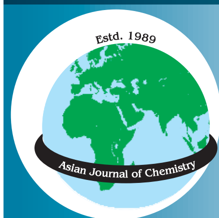

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Study on Interaction of Histamine, Tyramine and Phenethylamine with Bovine Serum Albumin Using Isothermal Titration Calorimetry

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The interaction between histamine, tyramine or phenethylamine and bovine serum albumin (BSA) were investigated by nano-Watt-scale isothermal titration calorimetry (ITC). From the analysis of the isothermal titration calorimetry data, the binding characteristics and thermodynamic properties of three systems were obtained and the binding mechanisms were discussed. It was found that the experimental data fit well with the Langmuir's binding theory. Bovine serum albumin has two classes of binding sites binding to histamine or tyramine. In histamine-BSA system, high affinity does not occur spontaneously. Low affinity binding is an enthalpy-entropy driven collaborative process in which enthalpy-driven was dominant. The low affinity binding is dominant, the reaction can proceed spontaneously. In tyramine-BSA system, the two classes of binding model act synergistically and formed stable tyramine-BSA complex. The reaction can proceed spontaneously. Differ from the two systems, phenethylamine (PEA) binding to bovine serum albumin by a single site or high affinity mode and form stable PEA-BSA complex. The reaction can also proceed spontaneously.

Keywords: Isothermal titration calorimetry, Bovine serum albumin, Biogenic amine, Interaction.

INTRODUCTION

Biogenic amines (BA), an endogenous harmful substance in food, is a kind of nitrogen-containing aliphatic, aromatic or heterocyclic organic compounds [1,2]. Including putrescine, cadaverine, spermine, spermidine, tyramine, phenylethylamine, histamine, serotonin and so on. A quantification of biogenic amine has a prominent role in regulating the growth, improving metabolic activity and controlling the blood pressure in the physiological activity of living body. Histamine and tyramine play an important role in hormones-mediated of human and animals, while dopamine and serotonin are very significant in the central nervous system transmitting [3-5]. Although biogenic amines has very important physiological functions in the living body, it can cause headache, changes in blood pressure, nausea and other allergic reactions or even life threatening when the intake is excessive in body [6]. Among biogenic amines, histamine has the biggest effects on human health, followed by tyramine [7]. In this paper, we selected histamine, tyramine and phenylethylamine as the titrant, analyzed the interaction between them and bovine serum albumin using isothermal titration calorimetry, which is of great significance in biochemistry and food area.

Serum albumin, accounting for about 50 % of the plasma proteins, is the major soluble protein in the circulatory system of vertebrate [8,9]. As the most abundant protein in serum of living body, serum albumin has many important physiological functions and also has vital influence on the storage, transport, distribution and metabolism process of small molecules and ions produces in the blood [10-14]. Bovine serum albumin (BSA), with multi-function, multi-purpose in blood plasma, contains 582 amino acid residues. The spatial structure of bovine serum albumin consists of three structural domains and each domain form cylinders structure by two subdomains in the form of rabbet abutment joint. Like most high abundant plasma proteins, bovine serum albumin has the characteristics of high stability and high solubility.

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 [15], have higher sensitivity and better reproducibility [16]. 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 to

characterize the interactions of biological molecules. Isothermal titration calorimetry has become a powerful tool for the study of binding reaction of the biological macromolecules [17,18].

In this paper, the binding reaction mechanisms of histamine-BSA, tyramine-BSA and PEA-BSA were investigated by calorimetry and the number of binding sites, binding constants, binding enthalpy change process, entropy change and the Gibbs free energy change of histamine-BSA, tyramine-BSA and PEA-BSA were determined. Furthermore, based on the thermodynamic parameters, the interaction between the three biogenic amines and bovine serum albumin were analyzed.

EXPERIMENTAL

Experimental Materials: Bovine serum albumin (BSA, purity ≥ 98.0 %, Shanghai Crystal Pure Reagent Company); histamine (purity ≥ 97 %, Shanghai vibration spectrum Biotechnology Company); tyramine (purity ≥ 98 %, Shanghai vibration spectrum Biotechnology Company); β-phenylethylamine (PEA, purity ≥ 98 %, colourless and transparent liquid at room temperature, Shanghai vibration spectrum Biotechnology Company); trihydroxy aminomethane (Tris, BR, Shanghai Sino-American Biotechnology company); HCl (0.1 mol/L), NaCl (AR, Xilong Chemical Company, Ltd.); experimental water is the second ultra-pure water from UPWS ultra-pure water treatment; Tris-HCl buffer, pH = 7.38, 1×10^{-5} mol/L bovine serum albumin solution, 1×10^{-3} mol/L histamine, tyramine and β-phenethylamine solutions were formulated in Tris-HCl buffer solution.

Experimental instrument: VP-ITC isothermal titration calorimetry instrument (GE, USA); ZD-2 precision pH meter (Shanghai Lei magnetic Instrument Factory); AB265-S METTLER TOLEDO electronic balance (Mettler-Toledo Changzhou company); UPWS ultra-pure water (desktop) (Hangzhou Yong Jie Da of purification Technology Company); 1 cm quartz cuvette; 25 mL and 50 mL volumetric flask, 1000 mL volumetric flask.

Isothermal titration calorimetry measurement: The isothermal titration experiment of binding process between histamine, tyramine or phenethylamine and bovine serum albumin were carried out on the VP-ITC isothermal titration calorimeter, with the repeatability of thermal power baseline within ± 20 nW. Bovine serum albumin solution with the concentration of 1×10^{-5} mol/L was injected into a 1.45 mL calorimeter cell and 250 μL of histamine, tyramine or β-phenethylamine solution with the concentration of 1×10^{-3} mol/L were absorbed into a syringe. The interval between two injections was 240 s and the stirrer in the ampoule was operated at a constant speed of 307 rpm. All the experiments were performed at 298.15 K and were started when the baseline was stable.

The data 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 bovine serum albumin and histamine, tyramine or β-phenethylamine were ascertained and the thermodynamics parameters (K, N, ΔH, ΔS and ΔG) were determined.

RESULTS AND DISCUSSION

Calorimeter model theory: There are a variety of binding sites models in the inbuilt Origin7.0 calculation program of MicroCal ITC (single-site binding model, two-site binding model, continuous multi-site binding model, *etc*.). Based on the Langmuir combination theory and calorimetric data, the fitting curve was obtained from non-linear least variance fitting principle and by use of Origin 7.0 software. From analysis of goodness of the fitting curve, the number of binding sites, binding constants, binding enthalpy change thermodynamic parameters of the process can be determined.

Binding reaction between protein and histamine, tyramine or β-phenethylamine may be expressed based on the following assumptions [19,20]: (1) The binding site of the protein molecule may bind the same ligand and all the congeneric sites are thermodynamically identical. (2) The congeneric 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. When we conduct the data fitting in this work, we found that Langmuir binding theoretical model [21-23] applied to the interaction system of BSA-BA. According to the langmuir combination theory, we have the following equations:

$$\theta_i = \frac{K_i C_L}{1 + K_i C_L} \tag{1}$$

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

where θ_i is the binding ratio, namely the average number of ligand molecules 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 ligand and protein. C_L is the unbound concentration of ligand.

Substituting formula (1) into (2):

$$C_{L,0} = C_L + C_{P,0}\left(\frac{N_1K_1C_L}{1+K_1C_L} + \frac{N_2K_2C_L}{1+K_2C_L}\right) \tag{3}$$

Then, a cubic equation of C_L can be obtained:

$$C_L^3 + pC_L^2 + qC_L + r = 0 \tag{4}$$

where

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

$$q = \frac{1}{K_1K_2} - C_{L,0}\left(\frac{1}{K_1} + \frac{1}{K_2}\right) + C_{P,0}\left(\frac{N_2}{K_1} + \frac{N_1}{K_2}\right) \tag{6}$$

$$r = -\frac{C_{L,0}}{K_1K_2} \tag{7}$$

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

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

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:

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

where V_{cell} is the volume of calorimeter cell, $\Delta\theta_i$ is the increment of binding ratio from injection j-1 to 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 isothermal titration calorimetry results.

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

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

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

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

Interaction between histamine, tyramine or phenethylamine and bovine serum albumin: To determine the experimental conditions, we primarily need to consider the solubility of the three biogenic amines. At room temperature, histamine, with the solubility of 2.05 g/0.095 L, is easily soluble in water. The solubility of β-phenethylamine is 4.6 g/L, while the solvent environment of bovine serum albumin, which comes from bovine serum is neutral. For these limitation, we take 25 °C (298.15 K), pH = 7.38 as the experiment condition.

Fig. 1 is the isothermal titration calorimetry curve for the binding of histamine, tyramine or β-phenethylamine to bovine serum albumin at 298.15 K and the curve of the binding heat *versus* molar ratio of histamine, tyramine or β-phenethylamine to bovine serum albumin at 298.15 K, by the non-linear fitting method with the two-site binding model (wherein the dilute heat of histamine dissolved in Tris-HCl buffer solution has been deducted). Moreover, the corresponding obtained results were given in Table-1.

From the fitting curve shown in Fig. 1, the binding interaction of histamine or tyramine to bovine serum albumin at 298.15 K is an exothermic reaction. The data of isothermal titration calorimetry were fitted using the built-in software of MicroCal ITC. Two-site binding model were found to be the optimal by the regression analysis of fitted curve and the fit of experimental points. That is to say, there are two types of binding sites when bovine serum albumin transport histamine or tyramine (m = 2). Table-1 illustrated that the two types of binding sites of histamine are the high-affinity sites with the number of binding sites N_1 = 35.5, the binding constant K_1 = 5.80 × 10^5 L/mol and the low-affinity sites with the number of binding sites N_2 = 46.47, the binding constant K_2 = 0.35 × 10^5 L/mol. While that of tyramine are the high-affinity sites with the number of binding sites N_1 = 67.20, the binding constant K_1 = 9.09 × 10^5 L/mol and the low-affinity sites with the number of binding sites N_2 = 78.51, the binding constant K_2 = 0.10 × 10^5 L/mol. From Table-1, it also can be clearly seen that enthalpy change of histamine-BSA at high-affinity sites ΔH_1 is -3.38 kcal/mol and that at low-affinity sites ΔH_2 is -161.33 kcal/mol; Gibbs free energy change at high-affinity sites ΔG_1 is 2.79 kcal/mol and that at low-affinity sites ΔG_2 is -177.07 kcal/mol. Both the Gibbs free energy changes (ΔG) of the two classes of binding sites is negative. It demonstrated that two classes of binding process of histamine-BSA under the present experimental condition can be spontaneous. The absolute values of ΔG at low-affinity sites is far large than that of high-affinity sites, which indicated that low-affinity sites is dominant. Enthalpy change of tyramine-BSA at high-affinity sites ΔH_1 is -30.52 kcal/mol and that at low-affinity sites ΔH_2 is -29.16 kcal/mol; Gibbs free energy change at high-affinity sites ΔG_1 is -55.62 kcal/mol and that at low-affinity sites ΔG_2 is -52.86 kcal/mol. Both the Gibbs free energy changes ΔG of the two classes of binding sites are negative. It demonstrated that two classes of binding process under the present experimental condition can be spontaneous and formed stable complex.

From Fig. 1, the binding interaction of β-phenethylamine to bovine serum albumin at 298.15 K is also an exothermic reaction. The data of isothermal titration calorimetry were fitted using the built-in software of MicroCal ITC. Single-site bonding model were found to be the optimal by the regression analysis of fitted curve and the fit of experimental points. That is to say, there is only one type of binding sites when bovine serum albumin transport β-phenethylamine (m = 2). Table-1 illustrates that the type of binding sites is the high-affinity sites with the number of binding sites N = 11.7, the binding constant K = 3.34 × 10^5 L/mol, the enthalpy change ΔH is -45.31 kcal/mol and Gibbs free energy change ΔG is -56.27 kcal/mol. The thermodynamic parameters $\Delta G < 0$, $\Delta S > 0$ demonstrated that the binding process of β-phenethylamine and bovine serum albumin under the present experimental condition can be spontaneous and accompanied by a significant increase in entropy. This may because β-phenethylamine fell into the hydrophobic region of bovine serum albumin (*i.e.* binding sites) and the direct hydrophobic interaction lead to the decrease of systematic energy (*i.e.* exothermic process).

TABLE-1
STOICHIOMETRIC DATA AND BINDING CONSTANT FOR THE BINDING OF HISTAMINE, TYRAMINE AND β-PHENETHYLAMINE TO BOVINE SERUM ALBUMIN

Amino acid	Binding sites	N_i	K_i (× 10^5 L/mol)	ΔG_i (kcal/mol)	ΔH_i (kcal/mol)	ΔS_i (cal/(mol K))
Histamine	High-affinity sites (i = 1)	35.50 ± 0.28	5.80 ± 0.38	2.79	-3.38	-20.70
	Low-affinity sites (i = 2)	46.47 ± 0.06	0.35 ± 0.01	-177.07	-161.33	52.90
Tyramine	High-affinity sites (i = 1)	67.20 ± 0.67	9.09 ± 0.08	-55.62	-30.52	84.20
	Low-affinity sites (i = 2)	78.51 ± 0.90	0.10 ± 0.01	-52.86	-29.16	79.50
β-Phenethylamine	High-affinity sites (i = 1)	11.70 ± 0.07	3.34 ± 0.04	-56.27	-45.31	36.80

Note: The temperature of the measurement is 298.15 K.

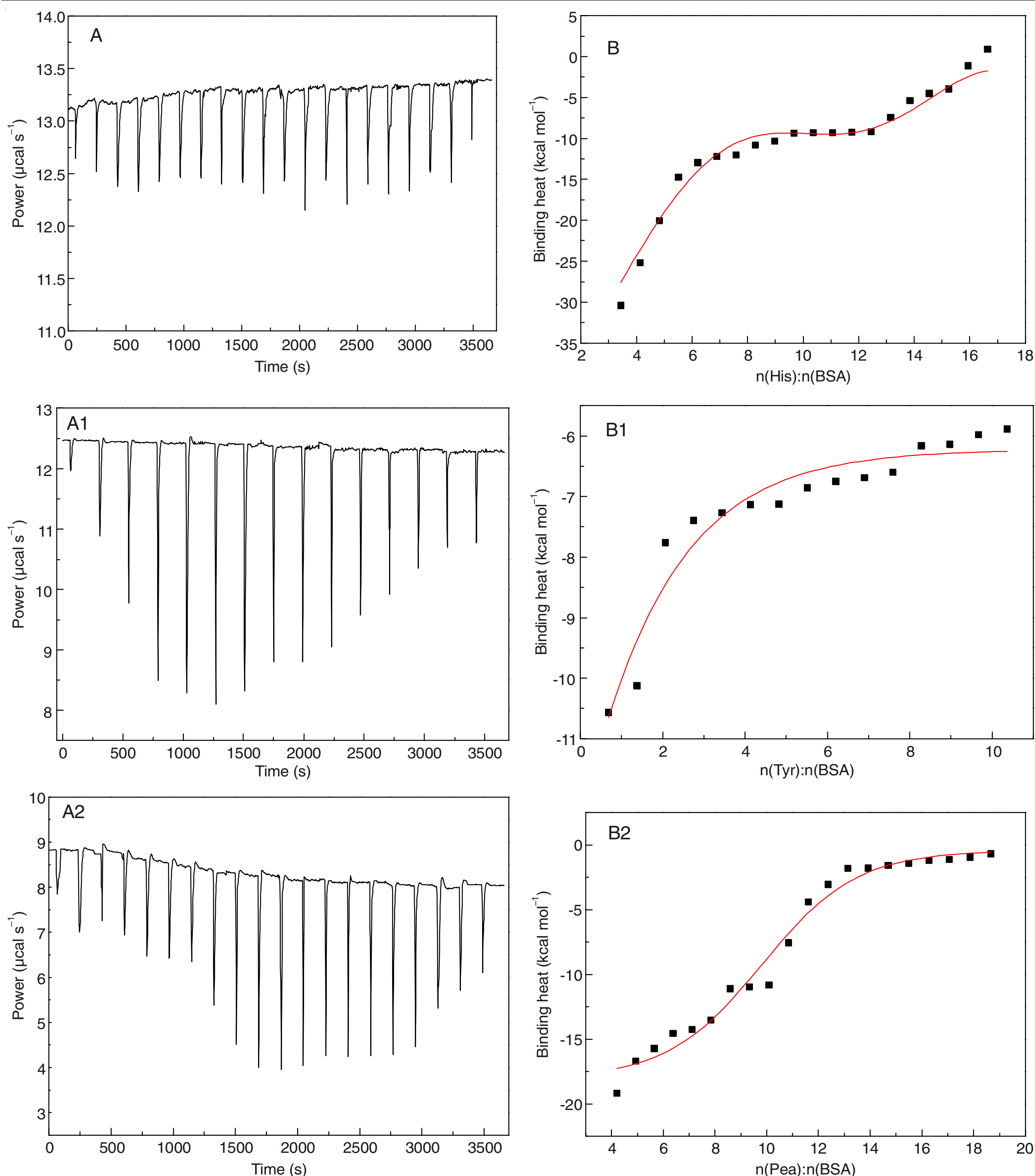

Fig. 1. Isothermal titration calorimetry profile for binding of histamine (A), tyramine (A1) and phenethylamine (A2) to bovine serum albumin. Non-linear fitting curve of the binding heat *versus* the molar ratio of histamine (B), tyramine (B1) and phenethylamine (B2) to bovine serum albumin between 2 °C to 80 °C. $C_{histamine} = C_{tyramine} = C_{PEA} = 1 \times 10^{-3}$ mol/L, $C_{BSA} = 1 \times 10^{-5}$ mol/L, T = 298.15 K

Conclusion

In this work, the interaction of histamine, tyramine, β-phenethylamine and bovine serum albumin were studied by isothermal titration calorimetry respectively at the experimental condition of T = 298.15 K, pH = 7.38, Tris-HCl buffer solution. We measured the binding sites and binding constants and also obtained the thermodynamic criterion of driving force in the binding process by analyzing the enthalpy and entropy change characteristics of systems, the results were as follows:

• Bovine serum albumin has two classes of binding sites binding to histamine or tyramine, *i.e.* high affinity sites and low affinity sites. In histamine-BSA system, high affinity sites with $\Delta H < 0$ (exothermic), $\Delta S < 0$ (entropy reduction), $|\Delta H| < T|\Delta S|$, indicating that the reaction is an entropy-driven

process or physical reaction. Entropy reduction effect is so obvious that the ΔG > 0, indicating that the reaction does not occur spontaneously. Low affinity sites with ΔH < 0 (exothermic), ΔS > 0 (entropy increase), | ΔH |> T | ΔS |, demonstrating the reaction is an enthalpy-driven process or a chemical reaction. Heat release and entropy increase effects lead to ΔG < 0 in this process, so the reaction is an enthalpy-entropy driven collaborative process in which enthalpy-driven was dominant [24,25]. These also indicate that in the binding mechanism of bovine serum albumin and histamine, the second binding site or low affinity sites is dominant. In the binding mechanism of bovine serum albumin and tyramine, the two types of binding sites are equally multiple. According to the analysis of thermodynamic data, two classes of binding sites showed ΔH < 0 (exothermic), ΔS > 0 (entropy increase), | ΔH | > T | ΔS | at 298.15 K, revealing that the reaction is an enthalpy-entropy driven process and this process is mainly driven by enthalpy. The reaction can proceed spontaneously for ΔG < 0. In the binding process of tyramine and bovine serum albumin, the two classes of binding mode act synergistically and formed stable tyramine-BSA complex.

• Differ from the two systems, β-phenethylamine binding to bovine serum albumin by a single site. Thermodynamic parameters ΔH < 0 (exothermic), ΔS > 0 (entropy increase), | ΔH | > T | ΔS |. The effects of heat release and entropy increase lead to ΔG < 0 in this process, indicating that the reaction can proceed spontaneously. The combination mode of β-phenethylamine and bovine serum albumin is high affinity mode and form stable PEA-BSA complex.

Microcalorimetry applied in this study was proved to be an accurate and rapid measuring method for indicating the interaction mechanism of biogenic amine with protein molecules, which has certain reference significance for future researches.

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