Human Serum Albumin Binding with Erbium (III) Chloride

Rezaei Behbehani G, and Shalbafan M

***Abstract*---**Erbium (III) Chloride-induced Conformational changes of human serum albumin, HSA, in phosphate buffer, $10\,\text{mM}$ at $p\text{H}\,7.4$ were investigated, using isothermal titration calorimetry, ITC, UV and fluorescence emission spectroscopy. The results indicate that $ErCl_3$, Er^{3+}, induces irreversible denaturation of the HSA structure. The UV absorption intensity of $HSA + Er^{3+}$ shows the slight blue shift in the absorbance wavelength with increasing Er^{3+} concentration. The fluorescence intensity was increased regularly and the slight red shift was observed in the emission wavelength. The $HSA + Er^{3+}$ complex increases the fluorescence of HSA and changes the microenvironment of tryptophan residue, suggesting the loss of the tertiary structure of HSA. The results obtained from the ITC data are in agreement with the spectroscopic methods. The strong negative cooperativity of Er^{3+} binding with HSA recovered from the extended solvation model, indicates that HSA has been denatured as a result of its interaction with Er^{3+} ions.

***Keywords*---**Human Serum Albumin, Isothermal titration calorimetry, Fluorescence spectroscopy, Erbium (III) chloride, UV spectroscopy.

I. Introduction

IT is well known that unfolding of some small proteins presents two-state behavior, while unfolding of multi domain 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]. The interactions between ions and residues in proteins can enhance the stability of some proteins. This actually suggests that specific binding of ions with residues in a protein has important implications for the biological functions of 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 10), namely site I and site II [5]-[10]. HSA has high affinity metal binding site of the N-terminus. We have reported the Er^{3+} ions induced unfolding event of the most abundant protein contained in the plasma, human serum albumin. The spectroscopic studies show that the HSA unfolding process is consistent with our previously reported ITC results, suggesting irreversible denaturation of HSA by Er^{3+} ions.

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II. Material and Methods

HSA was obtained from Sigma and $ErCl_3$ was purchased from Merck. Protein concentrations were determined from absorbance measurements at $279\,\text{nm}$ in 1 cm quartz cuvettes. All other materials and reagents were of analytical grade, and solutions were made in $50\,\text{mM}$ buffer phosphate using double-distilled water. The UV spectra is recorded on Unico 4802 double beam spectrophotometer with protein concentration of $20\mu\text{M}$. All fluorescence spectra and intensities were recorded using a PERKIN Elmer Bio 100 spectrofluorimeter equipped with a xenon lamp source and 1.0cm quartz cells, excitation and emission wavelengths were 295 and $334\,\text{nm}$, respectively and excitation and emission bandwidths were both $5\,\text{nm}$. The $HSA + Er^{3+}$ binding investigation was carried out by fluorimetric titration of HSA according to following titration procedure (temperature was maintained by a thermostat bath with circulating water throughout all the experiments): a cuvette of 1cm was filled with $400\mu\text{L}(20\mu\text{M})$ HSA solution. After recording the fluorescence intensities of HSA , $50\mu\text{L}$ of $ErCl_3$ stock solution ($2000\mu\text{M}$) was added consecutively. The isothermal titration calorimetric experiments were carried out on a VP-ITC ultra-sensitive titration calorimeter (Micro Cal, LLC, Northampton, MA). The micro calorimeter consists of a reference cell and a sample cell of 1.8mL in volume, 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 ($40\,\mu\text{M}$) and the reference cell contained buffer solution. The solution in the cell was stirred at 307rpm by the syringe (equipped with micro propeller), filled with $ErCl_3$ solution ($500\,\mu\text{M}$) to ensure rapid mixing. The titration of HSA with $ErCl_3$ solution involved 30 consecutive injections of the ligand solution, the first injection was $5\mu\text{L}$ and the remaining ones were $10\mu\text{L}$. In all cases, each injection was done 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. The measurements were

performed at a constant temperature of $27.0 \pm 0.02°C$ and the temperature was controlled using a Poly-Science water bath.

III. Results and Discussion

Results of study have been shown in figures from I through XI. It is well known that *HSA* is a monomeric protein comprising 585 amino acids and its secondary structure is mainly α-helix and 17 disulfide bridges. The initial crystal structure analyses have revealed that the principal regions of ligand binding sites of albumin are located in hydrophobic cavities in subdomains IIA and IIIA, and the tryptophan residue (Trp-214) is in subdomain IIA [10]-[15]. The UV absorption intensity of *HSA* decreased regularly with the addition of Er^{3+} ion concentration and the slight blue shift was observed in the wavelength with increasing Er^{3+} ion concentration (Figures I to IV).

UV spectrum of HSA shows two maximum at 279 and $205\,\text{nm}$. The band in $279\,\text{nm}$ is related to $\pi \to \pi^*$ transition of aromatic amino acids of tyrosine, phenylalanine and tryptophan and the absorption at 205 nm is related to the $\pi \to \pi^*$ transition of amide groups of peptide bonds.

The intrinsic fluorescence of *HSA* is almost contributed to tryptophan, because phenylalanine has a very low quantum yield and the fluorescence of tyrosine is almost totally quenched [11]. The change of fluorescence intensity of *HSA* is related to the tryptophan residue when the small molecules are added to HSA. The fluorescence spectra of HSA at various concentrations of Er^{3+} ions are shown in figure 5. The fluorescence intensity of *HSA* increased regularly and the slight red shift was observed in the emission wavelength with increasing Er^{3+} ion concentration, indicating that the $HSA + Er^{3+}$ complex was formed. Therefore, we concluded that the binding took place near Trp-214 and led to a conformational change with a local perturbation of the IIA binding site on *HSA* (figure X). Figures X to XII represent fluorescence emission spectra of the $HSA + Er^{3+}$ system obtained in $0.05M$ phosphate buffer, $p\text{H}\ 7.4$, ionic strength 0.1, at $298K$. The fluorescence intensities of *HSA* increased regularly and the slight red shift was observed for the emission wavelength with increasing Er^{3+} ion concentration, indicating that the microenvironment of tryptophan residue was changed. Therefore, we conclude that the binding took place near Trp-214 and led to a conformational change with a local perturbation of the IIA binding site in *HSA*. This increasing of emission shows the unfolding of the tertiary structure of human serum albumin.

The standard Gibbs free energy of denaturation, ΔG_D^0, is determined based on two state process as follows:

$$Native\ (N) \leftrightarrow Denaturerd\ (D) \tag{1}$$

The denaturation process can be monitored through the changes of the emission intensity at $334\,\text{nm}$. Assuming a two state mechanism, the denatured fraction of protein (F_D) and the dissociation equilibrium constant of the process (K_D) could be calculated using the following equations:

$$F_D = \frac{(Y_N - Y_{obs})}{(Y_N - Y_D)} \tag{2}$$

$$K_D = \frac{F_D}{1 - F_D} = \frac{(Y_N - Y_{obs})}{(Y_{obs} - Y_D)} \tag{3}$$

Y_N and Y_D are the intensity emission of a fully native and denatured conformation of HSA, while Y_{obs} is the emission intensity in every stage of the unfolding process, respectively. Then the standard Gibbs free energy of denaturation (ΔG_D^0) can be obtained as follows:

$$\Delta G_D^0 = RT \ln K_D \tag{4}$$

$$\Delta G_D^0 = \Delta H_D - T\Delta S_D \tag{5}$$

Where R is the universal gas constant and *T* is the absolute temperature. Figures 8 and 9 show the free energy changes versus temperature at $p\text{H}\,7.4$. We have shown previously that the heats of the $HSA + Er^{3+}$ interactions, *q*, in the aqueous solvent system can be reproduced using the following equation [11]-[15]:

$$q = q_{\max} x'_B - \delta_A (x'_A L_A + x'_B L_B) - (\delta_B - \delta_A)(x'_A L_A + x'_B L_B) x'_B \tag{6}$$

The parameters of δ_A and δ_B reflect to the net effect of Er^{3+} ions on the *HSA* stability in the low and high Er^{3+} 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. 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:

$$x'_B = \frac{p x_B}{x_A + p x_B} \tag{7}$$

x'_B is the fraction of bound Er^{3+} and $x'_A = 1 - x'_B$ is the fraction of unbound Er^{3+}. We can express x_B fractions, as the Er^{3+} concentrations, $[Er^{3+}]$, after every injection divided by the maximum concentration of the Er^{3+} upon saturation of all HSA, $[Er^{3+}]_{\max}$ as follows:

$$x_B = \frac{[Er^{3+}]}{[Er^{3+}]_{\max}} \tag{8}$$

L_A and L_B are the relative contributions of unbound and bound Er^{3+} in the dilution heats of Er^{3+} in the absence of HSA. The heats of $HSA + Er^{3+}$ interactions were fitted to equation 6 over the whole Er^{3+} compositions. During the procedure the only adjustable parameter (p) was changed until the good agreement between the experimental and calculated data were

approached. The binding parameters for $HSA + Er^{3+}$ interactions recovered from equation 6 are listed in table I. The agreement between the calculated and experimental results (figureXI) gives considerable support to the use of equation 6. The results obtained from ITC data are in agreement with the spectroscopic methods. The strong negative cooperativity of Er^{3+} binding with HSA recovered from the extended solvation model (p=0.46 in table I), indicates that HSA denatured as a result of its interaction with Er^{3+} ions. Destabilization of HSA by Er^{3+} ions indicates that Er^{3+} ions bind preferentially to the unfolded or partially denatured HSA. Such effects are characteristic of nonspecific interactions, in that Er^{3+} ions bind weakly to many residues of the protein, so that binding becomes a function of Er^{3+} ions, which is increased through the denaturation process. As it is clear in figure XI, there is a large structural change in a tiny range of Er^{3+} ion concentrations, suggesting a quick denaturation of *HSA*. These results strongly suggest that the nonspecific interactions are dominant. The positive and small value for δ_A indicates preferential binding of Er^{3+} ions with the unfolded (or partially folded) states of HSA. This is opposed to the specific interactions that could be defined as preferential interactions between ligands and the native folded state of a protein.

Therefore, both spectroscopic and calorimetric results points toward nonspecific interactions between HSA and Er^{3+} ions, suggesting irreversible denaturation of HSA by Er^{3+} ions. Erbium compounds have a high magnetic moment of 9.3 Bohr magnetons, thereby, bind to the cell surface of the microorganisms by ionic forces.

TABLE I

THERMODYNAMIC PARAMETERS FOR $HSA + Er^{3+}$ INTERACTIONS IN Er^{3+} SOLUTION WITH WATER VIA EQUATION 6. STRONG NEGATIVE COOPERATIVITY SHOWS IRREVERSIBLE DENATURATION OF HSA BY Er^{3+} IONS.

Parameters	$HSA + Er^{3+}$
p	0.46
δ_A	0.82
δ_B	-0.19
ΔG^0 / kJmol^{-1}	31.74
ΔH_D^0 / kJmol^{-1}	142.00
ΔS_D^0 / kJmol^{-1} K^{-1}	0.37

TABLE II

T_m FOR $HSA + Er^{3+}$ COMPLEX IS LESS THAN THAT OF PURE HSA, SUGGESTING NONSPECIFIC UNFOLDING OF HSA TOWARD IRREVERSIBLE DENATURATION OF HSA.

	T_m / K
HSA	344
$HSA + Er^{3+}$	335

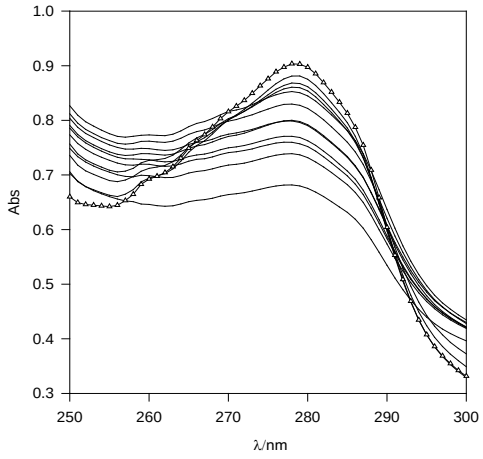

Fig. 1 The effect of Er^{3+} ions on UV spectrum of HSA (Δ) at $298K$. From up to down: 0.1cc of $ErCl_3$ injected to HSA solution (lines). The UV absorption intensity of HSA decreased regularly with the variation of Er^{3+} ion concentration and the slight blue shift was observed in the absorbance wavelength with increasing Er^{3+} ion concentration. Possibly, the positive charge of Er^{3+} ions interacts with protein negative charge and formed a complex in the aqueous buffer solution (50 mM, *p*H 7.4).

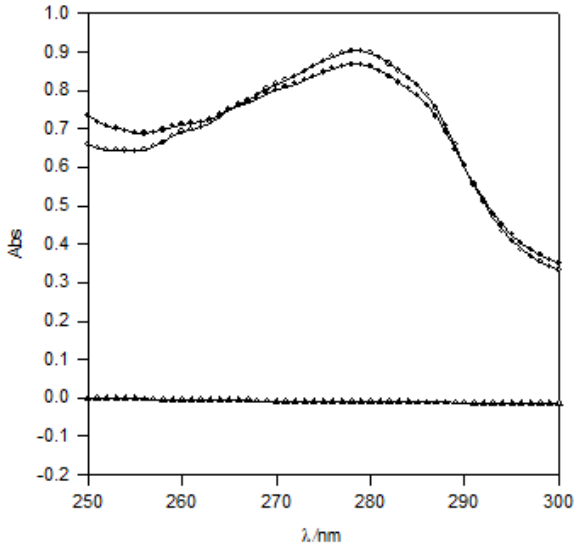

Fig. 2 Comparison between absorption spectrum of $[HSA] = 20\,\mu$M mixture of HSA and $[Er^{3+}] = 0.46$ mM. The absorption peak of Er^{3+} ions (Δ), the absorption peak of HSA ($\circ$) and the experimental absorptions for $HSA + Er^{3+}$ complexes($\bullet$). The UV absorption intensities of $HSA + Er^{3+}$ complex less than peak of HSA, suggesting destabilization of $[HSA]$. A slight blue shift was observed in the wavelength with increasing Er^{3+} ion concentration.

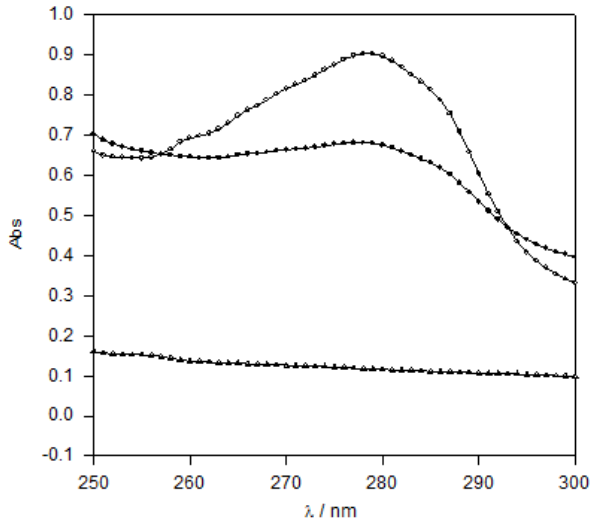

Fig. 3 Comparison between absorption spectrum for $[HSA] = 20\,\mu\text{M}$ and $[Er^{3+}] = 1.66\,\text{mM}$ mixtures. The absorption peak of Er^{3+} ions (Δ), the absorption peak of HSA (○) and the experimental absorptions for $HSA + Er^{3+}$ complexes (●). The UV absorption intensities of $HSA + Er^{3+}$ complexes are less than the peak of HSA and the more blue shift was observed in higher concentration of Er^{3+} ions.

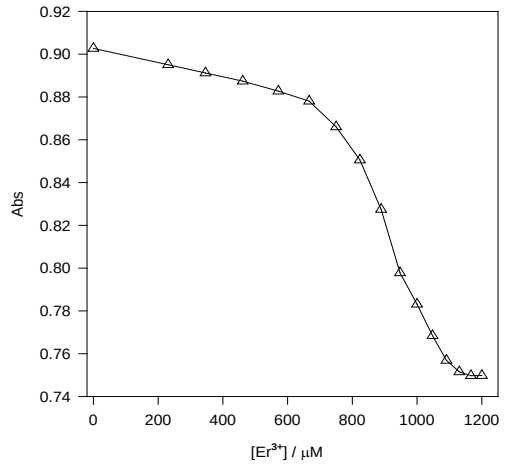

Fig. 4 UV spectrum of HSA ($T = 298K$).The Effect of concentration of Er^{3+} ions on HSA denaturation. $[HSA] = 2.0\times10^{-5}$ M and $[ErCl_3] = 0$, 181.18, 333.33, 461.53, 571.42, 750, 823.52, 888.88, 947.36, 1090.9, 1166.67 and 1200.56 μM).

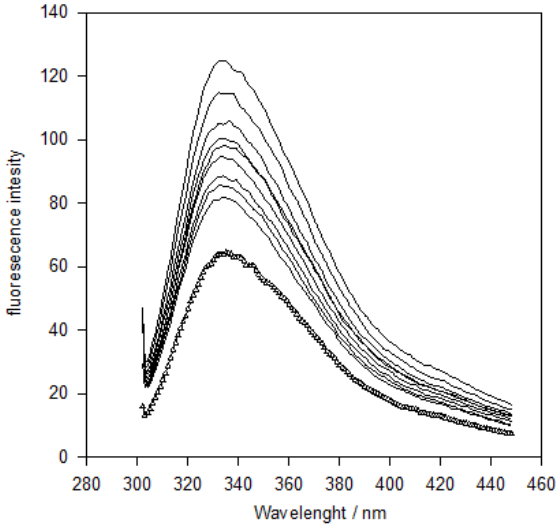

Fig. 5 Fluorescence emission spectra of the $HSA + Er^{3+}$ complexes obtained in $0.05M$ phosphate buffer, pH 7.4, ionic strength 0.1, at $298K$. The excitation wavelength was $295nm$. The concentration of HSA was fixed as: $[HSA] = 20\times10^{-6}$ M and $[ErCl_3] = A\times10^{-4}M$ (A= 2.2, 5.4, 6.6, 7.6, 8.5, 9.3 and 1) respectively (down to up). The fluorescence intensity of HSA increased regularly and slight red shift was observed in the emission wavelength ($335nm$) with increasing $ErCl_3$ concentration, indicating that a $HSA + Er^{3+}$ complex increases the fluorescence intensity of HSA and changes the microenvironment of tryptophan residue.

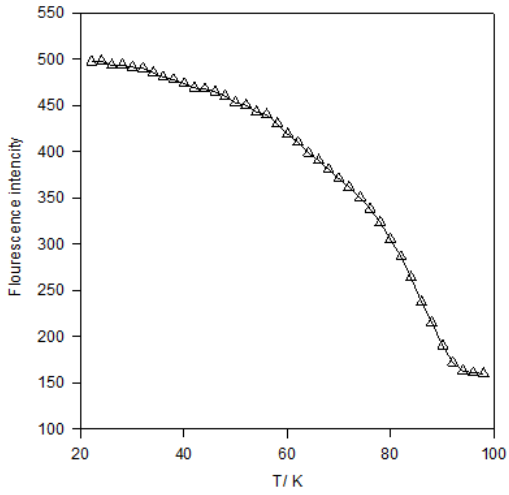

Fig. 6 The effect of increasing temperature on fluorescence quenching of $[HSA] = 20\,\mu\text{M}$. Thermal denaturation induces a modification of the environment of the albumin tryptophan. Denaturation is due to a redistribution of the water around the newly exposed hydrophobic residues of the protein. The reason for unfolding of *HSA* is the exposure of non-polar residues to a polar environment.

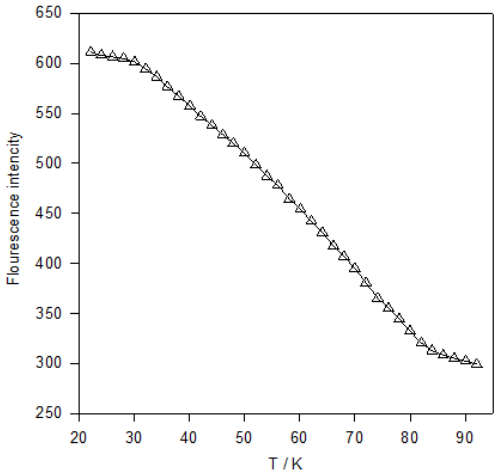

Fig. 7 Fluorescence quenching, indicating the denaturation of $[HSA] = 20\mu\mathrm{M}$ as a result of its interaction with $[ErCl_3] = 1\mathrm{mM}$.

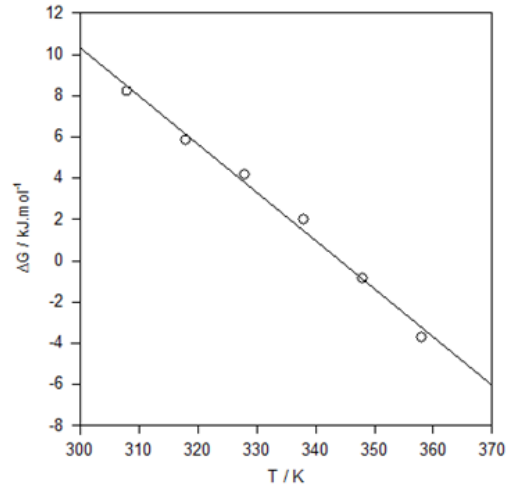

Fig. 8 Thermal denaturation curves of $[HSA] = 20\mu\mathrm{M}$ obtained from temperature scanning of fluorescence. The obtained T_m is $344K$, which can be determined on curves where $\Delta G = 0$.

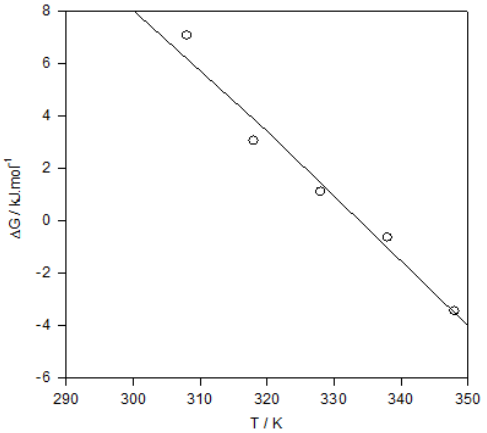

Fig. 9 Thermal denaturation of $[HSA] = 20\mu\mathrm{M}$ + $ErCl_3[1mM]$ obtained from thermal scanning of fluorescence. The calculated T_m is $335K$. indicating that Er^{3+} ions destabilize the HSA native structure.

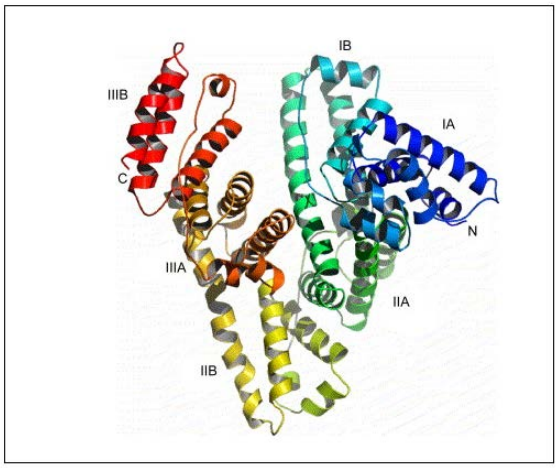

Fig. 9 The three-domain structure of HSA. The protein's secondary structure is shown in different colors. (N-and C-termini are marked as N and C, respectively)

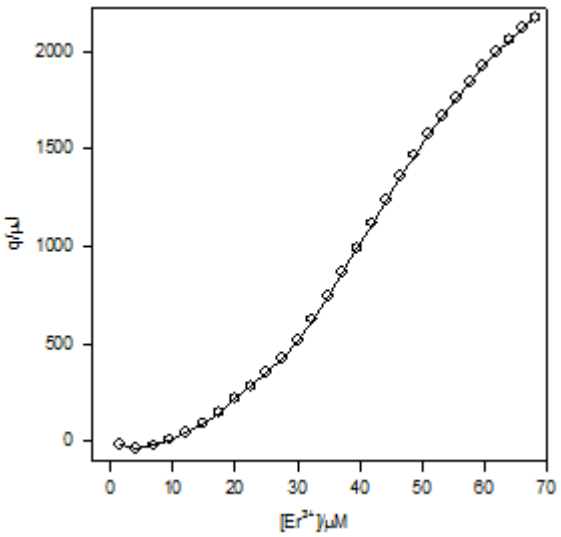

Fig. 9 Comparison between the experimental heats, q, for $HSA + Er^{3+}$ interactions (o) and calculated data (lines) via equation 6. The sharp slope of q against Er^{3+} concenteration during a tiny range of Er^{3+} concentration is indicative of irreversible nonspecific interaction.

IV. Conclusion

Denaturation of *HSA* structure is a very bad side effect of using Er^{3+} compounds as antiviral, cell labeling and Magnetic Resonance Image.

Acknowledgment

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References

[1] Abu Teir M.M., Ghithan SJH., Darwish S., Abu-Hadid M.M. (2011): Study of Progesterone interaction with Human Serum Albumin: Spectroscopic Approach. J. Appl. Biol. Sci. 5 (13), 35-47.

[2] Abu Teir M.M., Ghithan SJH., Darwish S., Abu-hadid M.M. (2012): Multi-spectroscopic investigation of the interactions between cholesterol and human serum albumin. J. Appl. Biol. Sci. 6(3), 45-55.

[3] Ahmad B., Parveen S., Khan R.H. (2006): Effect of albumin conformation on the binding of ciprofloxacin to human serum albumin. Biomacromolecules. 7, 1350-1356. http://dx.doi.org/10.1021/bm050996b

[4] Artali R., Bombieri G., Calabi L., Del Pra A. (2005): A molecular dynamics of human serum albumin binding sites. Il Farmaco 60, 485-495. http://dx.doi.org/10.1016/j.farmac.2005.04.010
[5] Bian Q, Xu LC, Wang SL, Xia YK, Tan LF, Chen JF, Song L, Chang HC, Wang XR (2004). Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. Occup. Environ. Med. 61:999-1005. http://dx.doi.org/10.1136/oem.2004.014597
[6] Cui F, Qin L, Zhang G, Liu X, Yao X, Lei B (2008). A concise approach to 1,11-didechloro-6-methyl-40-O-demethyl rebeccamycin and its binding to human serum albumin: Fluorescence spectroscopy and molecular modeling method. Bioorg. Med. Chem.16:7615-7621. http://dx.doi.org/10.1016/j.bmc.2008.07.017
[7] Cui F, Wang J, Cui Y, Yao X, Qu G, Lu Y (2007). Investigation of interaction between human serum albumin and N6-(2-hydroxyethyl) adenosine by fluorescence spectroscopy and molecular modelling. Luminescence. 22:546-553. http://dx.doi.org/10.1002/bio.999
[8] L. Shang, Y.Z. Wang, J.G. Jiang, S.J. Dong, Langmuir 23 (2007), 2714–2721. http://dx.doi.org/10.1021/la700700e
[9] J.A.O. Rumfeldt, P.B. Stathopulos, A. Chakrabarrty, J.R. Lepock,E.M. Meiering, J. Mol. Biol. 355 (2006) 106–123. http://dx.doi.org/10.1016/j.jmb.2005.10.042
[10] B.Haghighi, M.M. Papari and D. Shahidi.J.Chin.Chem.Soc.55(2008)245.
[11] J.Q. Liu, J.N. Tian, J.Y. Zhang, Z.D. Hu, X.G. Chen, Anal. Bioanal. Chem. 376 (2003) 864–867. http://dx.doi.org/10.1007/s00216-003-2017-8
[12] J.N. Tian, J.Q. Liu, J.Y. Zhang, Z.D. Hu, X.G. Chen, Chem. Pharm. Bull. 51 (2003) 579–582. http://dx.doi.org/10.1248/cpb.51.579
[13] C.Q. Jiang, M.X. Gao, X.Z. Meng, Spectrochim. Acta A 59 (2003) 1605–1610. http://dx.doi.org/10.1016/S1386-1425(02)00362-1
[14] A. Sulkowska, J. Mol. Struct. 614 (2002) 227–232. http://dx.doi.org/10.1016/S0022-2860(02)00256-9
[15] Pace CN. Measuring and increasing protein stability. Trends Biotechnol 1990, 8: 93-8. http://dx.doi.org/10.1016/0167-7799(90)90146-O
[16] Pace CN, Trevino S, Prabhakaran E, Scholtz JM. Protein structure, stability and solubility in water and other solvents.Philos Trans R SocLond B Biol Sci. 2004, 359: 1225-34. http://dx.doi.org/10.1098/rstb.2004.1500
[17] G. Rezaei Behbehani, M. mehreshtiagh, L. Barzegar, A. A. Saboury, J. Serb. Chem. Soc. 78 (2) 255–263 (2013).
[18] G. Rezaei Behbehani, A. Divsalar, A. A. Saboury, A. A. Hekmat, J. Solution Chem. 38 (2009) 219. http://dx.doi.org/10.1007/s10953-008-9360-5
[19] G. Rezaei Behbehani, M. Mirzaie, J. Therm. Anal. Calorim. 96 (2009) 631. http://dx.doi.org/10.1007/s10973-008-9311-9
[20] G. Rezaei Behbehani, A. A. Saboury, M. Mohebbian, S. Tahmasbi Sarvestani, M. Poorheravi, Chin. Chem. Lett. 20 (2009) 1389 http://dx.doi.org/10.1016/j.cclet.2009.05.029
[21] G. Rezaei Behbehani, A. A. Saboury, A. Fallah Baghery, A. Abedini, J. Therm. Anal. Calorim. 93 (2008) 479 http://dx.doi.org/10.1007/s10973-007-8674-7