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Thermal investigation of Human Serum Albumin upon Interaction with Ytterbium (III)

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ABSTRACT

In this paper complexation reaction between Yb3+ and Human serum albumin is examined using isothermal titration calorimetry (ITC). The extended solvation model was used to reproduce the enthalpies of HAS+Yb3+ interactions over the whole range of Yb3+ concentrations. The binding parameters recovered from this model were attributed to the structural change of HSA. The results show that Yb3+ ions bind to HSA with three equivalent affinity sites. It was found that in the high concentrations of the ytterbium ions, the HSA structure was destabilized.

1. Introduction

When blood is unavailable, plasma expanders are commonly used to treat patients with significant blood loss by restoring their circulatory volume [1-5]. HSA is naturally produced in the liver and secreted into the bloodstream at a high concentration [6, 7], where it binds a variety of molecules[8]. The protein is composed of three homologous domains (I-III); each domain has two subdomains (A and B) possessing common structural elements[9]. It transports metals, fatty acids, cholesterol, bile pigments, and drugs. In general, albumin represents the major and predominant antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress. A large proportion of total serum antioxidant properties can be attributed to albumin [10-12].

The study of lanthanide series interactions with HSA protein in particular, indicates the importance of the molecular shape of the complexes in addition to the suggested hydrophobic, van der Waals and electrostatic contributions. Ytterbium is a member of lanthanide series, originally known as rare earth

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metals. Ytterbium complex of tetraphenylporphyrin was used as fluorescence label of HSA [13-15]. In this work, we present the most comprehensive study on the interactions of Yb^{3+} ions with HSA for further understanding of the effects of Yb^{3+} ions on the stability and the structural changes of the HSA molecules.

2. Materials and method

Human Serum Albumin (HSA; MW=66411 gr/mol), Tris salt and Yb^{3+} ions obtained from sigma chemical Co. The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system. Yb^{3+} solution (2 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL HSA (75.2 μM at 300 K and 69.7 μM at 310 K) Injection of Yb^{3+} solution into the perfusion vessel was repeated 29 times, with 10 μL per injection. The calorimetric signal was measured by a digital voltmeter that was part of a computerized recording system. The heat of each injection was calculated by the ‘‘Thermometric Digitam 3’’ software program. The heat of dilution of the Yb^{3+} solution was measured as described above except HSA was excluded. The microcalorimeter was frequently calibrated electrically during the course of the study.

3. Results and discussion

We have shown previously that the heats of the ligand + HSA interactions in the aqueous systems can be calculated via the following equation [15-20]:

$$q = q_{\max} x'_B - \delta^{\theta}_A (x'_A L_A + x'_B L_B) - (\delta^{\theta}_B - \delta^{\theta}_A)(x'_A L_A + x'_B L_B) x'_B \tag{1}$$

q is the heat of Yb^{3+} + HSA interaction and $q_{\max}$ represents the heat value upon saturation of all HSA. The parameters δ^{θ}_A and δ^{θ}_B are the indexes of HSA stability in the low and high Yb^{3+} concentrations respectively. Cooperative binding requires that the macromolecule has more than one binding site, since cooperativity results from the interactions between identical binding sites with the same ligand. If the binding of a ligand at one site increases the affinity for that ligand at another site, then the macromolecule exhibits positive cooperativity. Conversely, if the binding of a ligand at one site lowers the affinity for that ligand at another site, then the enzyme 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 a macromolecule for binding with a ligand, respectively; $p = 1$ indicates that the binding is non-cooperative. x'_B can be expressed as follows:

$$x'_B = \frac{p x_B}{x_A + p x_B} \tag{2}$$

We can express x_B fractions, as the total Yb^{3+} concentrations divided by the maximum concentration of the Yb^{3+} upon saturation of all HSA as follows:

$$x_B = \frac{[Yb^{3+}]}{[Yb^{3+}]_{\max}}, \quad x_A = 1 - x_B \tag{3}$$

$[Yb^{3+}]$ is the concentration of Yb^{3+} and $[Yb^{3+}]_{\max}$ is the maximum concentration of the Yb^{3+} upon saturation of all HSA. In general, there will be "g" sites for binding of Yb^{3+} per HSA molecule and ν is defined as the average moles of bound Yb^{3+} per mole of total HSA. L_A and L_B are the relative contributions due to the fractions of unbound and bound metal ions in the heats of dilution in the absence of HSA and can be calculated from the heats of dilution of Yb^{3+} in the buffer solution, q_{dilut}, as follows:

$$L_A = q_{dilut} + x_B\left(\frac{\partial q_{dilut}}{\partial x_B}\right), \qquad L_B = q_{dilut} + x_A\left(\frac{\partial q_{dilut}}{\partial x_B}\right) \tag{4}$$

The heats of Yb^{3+}+HSA interactions, q, were fitted to Eq. 1 across the whole Yb^{3+} compositions. In the fitting procedure, *p* was changed until the best agreement between the experimental and calculated data was approached (Fig. 1). The optimized δ_A^θ and δ_B^θ 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 (0.999) support the method. The binding parameters for Yb^{3+}+HSA interactions recovered from Eq. 1 were listed in Table 2. The agreement between the calculated and experimental results (Fig. 1) is striking, and gives considerable support to the use of Eq. 1. δ_A^θ value for Yb^{3+}+HSA interactions in the low concentrations of the metal ions at 300 and 310 K is positive, indicating that in the low concentrations of the metal ions the HSA structure is stabilized. δ_B^θ value for Yb^{3+}+HSA interactions in the high concentrations of the metal ions at 300 and 310 K is negative, indicating that in the high concentrations of the metal ions the HSA structure is destabilized, resulting in an decrease in its activity. Destabilization by a ligand indicates that the ligand binds preferentially (either at more sites or with higher affinity) to the unfolded (denatured) enzyme or to a partially folded intermediate form of the enzyme. Such effects are characteristic of nonspecific interactions, in that the nonspecific ligand binds weakly to many different groups at the protein, so that binding becomes a function of ligand concentration, which is increased through unfolding events. *p* value for Yb^{3+}+HSA interactions at 300 and 310 K is 1, indicating that the interaction is non-cooperative.

According to the recently data analysis method, a plot of $(\frac{\Delta q}{q_{\max}})M_0$ versus $(\frac{\Delta q}{q})L_0$ should be a linear plot by a slope of 1/g and the vertical-intercept of $\frac{K_d}{g}$, which *g* and K_d can be obtained [15-21].

$$\frac{\Delta q}{q_{\max}} M_0 = (\frac{\Delta q}{q})L_0 \frac{1}{g} - \frac{K_d}{g} \tag{5}$$

Where *g* is the number of binding sites, K_d is the dissociation equilibrium constant, M_0 and L_0 are concentrations of HSA and Yb^{3+}, respectively, $\Delta q = q_{\max} - q$, *q* represents the heat value at a certain Yb^{3+} ion concentration and q_{max} represents the heat value upon saturation of all HSA. If *q* and q_{max} are calculated per mole of HSA then the molar enthalpy of binding for each binding site (ΔH) will be $\Delta H = q_{max}/g$. Dividing the q_{max} amount of (12500 and 17200 μJ equal to 92.34 and 137.09 $kJmol^{-1}$) by *g* =3, therefore, gives ΔH= 30.78 and 45.7 $kJmol^{-1}$ at 300 and 310 K, respectively.

To compare all thermodynamic parameters in metal binding process for HSA, the change in standard Gibbs free energy (ΔG°) should be calculated according to the equation (6), which its value can use in equation (7) for calculating the change in standard entropy (ΔS°) of binding process.

$$\Delta G^\circ = -RT \ln K_a, \tag{6}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ, \tag{7}$$

where K_a is the association binding constant (the inverse of the dissociation binding constant, K_d). The K_a value are obtained 5.59×10^5 and 9.17×10^5 $L.mol^{-1}$ at 300 and 310 K, respectively, Hence:

T=300 K ΔG° = -33 kJ mol^{-1} ΔS° = 0.21 kJ mol^{-1} K^{-1}

T=310 K $\Delta G^{\circ} = -34.24$ kJ mol^{-1} $\Delta S^{\circ} = 0.25$ kJ mol^{-1} K^{-1}

It means that the binding process is spontaneous resulted by entropic driven. All thermodynamic parameters for the interaction between HSA and Yb^{3+} ion have been summarized in Table 1. All thermodynamic parameters of the complex formation including ΔG°, ΔH°, ΔS°, indicate that the process is endothermic and entropy driven. This issue shows the predominant role of hydrophobic forces in the interaction between Yb^{3+} and HSA. Structure of HSA have a hydrophobic core in which side chains are concealed from water, which stabilizes the folded state, and polar side chains interact with surrounding water molecules

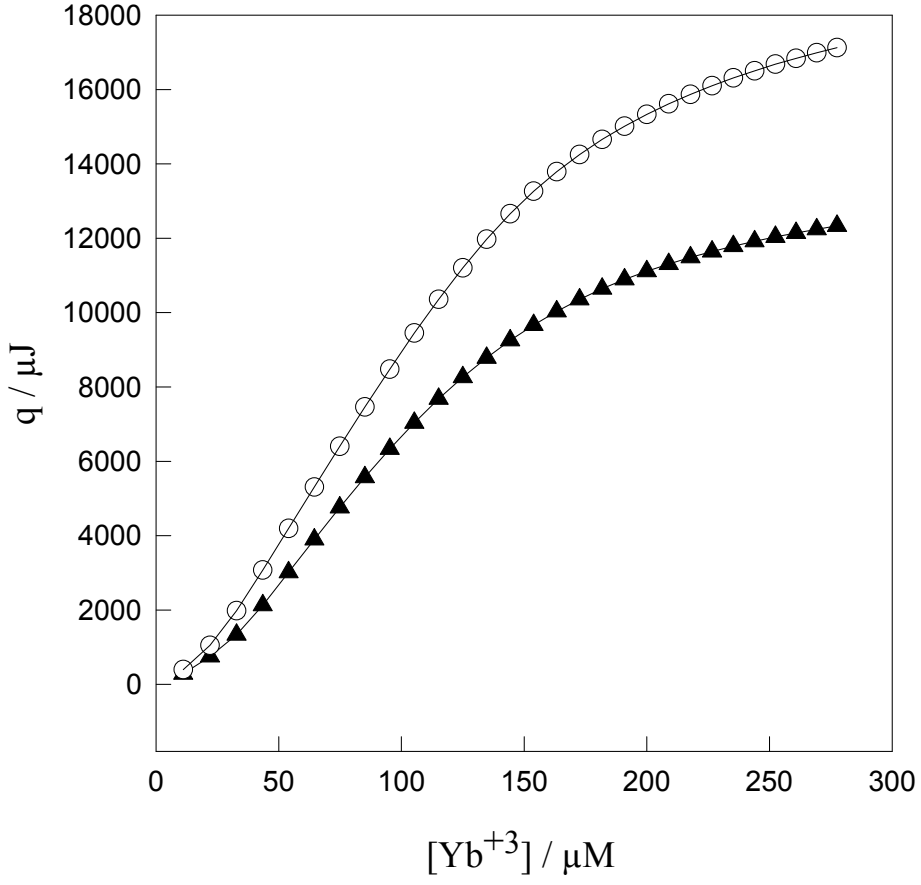

Fig. 1. Comparison between the experimental heats (▲) at 300 K, (○) at 310 K for Yb^{3+} + HSA interactions and the calculated data (lines) via Eq. 1. The [Yb^{3+}] are the concentrations of [$YbCl_3$] solution in μM.

Table 1 Binding parameters for HSA+Yb^{3+} interactions. The big association equilibrium constant values show that there is strong interaction between Yb^{3+} and HSA. The entropy driven of the interaction indicates, minimizing the number of hydrophobic side chains exposed to water is the principal driving force behind the folding process. The large and negative values of δ_A^θ and δ_B^θ show that HSA has been destabilized in the low and high concentrations of Yb^{3+} ions

parameters	T=300K	T=310K
$K_a / Lmol^{-1}$	$5.59\times10^5\pm210$	$9.17\times10^5\pm210$
P	1±0.01	1±0.01
δ_A^θ	29.04±0.14	14.05±0.14
δ_B^θ	-53.22±0.16	-71.57±0.16
ΔH / kJmol^{-1}	30.78±0.09	45.7±0.09
ΔG / kJmol^{-1}	-33 ±0.07	-34.24±0.07
ΔS / kJmol^{-1}K^{-1}	0.21±0.02	0.25±0.02

4. Conclusion

The large association equilibrium constant values show that the interaction between Yb^{3+} and HAS is so strong. The entropy driven of the interaction indicates, minimizing the number of hydrophobic side chains exposed to water is the principal driving force behind the folding process. The large and negative values of δ_A^θ and δ_B^θ show that HSA has been destabilized in the low and high concentrations of Yb^{3+} ions. Precise binding parameters for HSA+Yb^{3+} interactions support the predictive extended solvation model.

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