Study on the Interaction of Zinc Ion Binding with Human Serum Albumin using Isothermal Titration Calorimetry

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Summary: The interaction between zinc ion and human serum albumin (HSA) was investigated by nano-Watt- scale isothermal titration calorimetry (ITC). From the analysis of the ITC data, the binding characteristics and thermodynamic properties of the system were obtained and the binding mechanism was discussed. It was found that the experimental data fit well with the Langmuir's binding theory and the system behaved as a system with two classes of binding sites (high-affinity and low-affinity binding site). The binding number of high-affinity binding site (N_1) is 1.40 and the binding constant (K_1) is 2.72×10^5 L/mol. For the low-affinity binding site, the binding number (N_2) is 1.55 and the binding constant (K_2) is 3.78×10^3 L/mol. Moreover, it was indicated by the thermodynamic analysis that the binding processes of both types of binding sites were exothermic and spontaneous. The high-affinity binding was an enthalpy-entropy synergically driven process and the electrostatic interaction was the main force, while the low-affinity binding was an enthalpy-driven process and this process was mainly driven by the van der Waals forces.

Key words: Isothermal titration calorimetry, Human serum albumin, Zinc ion, Interaction.

Introduction

Metal ions play an irreplaceable role in the process of maintaining normal physiological function of living organisms. Zinc ion is one of the essential trace elements in human body, second only to iron. To be exerted its efficacy, zinc ion must be transported to the target site through blood. Human serum albumin (HSA), the most abundant protein with multi-function, multi-purpose in human blood plasma, contains 585 amino acid residues, 17 disulfide bonds and its molecular mass is about 67 kD. The only tryptophan residue (Trp) locates at 214, consisting of three structural domains: domain I, domain II and domain III and the structure of each domain is divided into A, B two subdomains, each subdomain is made up of three spiral cylinder structures in the form of rabbet abutment joint [1,2]. It is the key carrier protein in blood plasma and can combine with many endogenous and exogenous substances and plays an important role in storage and transport of metal ions in body [3]. Therefore, studies on the binding of metal ions to serum albumin can provide effective information to understanding of the metabolic process and biological effects of metal ions [4-7].

According to the present literatures, it can be known that studies of the interaction between metal ions and serum albumin are mainly performed by means of spectroscopy, electrochemistry and dialysis [8-11]. However, the binding interactions have rarely been investigated by the calorimetric method [12]. Moreover, information of the interaction of zinc ion and serum albumin obtained by calorimetry has not been reported. Therefore, the investigation of the binding of zinc ion to serum albumin by calorimetry is very essential, which may assist in deep understanding of the binding process of metal ions and serum albumin.

Isothermal titration calorimetry(ITC), a non-destructive method for biochemical thermodynamics, is believed to be the most accurate and valuable thermal-chemical method at present, with higher sensitivity and better reproducibility [13,14]. Using this method, the heat changes in the titration reaction process can be detected quantitatively and directly and the thermodynamic parameters such as binding constant K, number of binding sites (N), enthalpy change (ΔH), entropy change (ΔS) and Gibbs free energy (ΔG) can be determined. The interactions of binding systems can be characterized by ITC, irrespective of the nature of solvents, spectroscopic and electrical property of the reactive system and none of the additives was required, thus this method does not interrupt normal functions of the body. ITC has been a powerful tool for the study of binding reaction of the biological macromolecules with drugs or ions [15, 16], such as fluoroquinolone binding to

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serum albumin. In this work, the interaction of zinc ion and HSA was investigated by the ITC method and the thermodynamic parameters of K, N, ΔH, ΔS and ΔG were determined. Furthermore, based on the thermodynamic analysis, the mechanism of interaction between zinc ion and HSA was discussed.

Experimental

Materials

Human serum albumin (HSA, purity≥96.0%) was purchased from Sigma Chemical Company. Zinc chloride ($ZnCl_2$, purity≥98.0%) and trihydroxy aminomethane (Tris, BR) were provided by Shantou Xilong Chemical Corporation of Guangdong and Sino-American Biotechnology Company of Shanghai, respectively. The other reagents, i.e., HCl (mass fraction of 36% to 38%) and NaCl were analytical grade and made in China. Solutions of HSA (1×10^{-5} mol/L) and $ZnCl_2$ (1×10^{-3} mol/L) were prepared with Tris-HCl buffer solution (pH = 7.4).

ITC Measurement

The isothermal titration experiment of binding process of zinc ion and HSA was carried out on the VP-ITC isothermal titration calorimeter [17, 18], with the repeatability of thermal power baseline within ±20nW. HSA solution with the concentration of 1×10^{-5} mol/L was injected into a 1.45 mL calorimeter cell and 250 μL of zinc chloride solution with the concentration of 1×10^{-3} mol/L was absorbed into a syringe. The interval between two injections was 240s and the stirrer in the ampoule was operated at a constant speed of 307 rpm. All the experiments were performed at 298.15K and were started when the baseline was stable. To determine the heat of dilution of HSA and zinc chloride solution, titration experiments of zinc chloride solution injected into buffer solution and buffer solution into HSA solution were performed. The later ones proved that dilution was a very weak endothermic process and heat of dilution is less than 0.3 *k*J/mol, which can be ignored [19].

The dates were collected automatically and subsequently analyzed with a one-site, two site or sequential sites binding model etc. by the Windows-based Origin software package (version 8.5) supplied by MicroCal. The fitting curve was obtained from nonlinear least variance fitting principle and by use of Origin software. From analysis of goodness of the fitting curve compared with the experimental data points, the binding model between HSA and zinc ion was ascertained and the thermodynamics parameters (K, N, ΔH, ΔS and ΔG) were determined. Here need to explain is that the ITC only gives the best model's results by processing experimental data, the software can not provided the detail of the analysis and comparison of the different models results.

Results and Discussion

Thermodynamic Assumptions of the Binding of Zinc Ion to HSA

The binding process of HSA (receptor) and zinc ion (ligand) in the system may be expressed based on the following basic assumptions [20]: (1) One protein (HSA) molecule has i classes of binding sites, which can bind the same ligand. All sites within one class of binding are thermodynamically identical. (2) The i classes of binding sites are assumed to be mutually independent, so that the binding ratio on one class of site does not dependent on that of the other. In this work, it should be stressed that two classes of binding sites existed in the system according to results shown in the following section. So from the above basic assumptions and the well-known Langmuir's binding theory [20-23], we have the following equations:

$$\theta_i = \frac{K_i C_L}{1 + K_i C_L} \quad (1)$$

$$C_{L,0} = C_L + C_{P,0}(N_1\theta_1 + N_2\theta_2) \quad (2)$$

where θ_i (i = 1 or 2) is the binding ratio, namely the average number of ligand molecules (zinc ion) bound per protein molecule. N_i and K_i are number of binding sites, and binding constant of the i class of binding site, $C_{L,0}$ and $C_{P,0}$ are the original concentration of zinc ion and HSA . C_L is the unbound concentration of zinc ion.

Substituting formula (1) into (2):

$$C_{L,0} = C_L + C_{P,0}\left(\frac{N_1 K_1 C_L}{1 + K_1 C_L} + \frac{N_2 K_2 C_L}{1 + K_2 C_L}\right) \quad (3)$$

Then, a cubic equation of C_L can be obtained:

$$C_L^{\,3} + pC_L^{\,2} + qC_L + r = 0 \quad (4)$$

where: $$p = \frac{1}{K_1} + \frac{1}{K_2} - C_{L,0} + (N_1 + N_2) \quad (5)$$

$$q = \frac{1}{K_1 K_2} - C_{L,0}\left(\frac{1}{K_1} + \frac{1}{K_2}\right) + C_{P,0}\left(\frac{N_2}{K_1} + \frac{N_1}{K_2}\right) \quad (6)$$

$$r = -\frac{C_{L,0}}{K_1 K_2} \quad (7)$$

From formula (4), C_L with physical meaning is given:

$$C_L = \frac{2\sqrt{p^2 - 3q}\cos\left(\frac{\theta}{3}\right) - p}{3} \quad (8)$$

where

$$\theta = \arccos\frac{-2p^3 + 9pq - 27r}{2\sqrt{(p^2 - 3q)^3}} \quad (9)$$

The heat, Q_j, for the jth injection in an experimental trail can be expressed as:

$$Q_j = C_{P,0} V_{cell}\left(N_1 \Delta\theta_1 \Delta H_1 + N_2 \Delta\theta_2 \Delta H_2\right) \quad (10)$$

where V_{cell} is the volume of calorimeter cell, $\Delta\theta_i$ is the increment of binding ratio from injection j-1to j and ΔH_i is the binding enthalpy of i class of binding site. From the formula (3) to (9), it can be seen that in the case of $C_{L,0}$ and $C_{P,0}$ being known, C_L is the function of N_i and K_i. Therefore, the maximum likelihood values of six parameters of N_1, N_2, K_1, K_2, ΔH_1, ΔH_2 in equation (10) can be obtained by nonlinear minimum variance fitting based on the ITC results.

Moreover, based on the basic thermodynamic formulas (11) and (12):

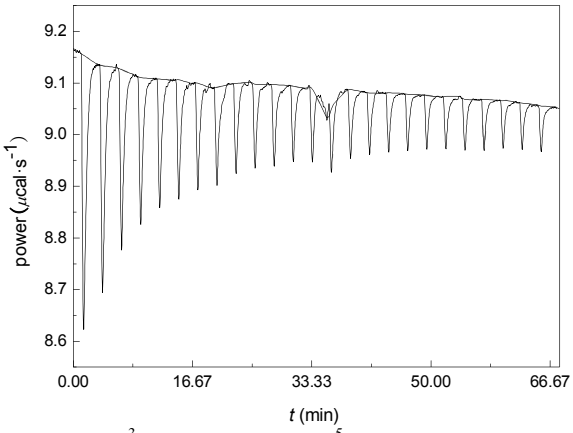
$$\Delta G^\circ = -RT \ln K \quad (11)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (12)$$

The change of Gibbs free energy (ΔG) and entropy (ΔS) of binding reaction also can be calculated.

Binding Parameters and Thermodynamic Properties of the Binding System

The calorimetric data are shown in Fig.1.



$_{Zn}2+ = 1\times10^{-3}$ mol/L, $C_{HSA} = 1\times10^{-5}$ mol/L, T=298.15K

Fig. 1: ITC dates for the binding of zinc ion to HAS.

The raw ITC data for the binding of zinc ion to HSA at 298.15K were shown in Fig.1. It was illustrated that the binding of zinc ion to HSA is an exothermic process, where heat of dilution of the zinc ion dissolved in Tris-HCl buffer solution has been deducted. Treated by software of MicroCal ITC, the experimental data points were found to fit well with the two-site bonding model. This best fitting suggested that two classes of binding sites existed in the binding process of zinc ion to HSA. The nonlinear fitting curve of the binding heat versus molar ratio of zinc ion to HSA at 298.15K was presented in Fig. 2, and the line is the result of simulation with the two-site bonding model and the points were obtained from experiment. Moreover, the corresponding obtained results were given in Table-1.

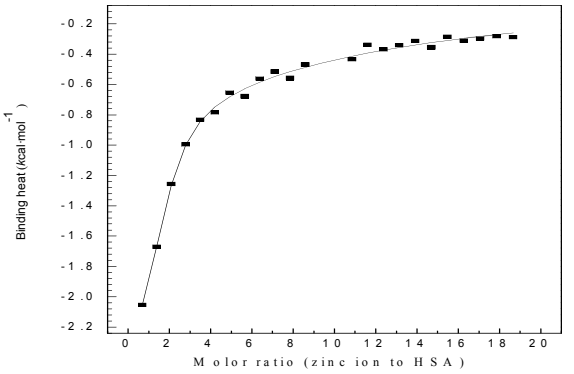


Fig. 2: The curve of the binding heat versus molar ratio of zinc ion to HSA at 298.15K, by the non linear fitting method with the two-site bonding model.

Table-1: Thermodynamic and dynamics parameters of the HSA-Zn binding system.

Parameters	Values	Parameters	Values
N_1	1.40±0.11	N_2	1.55±0.33
K_1 (L·mol^{-1})	(2.72±0.58) ×10^5	K_2 (L·mol^{-1})	(3.78±0.73) ×10^3
ΔG_1 (*k*J·mol^{-1})	-30.97	ΔG_2 (*k*J·mol^{-1})	-20.42
ΔH_1 (*k*J·mol^{-1})	-10.88	ΔH_2 (*k*J·mol^{-1})	-60.49
ΔS_1 (J·mol^{-1}·K^{-1})	67.39	ΔS_2 (J·mol^{-1}·K^{-1})	-134.37

When data are expressed as mean ±S.D, the measurement frequency n=3.

From the goodness of the fitting curve shown in Fig.2, the binding interaction of zinc ion to HSA can be accurately determaained with the two-site bonding model. Table-1 illustrated that the two types of binding sites are the high-affinity sites with the number of binding sites N_1 = 1.40, the binding constant $K_1 = 2.72 \times 10^5$ L/mol and the low-affinity sites with the number of binding sites N_2 = 1.55, the binding constants $K_2 = 3.78 \times 10^3$ L/mol. Based on the comparative analysis of the above values, the information during the binding process of HSA with zinc ions can be indicated. On the one hand, as $K_1 > K_2$, the high-affinity sites of HSA is higher strength binding with zinc ions than the low-affinity sites. On the other hand, as $N_1 < N_2$, the low-affinity sites of HSA bound to more amount of zinc ions, which implied that the low-affinity sites(N_2) played the dominant role in the binding process . In addition, the meaning of the resulting fractional number of binding sites (1.40, 1.55) presented in the text should be mentioned. Here, the binding number does not mean the fractional number of every macromolecule of the protein binding zinc ions strongly at the molecular level. On the contrary, it means that in the solution some molecules bound only one zinc cation (per protein molecule) with high affinity, but some other bound strongly two zinc cations and thus the mean number of bound ions which we observed for this statistical population is the fraction [14, 24-27].

From Table-1, it also can be clearly seen that enthalpy change at high-affinity sites ΔH_1 is -10.88 *k*J/mol and that at low-affinity sites ΔH_2 is -60.49 *k*J/mol; Gibbs free energy change at high-affinity sites ΔG_1 is -30.97 *k*J/mol and that at low-affinity sites ΔG_2 is -20.42 *k*J/mol. Both the Gibbs free energy changes ΔG of the two classes of binding sites are negative and the absolute values are large. It demonstrated that two classes of binding process under the present experimental condition can be spontaneous and thus the HSA-Zn complex is relatively stable.

Discussion of the Interaction Mechanism of Zinc ion and HSA

Weak interaction forces existed in the system of metal ions binding with the biological macromolecules, such as hydrophobic interaction, hydrogen bonding and electrostatic forces [28]. The interaction between zinc ion and HSA can be analyzed based on the obtained changes of thermodynamic properties according to Ross's viewpoints [26]. For the high-affinity binding, as the $\Delta H_1 < 0$ (exothermal) and $\Delta S_1 > 0$ (entropy increase), the interaction between zinc ion and HSA is mainly electrostatic force. As $\Delta G_1 = \Delta H_1 - T\Delta S_1 < 0$ and both exothermic heat and the entropy increase effects result in $\Delta G_1 < 0$, this binding was the process with synergistic driving force of enthalpy-entropy. For the low-affinity binding, due to $\Delta H_2 < 0$ (exothermal) and $\Delta S_2 < 0$ (entropy decrease), the interaction between zinc ion and HSA is mainly the van der Waals forces. Because $|T\Delta S_2| < |\Delta H_2|$, $\Delta G_2 = \Delta H_2 - T\Delta S_2 < 0$, and the enthalpy change led to $\Delta G_2 < 0$, this binding was the enthalpy-driven process [29, 30].

Moreover, to make the experimental condition get close to physiological properties, the pH of the bulk solvent was selected as pH=7.4. A series of temperatures (298.15K, 303.15K, 308.15K, 310.15K and 313.15K) were also set to perform the preliminary experiments. Although the obtained binding parameters by the ITC experiment were different in some extent, the data tendencies were in accordance with the results obtained at 298.15K. Therefore, we only chose the results obtained at 298.15K shown in the text. The influence of pH and temperatures on the binding reaction between the metal ions and serum albumin has been studied by a few researchers [31, 32], which can offer a useful reference and avoid the replicate tests in this paper.

Conclusion

Calorimetry has contributed a great deal to our current understanding of the mechanisms of regulation and control of biological structures and processes at the molecular level [33-35]. Its advantage is that the method directly measures the heat signal associated with the binding process and thereby avoids the need to partition the free and bound ligands. The method has been used widely in the determination of thermodynamic parameters [36-38]. In fact, all biological processes depend critically on the binding of ligand by specific protein. Because nearly all binding interactions are accompanied by a change in enthalpy and all reactions will produce a calorimetric signal, the calorimetry offers the possibility of determining directly not only the enthalpy, but also the free energy and the entropy in a single experiment.

In this work, the interaction of zinc ion and HSA was studied by isothermal titration calorimetry. The results show that HSA has two classes of binding sites binding to zinc ions. The high-affinity binding is the enthalpy-entropy synergically driven process and the electrostatic interaction is the main force. The low-affinity binding is an enthalpy-driven process and this process is mainly driven by the van der Waals forces. Both classes of binding reaction are exothermic and spontaneous process. Microcalorimetry applied in this study was proved to be an accurate and rapid measuring method for indicating the interaction mechanism of metal ions with protein molecules, which has certain reference significance for future researches.

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