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Thermodynamic study on the interaction between anti-tumor drug tegafur and human serum albumin

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Abstract

The changes of thermodynamic properties of the system on interaction between tegafur and human serum albumin (HSA) and the changes of secondary structure units of HSA in the system at 298.15 K have been investigated by the Nano-Watt-Scale isothermal titration calorimetry (ITC), the Langmuir's binding model and the circular dichroism (CD) spectrometry.

Keywords: Tegafur; Human serum albumin; Isothermal titration calorimetry; Circular dichroism

The investigation of the interaction between anti-tumor drug and biological macromolecule protein, which is a chemical substance composition in cell, is a basic path to approach the mechanism of tumor cell apoptosis induced by the drug, and it is also an important content of study on cell apoptosis intervention, which is a developing therapeutical method of disease [1]. Tegafur is an anti-metabolism drug, which can prevent formation of pyrimidine-nucleotide in tumor cell. The serum albumin is the most abundant and the key carrier protein in blood plasma. It can combine with many endogenous and exogenous substances, and has the wide actions to storage, transportation, performs pharmaceutical action on acceptor site, and controls of acting time and concentration of drug in body [2]. Therefore, the study on binding model between the serum albumin and medicinal molecule can provide a lot of effective information to approaching to the above-mentioned subjects [3]. We investigated the interactions between tegafur and HSA by the isothermal titration calorimetry, the Langmuir's binding theory and the circular dichroism spectrometry for the first time.

1. Experimental

HSA (98%, in purity) and tegafur (99%, in purity) were purchased from Huamei Biological Engineering Co. (China) and Bailing Chemical Technology Limited Co. (China), respectively. The other reagents were analytical grade and made in China. All solutions of tegafur and HSA were prepared with Tris–HCI buffer solution (pH 7.4). The isothermal titration experiment of binding process of tegafur and HSA at 298.15 K was performed by the Nano-Watt-Scale microcalorimeter (TAM 2277, Sweden), and the original concentrations of tegafur and HSA solutions are 14.30 mmol/L and 47.20 μmol/L, respectively. The detailed operations have been described in early report [4]. The

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conformational transitions of HSA on two types of binding sites in the system were determined from the CD data with Chen and Yang's fitting method [5] by the Jasco J-810 spectropolarimeter (Japan), and the original concentration of HSA solution was 2 μmol/L.

2. Results and discussion

2.1. Thermodynamic assumptions of the binding system

The basic assumptions on binding process of HSA and tegafur (or called ligand) in the system may be expressed as reported in [6,7]. (a) One protein (HSA) molecule may have *i* classes of binding sites, which can bind same ligand. (b) All sites within one class of binding thermodynamically are identical. (c) 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. From above assumptions and Langmuir's binding theory [8,9], we have the following equations:

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

$$c_{L,0} = c_L + c_{P,0} \sum_{i=1}^{m} N_i \theta_i \tag{2}$$

where $C_{L,0}$ and $C_{P,0}$ are the original concentrations of tegafur ligand and HSA, c_L the unbound concentration of tegafur, θ_i, K_i and N_i are the binding ratio, the binding constant and the binding site numbers of *i* class of binding site. The heat, Q_j, for the *j*th injection in an experimental trail may be expressed as

$$Q_j = c_{P,0} V_{cell} \sum_{i=1}^{m} N_i \Delta\theta_i \Delta H_i \tag{3}$$

where V_{cell} is the volume of the calorimeter cell, $\Delta\theta_i$ the increment of binding ratio from injection $j-1$ to injection j, and ΔH_i is the binding enthalpy of *i* class of binding site. The fitting curve in Fig. 1 is obtained from nonlinear least variance fitting principle and by use of software MATLAB 7.01. From analysis of goodness of fit of the curve compare with the experimental data points, two classes of binding sites can be determined rationally, and we may obtain maximum likelihood values for six parameters K_1, K_2, ΔH_1, ΔH_2, N_1 and N_2 in Eq. (3). Based on following thermodynamic formulas, we may also obtain the changes of binding Gibbs free energies (ΔG) and entropies (ΔS) of tegafur and HSA on two classes of binding sites in the system:

$$\Delta G = -RT \ln K \qquad \Delta G = \Delta H - T\Delta S \tag{4}$$

The obtained results were given in Table 1.

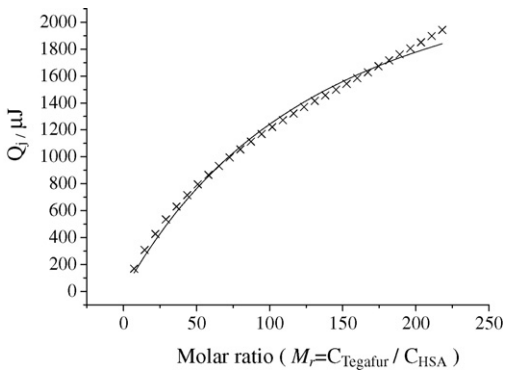

Fig. 1. The nonlinear fitting curve of Q_j vs. M_r in the binding system at 298.15 K. The line is the result of simulation and the points were obtained from experiment.