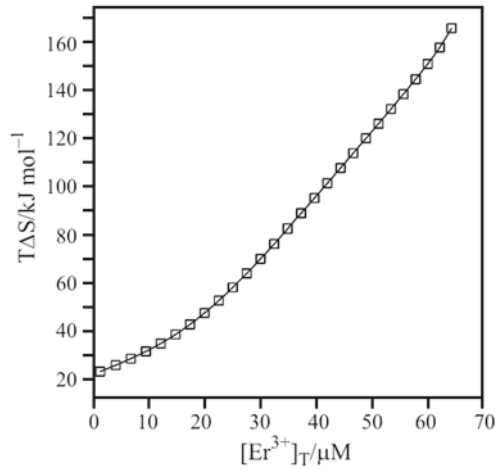

Fig. 3 Comparison between the □ – experimental entropies, $T\Delta S$, for HSA+Er^{3+} interactions and — – calculated data. $[Er^{3+}]_T$ is total concentrations of Er^{3+} solutions

A non-polar 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 δ_A^θ and increase in the stability of proteins. The δ_A^θ value reflects the hydrophobic property of HSA, leading to the enhancement of water structure. The greater the extent of this enhancement, the greater the stabilization of the HSA structure and the greater the value of δ_A^θ. δ_A^θ value (Table 3) for HSA+Er^{3+} interaction is much closed to 0 (Table 3), indicating that Er^{3+} has no significant effect on the HSA structure and its biological activity.

Table 2 The appearance association equilibrium constants, K_a, *vs*. the average number of occupied sites on HSA molecule, ν, and Er^{3+} concentrations.

$[Er^{3+}]/\mu M$	ν	$K_a/\mu M^{-1}$
1.385	0.020	0.004
4.132	0.062	0.004
6.849	0.105	0.001
9.537	0.150	0.0004
12.195	0.195	0.002
14.825	0.243	0.003
17.426	0.291	0.005
20.000	0.342	0.007
22.546	0.394	0.009
25.066	0.448	0.011
27.559	0.504	0.013
30.026	0.561	0.017
32.467	0.621	0.021
34.884	0.683	0.027
37.275	0.747	0.035
39.642	0.814	0.045
41.985	0.884	0.058
44.304	0.956	0.075
46.599	1.031	0.098
48.872	1.109	0.130
51.122	1.191	0.176
53.350	1.275	0.244
55.556	1.364	0.350
57.739	1.457	0.530
59.902	1.554	0.866
62.044	1.655	1.609
64.165	1.762	3.753
66.265	1.874	15.065
68.345	1.991	ERR

Table 3 Thermodynamic parameters for HSA+Er^{3+} interactions in Er^{3+} solution with water via Eq. (1)

[HSA]	*p*	δ_A^θ	δ_B^θ	ΔH_{max}/kJ mol^{-1}
40 μM	0.58	0.000	0.000	46.592

p value is less than one (p=0.58), which indicates that there is negative cooperativity in the two binding sites of HSA. Since the conformational changes of compounds on the carrier protein may be considered to be a deleterious effect of the designed ligand, then these results obtaining from the interaction of Er^{3+} with blood carrier protein of HSA probably provide useful information to design better metal anticancer complexes or metal based drugs with lower side effects in the future.

Conclusions

The new extended solvation model was used to reproduce the enthalpies for the interaction of erbium chloride(III) with HSA. We have calculated all thermodynamic functions, cooperativity parameters, equilibrium constants and stability prediction as a result of ligand interaction with HSA, just using Eqs (1) and (7) and it is a revolution in the ligand+macromolecule interactions.

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