Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
ISSN 1984-6428

| Vol 9 | | No. 1 | | January-March 2017 |

Full Paper

Lanthanide Chlorides Decrease the Antioxidant Property of Human Serum Albumin

Mahmood Payeghadr[a], Gholamreza Rezaei Behbehani[b]*, Monir Shalbafan[b]

[a]*Department of Chemistry, Payame Noor University, 19395-4697 Tehran, Iran*
[b]*Department of Chemistry, Imam Khomeini International University, Qazvin, Iran*

Article history: Received: 07 April 2016; revised: 11 January 2017; accepted: 12 January 2017. Available online: 13 February 2017. DOI: http://dx.doi.org/10.17807/orbital.v9i1.941

Abstract: Substantial conformational changes of human serum albumin, HSA, induced by Lanthanide chlorides (Ce^{3+}, La^{3+}, Er^{3+}) in phosphate buffer, 10 mmol L^{-1} at pH 7.4, was studied by Isothermal Titration Calorimetry, ITC. The strong negative cooperativity of Lanthanide chlorides interaction with HSA recovered from the extended solvation model, indicates that HSA denatures as a result of its interaction with toxic Lanthanide chlorides.

Keywords: human serum albumin; the extended solvation model

1. INTRODUCTION

It is well known that unfolding of some small proteins presents highly cooperative two-state behavior, while unfolding of multidomain proteins with populations of partially folded states involves a multi-stage process [1]. The protein stability and folding pathways are closely dependent on the various solvent ionic compositions [2]. Salts are known to affect these in a variety of ways, such as specific and non-specific binding of ions to the protein molecules and electrostatic shielding of charges [3]. The interactions between ions and residues in proteins can enhance the stability of some proteins [2-4]. HSA has one cysteine residue at position 34 (in domain I) with a free sulfhydryl group. HSA plays a special role in transporting metabolites and drugs throughout the vascular system. Aromatic and heterocyclic ligands were found to bind within two hydrophobic pockets in subdomains IIA and IIIA (Figure 1), namely site I and site II [1-4]. HSA has a high affinity metal binding site at the N-terminus.

The wide spread use of the lanthanide compounds and their applications in agriculture and medicine have raised great public concern regarding the toxicity of the lanthanide salts. Cerium oxalate was used as an antiemetic, especially in the vomiting during pregnancy and kinetoses, although its mechanism of action has never been clarified. Lanthanum carbonate is used as phosphate binders to reduce the absorption of phosphate, typically in people with chronic kidney failure [5, 6].

Erbium will gradually accumulate in soils and water soils and this will eventually lead to increasing its concentrations in human, animals and soil particle. Erbium ions are dangerous, due to the fact that damps and gasses can be inhaled with air. This can cause lung embolisms, especially during long-term exposure. Erbium ion is harmfull for the liver when it accumulates in the human body [6].

The results obtained from the most predictive theory of the extended solvation model, in this study, indicate a huge unfolding of HSA structure. The strong negative cooperativity, obtained from this powerfull model, were suggested the denaturation of HSA by toxic Lanthanide ions. Therfore, it is posssible to conclude that the treatment with lanthanide ions could increase the oxidative stresses because of HSA denaturation.

2. MATERIAL AND METHODS

2.1. Materials

HSA (MW=66411g mol^{-1}) was obtained from Sigma chemical Co. and Lanthanide salts ($CeCl_3$,

*Corresponding author. E-mail: grb402003@yahoo.com

$LaCl_3$, $ErCl_3$) were purchased from Merck. All other materials and reagents were of analytical grade, and solutions were made in 50 mmol L-1 buffer phosphate using double-distilled water.

2. 2. Methods

The micro calorimeter consists of a reference and a sample cell including 1.8 mL of the solutions, with both cells insulated by an adiabatic shield. All solutions were degassed, by stirring under vacuum, before being used. The sample cell was loaded with HSA solution (40 µmol L^{-1}) and the reference cell contained the buffer solution. The solution in the cell was stirred at 307 rpm by the syringe (equipped with micro propeller), filled with Lantanide solutions (500 µmol L^{-1}) to ensure rapid mixing. The titration of HSA with Lanthanide solution involved 30 consecutive injections of the ligand solution, the first injection was 5µL 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 Lanthanide salts dilution, control experiments were done, in which identical aliquots were injected into the buffer solution with the exception of HSA. The heats of interactions were measured by Isothermal Titration Calorymetery. The measurements were performed under constant temperature of 27.0 ± 0.02 ºC and the temperature was controlled using a Poly-Science water bath. The experimental heats of interactions have been shown graphycally in figure 2.

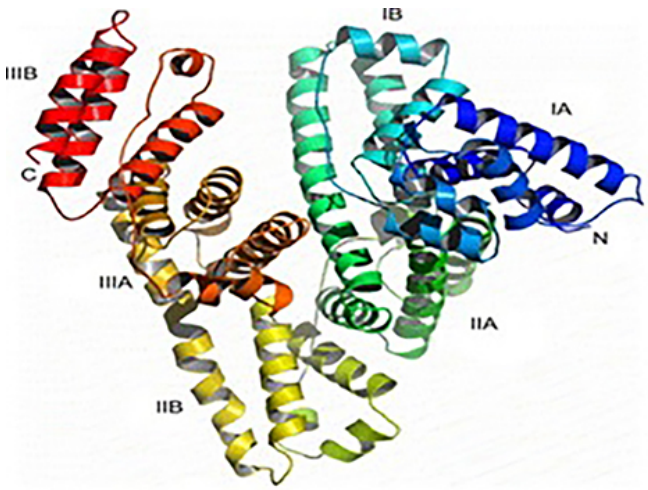

Figure 1. The three-domain structure of HSA.The protein secondary structure is shown in different colors (N- and C-termini are marked as N and C, respectively).

3. RESULTS AND DISCUSSION

We have shown previously that the heats of the biomolecules + ligands interactions (q), in the aqueous solvent system can be reproduced using the following equation [7-9].

$$q = q_{max}x'_B - \delta_A(x'_AL_A + x'_BL_B) - (\delta_B - \delta_A)(x'_AL_A + x'_BL_B)x'_B \qquad (1)$$

The parameters of δ_A and δ_B reflect to the net effect of Lanthanide ions on the HSA structural changes in the low and high ligands concentrations, respectively. The positive values for δ_A and δ_B indicate that the ligands stabilized the HSA structure, while the negative values of δ_A and δ_B show that HSA is destabilized as a result of its interaction with the ligand. 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 [7-9].

x'_B can be expressed as:

$$x'_B = \frac{px_B}{x_A + px_B} \qquad (2)$$

x'_B he fraction of bound ligand and $x'_A = 1 - x'_B$ is the fraction of unbound ligand. We can express x_B

fractions, as the ligand concentrations, after each injection divided by the maximum concentration of the ligand upon saturation of all HSA, [ligand]max as follows:

$$x_B = \frac{[\text{ligand}]}{[\text{ligand}]_{max}} \qquad (3)$$

LA and LB are the relative contributions of unbound and bound Ligand in the dilution heats of ligand in the absence of HSA. The heats of HSA+Ligand ions interactions were fitted to equation 1 over the whole ligand concentrations (Figure 2). During the procedure the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached. The binding parameters for HSA+Lanthanide chlorides complexes recovered from equation 1 were reported in Table 1.

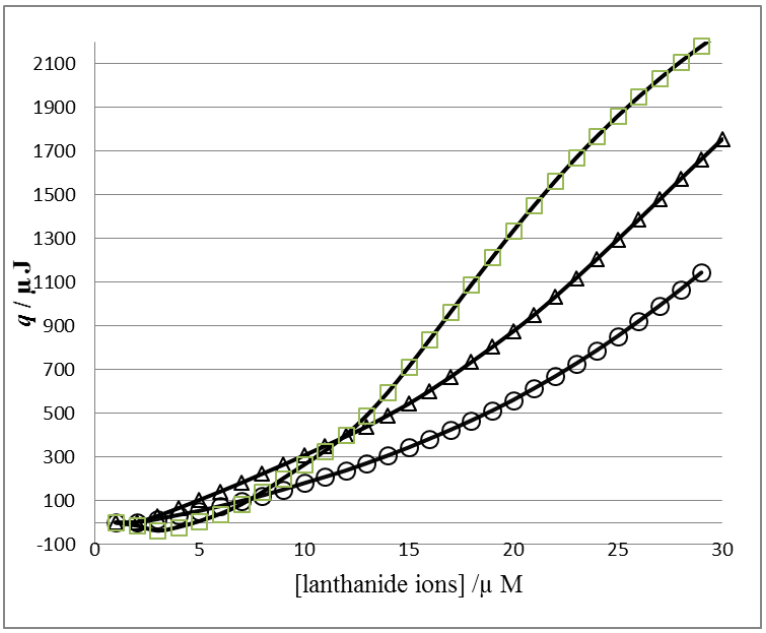

Figure 2. Comparison between the experimental heats, *q*, for HSA+Lanthanide ions [$ErCl_3$ (□), $LaCl_3$ (Δ) and $CeCl_3$ (O)] interactions and calculated data (lines) via equation 1.

Table 1 Thermodynamic parameters for HSA+Lanthanide chlorides interactions via equation 1. Strong negative cooperativity (p<<1) shows the irreversible denaturation of HSA by toxic Lanthanide ions.

HSA+Ligand	*p*	ΔG_D^0 / kJ mol^{-1}	ΔH_D^0 / kJ mol^{-1}	ΔS_D^0 / kJ mol^{-1}K^{-1}
HSA+Ce^{3+}	0.27	32.73	15.56	-0.06
HSA+La^{3+}	0.396	-19.85	70.65	0.30
HSA+Er^{3+}	0.58	31.74	142.00	0.37

The agreement between the calculated and experimental results is striking, and gives considerable support to the use of equation 1. The negative cooperativity shows that the lanthanid salts binding with HSA results in a loss of its native structure. The prediction of strong negative cooperativity of Lanthanide ions binding with HSA recovered from the extended solvation model (p<<1 in table 1), indicates that HSA denatured as a result of its interaction with the Lanthanide salts. The distinguish of negative cooperativity easily and its correlation to the protien denaturation that is hardly predicted by the previously introduced models, is the important aspect of the extended solvation model (equation 1). Denaturation of HSA by Lanthanide ions indicates that Lanthanide ions bind preferentially to the unfolded or partially denatured HSA. Such effects are characteristic of nonspecific interactions, in which the binding becomes a function of Lanthanide ions concenteration, indicating a large structural change in a tiny range of Lanthanide ions concentrations (evidenced by sharp slopes in figure 2), suggesting a quick denaturation of

HSA. The results recovered from the extended solvation model, are indicative of nonspecific interactions between HSA and Lanthanide ions, suggesting irreversible denaturation of HSA by Lanthanide ions. The results obtained from the extended solvation model, indicates that HSA have lost its antioxidant properties Denaturation of HSA structure is a very bad side effect of using toxic Lanthanide compounds as drugs.

4. CONCLUSION

The interaction of HSA with Lanthanide ions was studied by Isothermal Titration Calorimetry method. The results recovered from the extended solvation model, indicate that Lanthanide ions induces irreversible denaturation of the HSA structure. These conformational alterations are accompanied by a significant alteration of secondary structures. The negative cooperativity and its correlation to the protien denaturation that is hardly predicted by previous models [10, 11], is the new and important aspect of the extended solvation model.

5. ACKNOWLEDMENTS

The Universities of Imam Khomeini (Qazvin) and Payam noor are acknowledged for their financial supports of the work.

6. REFERENCES AND NOTES

[1] Artali R, Bombieri G, Calabi L, Del Pra A. *Farmaco*. **2005**, 60, 485-495.

[2] Shang L, Wang YZ, Jiang JG, Dong SJ. *Langmuir*. **2007**, 23, 2714-2721. [CrossRef]

[3] Rumfeldt JA, Stathopulos PB, Chakrabarrty A, Lepock JR, Meiering EM. *J Mol Biol.* **2006**, 355, 106-121. [CrossRef]

[4] Rosell FI, Mauk MR, Mauk AG. *Biochem*, **2005**, 44, 1872-1879. [CrossRef]

[5] Ouattara M, Cunha EB, Li X, Huang YS, Dixon DW. *Mol. Microbiol*. **2010**, 78, 739-756. [CrossRef]

[6] Honsa ES, Maresso AW. *Biometals.* **2011**, 24, 533-545. [CrossRef]

[7] Rezaei Behbehani G, Saboury AA, Taherkhani A, Barzegar L, Mollaaghazade A. *J Therm Anal Calorim*. **2011**, 105, 1081-1086. [CrossRef]

[8] Rezaei Behbehani G, Saboury AA, Barzegar L, Zarean O. *J Therm Anal Calorim.* **2010**, 101, 379-384. [CrossRef]

[9] Rezaei Behbehani G. Saboury AA.Taleshi E. *J Mol Recognit*. **2008**, 21, 132-135. [CrossRef]

[10] Westermark PO, Lansner A. *Biophys*. **2003**, J. 85:126–139. [CrossRef]

[11] Ortiz A. *Journal of Uncertain Systems.* **2013**, 7, 198-202.