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Study of characterization and application on the binding between 5-iodouridine with HSA by spectroscopic and modeling

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Abstract

The binding of 5-iodouridine with human serum albumin was investigated under the simulative physiological conditions. The fluorescence spectra in combination with UV absorption and modeling method were used in the present work. A strong fluorescence quenching reaction of 5-iodouridine to HSA was observed and the quenching mechanism was suggested as static quenching procedure. The binding constants (K) at different temperatures as well as thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS), were calculated. It showed that the hydrophobic interaction was a predominant intermolecular force in order to stabilize the complex, which was in agreement with the result of modeling study. The binding distance between 5-iodouridine and HSA was calculated on the basis of the theory of Föster energy transfer. The effects of other ions on the binding constants were also discussed. Synchronous fluorescence spectroscopy (SFS) technique were successfully applied to determine protein in the biological samples.

Keywords: 5-Iodouridine; Human serum albumin (HSA); Fluorescence quenching; Modeling; Synchronous fluorescence; Determination

1. Introduction

Protein is an important chemical substance in our life and one of the main targets of all medicines in organism. Serum albumin, the most abundant protein in the circulatory system, is one of the most extensively studied proteins because it can interact with many endogenous and exogenous substances (Cui et al., 2006). Binding of drugs to plasma protein is an important pharmacological parameter, since it frequently affects the distribution and elimination of a drug as well as the duration and intensity of its physiological action. The effect is especially significant for highly protein bound drugs, where only a small alteration in bound fraction can produce a profound change in the pharmacodynamically active free drug concentration (Seedher & Bhatia, 2006). The studies on this aspect can provide information of the structural features that determine the therapeutic effectivity of drugs, and have been an interesting research field in life science, chemistry, biochemistry and clinical medicine. The quantitative determination of protein is also considerably essential in biochemistry and clinical medicine because it is often used as a reference for the measurements of other components in biological systems and medical treatment (Cong, Guo, Wang, & Shen, 2001; Jia, Dong, Li, Chen, & Hu, 2002).

Nucleosides and their derivatives have a wide range of biological activities, such as antitumor, antiviral and antibacterial activities (Guo & Ye, 2000; Park, Kim, & Dordick, 2000). It is recognized that nucleosides have the most potential function to restrain virus (Wang & Huang, 1987), and have been an interesting research field in chemistry, biology and medicine. 5-iodouridine is an important compound of nucleosides. It was widely used to manufacture medicine of anticancer, antimicrobial and antiviral

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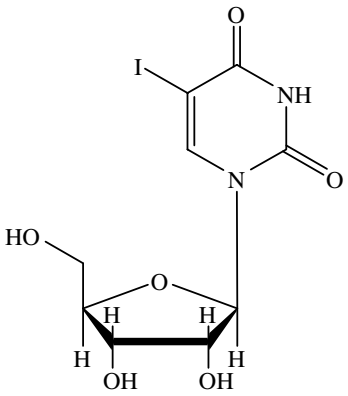

Fig. 1. The structure of 5-iodouridine ($C_9H_{11}IN_2O_6$, molecular weight 354.1).

(molecular structure shown in Fig. 1). Therefore, studying the interaction of 5-iodouridine with HSA has special biochemical importance and can be used as a model for elucidating the drug–protein complication.

Fluorescence spectroscopy has been the most widely used spectroscopic technique for monitoring drug binding to plasma albumin because of its sensitivity, accuracy, rapidity, and ease of use (Seedher & Bhatia, 2006). The fluorescence measurements can give some information of the binding of small molecule substances to protein, such as the binding constants, binding mechanism, binding mode, binding sites, intermolecular distance, etc. (Sulkowska, 2002). While in the synchronous spectra, the sensitivity associated with general fluorescence is maintained while offering several advantages: spectral simplification, spectral bandwidth reduction and avoiding different perturbing effects (Chen, Huang, Xu, Zheng, & Wang, 1990). The synchronous fluorescence technology was an effective method for determination of HSA.

Fluorescence, UV absorption and modeling methods were used to study the interaction of 5-iodouridine with HSA in this work. The molecular interactions are often monitored by these methods because of they have advantages over conventional approaches such as affinity and size exclusion chromatography, equilibrium dialysis, ultrafiltration and ultracentrifugation, which suffer from lack of sensitivity or long analysis time or both and use of protein concentrations far in excess of the dissociation constant for the drug–protein complex and for drug–protein interaction studies (Kandagal, Ashoka, Seetharamappa, Vani, & Shaikh, 2006). Synchronous fluorescence technology was applied to determine the total protein in human body fluids including serum, urine and saliva samples with 5-iodouridine as probe with a satisfying result.

2. Materials and methods

2.1. Materials

HSA (Sigma) was directly dissolved in double distilled water to prepare the stock solution (2.0×10^{-5} mol/L HSA), and the stock solution was kept in the dark at 0–4 °C; 4.6×10^{-4} mol/L 5-iodouridine (synthesized by the methods of microwave-assisted without solvent) solution was obtained by dissolving it in double distilled water. 0.1 mol/L Tris–HCl buffer solution of pH 7.4, 0.5 mol/L NaCl working solution and 1.0 mg/mL coexistent ions solutions were prepared. Human serum samples were from the Hospital of Henan Normal University and stored at 0–4 °C. Unless otherwise mentioned, all chemicals were of analytical reagent grade and were used without further purification. Double distilled water was used throughout the experiment.

2.2. Apparatus and methods

All fluorescence measurements were carried out on an FP-6200 spectrofluorimeter (JASCO, Japan) and an RF-540 spectrofluorimeter (Shimadzu, Japan) equipped with a thermostat bath and 1.0 cm quartz cells, using 5/5 nm slit widths. T6 UV–vis spectrophotometer (Purkinje General Instrument Co. Ltd. Beijing, China) equipped with 1.0 cm quartz cells was used for scanning the UV spectra. PH values were measured with a pH-3digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass electrode.

2.3. Fluorescence measurements

2.0 mL Tris–HCl buffer solution at pH 7.4, 2.0 mL NaCl solution, appropriate amount of HSA and 5-iodouridine were added to a 10 mL standard flask, then diluted to the mark with double distilled water. Fluorescence quenching spectra of HSA were recorded from 300 to 450 nm at an excitation wavelength of 280 nm and the synchronous fluorescence spectra were from 280 to 350 nm with $\Delta\lambda = 20$ nm.

3. Results and discussion

3.1. Optimization of experimental conditions

In order to select the optimum conditions, various experimental parameters including medium, pH, ionic strength, temperature were studied with the concentration of 5-iodouridine being 0.92×10^{-5} mol/L in all cases. From the experimental results, 2.0 mL of 0.1 mol/L Tris–HCl buffer solution at pH 7.4 was considered to be the best choice; Ionic strength was tested by using NaCl and the concentration was selected at 0.1 mol/L; The binding constants between HSA with 5-iodouridine was determined at different temperatures (19, 29 and 39 °C); the binding constant at 19 °C was higher than those at other temperatures, so room temperature (19 °C) was chosen. We also tested the effect of addition sequence of reagents on fluorescence intensity of system according to the permutation and combination, and found that the sensitivity was higher if the

order was Tris–HCl → NaCl → HSA → 5-iodouridine. Thus, this sequence was adopted in the experiment.

3.2. Binding mechanism and binding constants

3.2.1. Fluorescence quenching spectra

HSA has three intrinsic fluorophores viz., tryptophan, tyrosine, and phenylalanine residues (Tang, Luan, & Chen, 2006). Actually, the intrinsic fluorescence of HSA is almost contributed by tryptophan alone and this viewpoint was supported by the experimental observation of Sulkowska (Sulkowska, 2002). The change of intrinsic fluorescence intensity of HSA is that of fluorescence intensity of tryptophan residue when small molecule substances are bound to HSA (Kandagal et al., 2006).

Fig. 2 is the fluorescence emission spectra of HSA in the absence and presence of 5-iodouridine. HSA has a strong fluorescence emission with a peak at 339 nm on excitation at 280 nm. The fluorescence intensity distinct decreases in the presence of 5-iodouridine and the maximum emission wavelength were shift from 339 to 348 nm, suggested that that it interacted with 5-iodouridine. And the microenvironment around the tryptophan residue was changed after the addition of 5-iodouridine (Trynda-Lemiesz, Keppler, & Koztowski, 1999).

3.2.2. Fluorescence quenching mechanisms and binding constants

The static quenching and dynamic quenching were studied at different temperatures. The quenching rate constants decreased with increasing temperature for the static quenching, while reversed effect was observed for the dynamic quenching (Chen et al., 1990). The possible quenching mechanism could be interpreted by the fluorescence quenching spectra of the proteins and the F_0/F–[Q] (Stern–Volmer) curves of HSA with 5-iodouridine at different temperatures (19, 29 and 39 °C) as shown in Fig. 3.

It could be found that the Stern–Volmer plots were linear and the slopes decreased with increasing temperature, which was consistent with the static type of quenching mechanism. The K_{sv} and correlation coefficient values, r (the linear relationship between F_0/F and [Q]) obtained at different temperatures are listed in Table 1. As a result, the possible quenching mechanism between 5-iodouridine and HSA was suggested as static quenching but not dynamic quenching. In order to confirm this point, the procedure was assumed to be dynamic quenching. The fluorescence quenching data were analyzed by the Stern–Volmer equation:

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensity in the absence and presence of quenchers, respectively. K_q, K_{sv}, τ_0 and [Q] are the quenching rate constant of the biomolecule, the Stern–Volmer quenching constant, the average lifetime of molecule without quencher and concentration of quencher, respectively. Obviously:

$$K_{sv} = K_q\tau_0 \quad (2)$$

Taking average lifetime of molecule fluorescence as around 10^{-8} s (Lakowicz & Weber, 1973), an approximate quenching constant (K_q, L/mol s) could be obtained according to the Eq. (2). The results are listed in Table 1.

The maximum scatter collision quenching constant of various quenchers with the biopolymer is 2.0×10^{10} L/mol s (Ware, 1962). Obviously, the rate constant of protein quenching procedure initiated by 5-iodouridine was greater than the K_q of the scatter procedure. This also indicated that the quenching was not initiated by dynamic collision but by the formation of complex. The static quenching equation is presented by:

$$(F_0 - F)^{-1} = F_0^{-1} + K^{-1}F_0^{-1}[Q]^{-1} \quad (3)$$

where K denotes the binding constant of 5-iodouridine and biomolecule, which can be calculated from the slope and intercept of Lineweaver–Burk curves as shown in Fig. 4 (K = intercept/slope). The results were listed in Table 2.

It could be found that there was a strong interaction of 5-iodouridine with HSA. The interaction was weakened

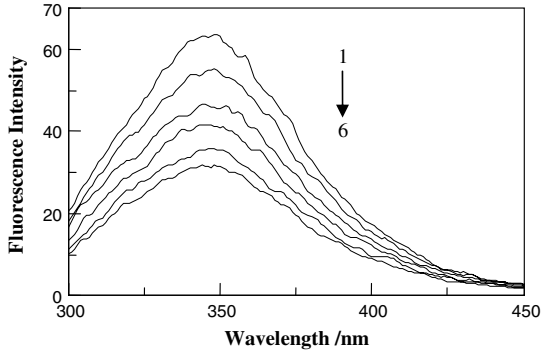

Fig. 2. The fluorescence quenching spectra of HSA by different concentrations of 5-iodouridine with the excited wavelength at 280 nm in Tris–HCl buffer solution (pH 7.4) at 19 °C, $C_{HSA} = 1.2 \times 10^{-6}$ mol/L. From 1 to 6 the concentration of 5-iodouridine was 0, 0.92, 1.84, 2.76, 3.68, 4.60×10^{-5} mol/L, respectively.

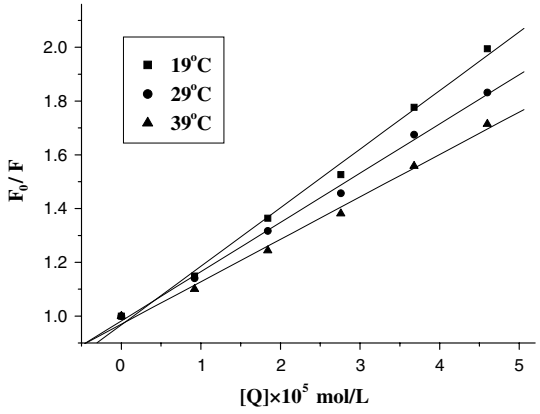

Fig. 3. The Stern–Volmer curves for quenching of HSA with 5-iodouridine at pH 7.40; $C_{5\text{-iodouridine}}$ and C_{HSA} are the same as those in Fig. 2.

Table 1
The quenching constants (L/mol s) of the 5-iodouridine–HSA system at different temperatures

T (°C)	Stern–Volmer equation	K_q (L/mol s)	R
19	$Y = 0.9752 + 2.1594 \times 10^4$ [Q]	2.1594×10^{12}	0.9973
29	$Y = 0.9819 + 1.8323 \times 10^4$ [Q]	1.8323×10^{12}	0.9982
39	$Y = 0.9968 + 1.3682 \times 10^4$ [Q]	1.3682×10^{12}	0.9968

Table 2
The binding constants (L/mol) of the 5-iodouridine–HSA system at different temperatures

T (°C)	Lineweaver–Burk equation	K (L/mol)	R
19	$Y = 0.01248 + 0.0876 \times 10^{-5}$ 1/[Q]	1.4245×10^4	0.9996
29	$Y = 0.01045 + 0.1096 \times 10^{-5}$ 1/[Q]	0.9538×10^4	0.9989
39	$Y = 0.00842 + 0.1487 \times 10^{-5}$ 1/[Q]	0.5662×10^4	0.9999

when the temperature rose, but the effect of temperature was small. Thus, the quenching efficiency of 5-iodouridine to HSA was not reduced obviously when experimental temperature rose.

3.3. UV absorption spectra

UV absorption measurement is a very simple method and applicable to explore the structure (Hu, Liu, Wang, Xiao, & Qu, 2004) and to know the complex formation (Bi et al., 2005; Kandagal et al., 2006). In order to prove the result of fluorescence study, The UV absorption spectra of the HSA in different ratios of 5-iodouridine to HSA under the simulative physiological conditions were measured (Fig. 5). As shown in Fig. 5, HSA had a strong absorbance with a peak at 208 nm and the peak intensity increased with the addition of 5-iodouridine. Meanwhile, the formation of 5-iodouridine–HSA resulted in the distinct shift of 5-iodouridine–HSA spectrum towards longer wavelength, which indicated the interaction between HSA and 5-iodouridine took place. The obvious enhancement of absorbency intensity (A) also indicated the formation of new complexes between HSA and 5-iodouridine.

3.4. Binding mode

The forces acting between a drug and a biomolecule are composed of weak interactions of molecules such as hydrogen bond formation, Van der Waals forces, electrostatic forces, and the hydrophobic interaction (Leckband, 2000). The thermodynamic parameters, enthalpy change

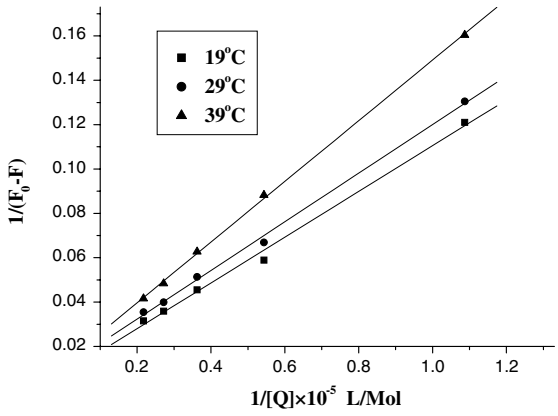

Fig. 4. The Lineweaver–Burk curves for quenching of HSA with 5-iodouridine, $C_{5\text{-iodouridine}}$ and C_{HSA} are the same as those in Fig. 2.

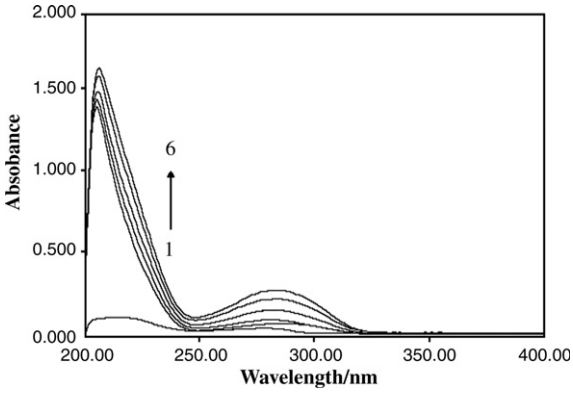

Fig. 5. The UV absorption spectra of 5-iodouridine–HSA in aqueous solution under the optimum conditions (pH 7.4, ionic strength 0.1 mol/L, 19 °C). 6, $C_{5\text{-iodouridine}} = 0.5 \times 10^{-5}$ mol/L, from 5 to 1, $C_{HSA} = 1.2 \times 10^{-6}$ mol/L; and the concentration of 5-iodouridine are 0, 0.92, 1.84, 2.76, 3.68, 4.60 $\times 10^{-5}$ mol/L, respectively.

(ΔH) and entropy change (ΔS) of binding reaction are the main evidence for confirming binding modes. From the thermodynamic standpoint, $\Delta H > 0$ and $\Delta S > 0$ implies a hydrophobic interaction; $\Delta H < 0$ and $\Delta S < 0$ reflects the van der Waals force or hydrogen bond formation; and $\Delta H \approx 0$ and $\Delta S > 0$ suggests an electrostatic force (Ross & Subramanian, 1981).

The temperature dependence of the binding constants was studied at three different temperatures (19, 29 and 39 °C) in order that HSA did not undergo any structure degradation. Because the temperature effect was very small, the interaction enthalpy change could be regarded as a constant if temperature range was not too wide. According to the following thermodynamic equations:

$$\ln K = -\Delta H/RT + \Delta S/R \quad (4)$$

$$\Delta G = \Delta H - T\Delta S = -RT\ln K \quad (5)$$

where K is the Lineweaver–Burk static quenching constant at corresponding temperature and R is the gas constant, where ΔH and ΔS of reaction could be determined from the linear relationship between $\ln K$ and the reciprocal absolute temperature. The free energy change (ΔG) could be calculated by the Eq. (5). The results were represented in Table 3.

As shown in Table 3, ΔG and ΔH were negative, while ΔS was positive. Therefore, the formation of 5-iodouridine–HSA coordination compound was spontaneous and exothermic reaction accompanied a positive ΔS value. According to the views of Orawlovel (Orwcora & Bochs,

Table 3
Thermodynamic parameters of 5-iodouridine–HSA interaction at pH 7.4

Complex	T (°C)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol K)
5-Iodouridine–HSA	19	−23.22	−9.94	40.0
	29	−23.01		
	39	−22.42		

1996) and Masaki Otagiri (Mohammed, Toru, Tomoko, Keishi, & Masaki, 1993), and Ross and Subramananian (Ross & Subramanian, 1981), the positive ΔS value is frequently taken as evidence for hydrophobic interaction. Furthermore, specific electrostatic interactions between ionic species in aqueous solution are characterized by a positive value of ΔS and a negative ΔH value. Accordingly, it was not possible to account for the thermodynamic parameters of 5-iodouridine–HSA compound on the basis of a single interaction molecular (Gonzalez, Acquotteh, & Cayre, 1992). It was more likely that hydrophobic and electrostatic interactions were involved in the binding process. However, 5-iodouridine might be considered to be largely unionized under the experimental conditions, as could be expected from its structure. Thus, electrostatic interaction could not play a major role in the binding, and 5-iodouridine bound to HSA was mainly based on the hydrophobic interaction.

3.5. The energy transfer of 5-iodouridine with HSA

According to Föster's non-radiative energy transfer theory (Förster, 1996), the rate of energy transfer depends on: (i) the relative orientation of the donor and acceptor dipoles, (ii) the extent of overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor and (iii) the distance between the donor and the acceptor. The energy transfer effect is related not only to the distance between the acceptor and the donor, but also to the critical energy transfer distance R_0, that is

$$E = 1 - F/F_0 = R_0^6/(R_0^6 + r^6) \quad (6)$$

where r is the distance between the acceptor and the donor and the R_0 is the critical distance when the transfer efficiency is 50%, which can be calculated by

$$R_0^6 = 8.8 \times 10^{-25} k^2 N^{-4} \phi J \quad (7)$$

where k^2 is the spatial orientation factor between the emission dipole of the donor and the absorption dipole of the acceptor. The dipole orientation factor, k^2, is the least certain parameter in calculation of the critical transfer distance, R_0. Although theoretically k^2 can range from 0 to 4, the extreme values require very rigid orientations. If both the donor and acceptor are tumbling rapidly and free to assume any orientation, then k^2 equals 2/3 (Yang, 1991). If only the donor is free to rotate, then k^2 can vary from 1/3 to 4/3 (Lakowicz, 1983 (chap. 10); Wu & Stryer, 1972), N is the refractive index of the medium, ϕ the fluorescence quantum yield of the donor and J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor, given by:

$$J = \sum F(\lambda)\epsilon(\lambda)\lambda^4 \Delta\lambda \Big/ \sum F(\lambda)\Delta\lambda \quad (8)$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor in wavelength λ and is dimensionless, $\varepsilon(\lambda)$ the molar absorption coefficient of the acceptor in wavelength λ and unit is cm/mol/L. It has been reported for HSA that $k^2 = 2/3$, $\phi = 0.118$ and $N = 1.336$ (Cui, Fan, Li, & Hu, 2004). From the relationships, J, E, and R_0 can be calculated.

Fig. 6 is the overlap of UV absorption spectra of 5-iodouridine with the fluorescence emission spectra of HSA. The value of the overlap integral was calculated. Based on the above data, it could be obtained the value of $R_0 = 2.24$ nm and $r = 3.01$ nm. The distance between 5-iodouridine and tryptophan residue in HSA is 3.01 nm, which were smaller than 7 nm, confirming the static quenching interaction of 5-iodouridine and HSA.

3.6. The effect of other ions on the binding constants

The molecule of protein contains the elements of S, P, Cu and Mn besides C, H, O and N. In addition, some of trace metal ions exist in the organism, and which have definite ability to bind proteins (He et al., 2005; Liang et al., 2001). The existence of metal ions can directly influence the binding force of drug with protein. Therefore, the effect of common ions on the binding constants was investigated at room temperature (19 °C), which can only be used as a model for study the binding of 5-iodouridine with HSA. The results are listed in Table 4.

It is shown that the binding constants between the protein and 5-iodouridine decreased in the presence of common ions, implying that the competition between the common ions and 5-iodouridine led to HSA-5-iodouridine binding constants and the binding force between serum albumin protein and pharmaceutical also decreased, short-

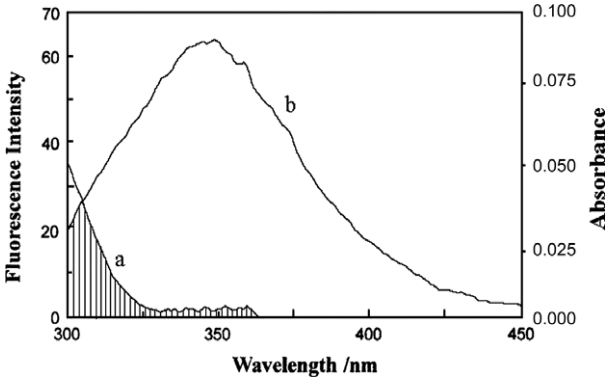

Fig. 6. The overlap of UV absorption spectrum of 5-iodouridine with the fluorescence emission spectrum of HSA. (a) The fluorescence emission spectrum of HSA (1.2×10^{-6} mol/L). (b) The UV absorption spectrum of 5-iodouridine (0.92×10^{-5} mol/L).

Table 4
The binding constants (L/mol) of 5-iodouridine with HSA (K_{HSA}) at 19 °C in the presence of common ions

Ions	K_{HSA} (10^4)	R_{HSA}	Ions	K_{HSA} (10^4)	R_{HSA}
K^+	1.0967	0.9997	Mg^{2+}	0.8533	0.9994
Ca^{2+}	1.2744	0.9995	Pb^{2+}	0.9539	0.9999
NH_4^+	0.6654	0.9988	Al^{3+}	1.1736	0.9982
Bi^{3+}	0.9565	0.9997	F^-	0.9667	0.9996
Cu^{2+}	1.0488	0.9999	SiO_3^{2-}	0.8766	0.9998
SO_3^{2-}	1.0854	0.9998	NO^{3-}	1.0765	0.9998
Co^{2+}	1.1220	0.9999	CO_3^{2-}	1.3076	0.9994
Ni^{2+}	1.1429	0.9997	PO_4^{3-}	0.9356	0.9998
SO_4^{2-}	1.4179	0.9973	$C_2O_4^{2-}$	1.3326	0.9990
Zn^{2+}	0.8796	0.9993	Cd^{2+}	0.9827	0.9999
Hg^{2+}	1.0213	0.9995	Mn^{2+}	1.0765	0.9995

ened the store time of pharmaceutical in blood plasma and improved maximum reaction intensity of pharmaceutical.

3.7. Molecular modeling

The complementary applications of molecule modeling have been employed by computer methods to improve the understanding of the interaction of 5-iodouridine and HSA. Descriptions of the 3-D structure of crystalline albumin revealed that HSA comprises of three homologous domains (denoted I, II, and III): I (residues 1–195), II (196–383), and III (384–585), and each with two subdomains, A and B possessing common structural motifs. It was suggested that the principal regions of ligand binding to HSA are located in hydrophobic cavities in subdomains IIA and IIIA, which were consistent with site I and site II, respectively, and one tryptophan residue (Trp-214) of HSA was in subdomain IIA (He & Carter 1992). There was a large hydrophobic cavity present in subdomain IIA that many drugs could bind into. The crystal structure of HSA in complex with warfarin was taken from the Brookhaven Protein Data Bank (entry codes 1 h9z) (Petitpas, Bhattacharya, Twine, East, & Curry, 2001).The potential of the 3-D structures of HSA was assigned according to the Amber 4.0 force field with Kollman-all-atom charges. The initial structures of all the molecules were generated by molecular modelling software Sybyl 6.9.1 (SYBYL, 2003). The geometries of the compounds were subsequently optimized using the Tripos force field with Gasteiger–Marsili charges. Then it was used to replace warfarin in the HSA-warfarin crystal structure. At last, Flexx program was used to establish the interaction mode between the 5-iodouridine and HSA.

The best energy ranked result was shown in Fig. 7. It was obvious that the 5-iodouridine molecule was situated within subdomain IIA hydrophobic cavity, and the 5-iodouridine was adjacent to hydrophobic residues LYS (195), LYS (199), GLU (292), HIS (288), ALA (291), ARG (218), ARG (222) ASP (451), etc., of subdomain IIA of HSA (site I). The results of molecular modeling suggested that the interaction between HSA and 5-iodouridine was dominated by hydrophobic force, which was in agreement with the binding mode proposed in thermodynamic analysis. In addition, there were some hydrogen bonds between 5-iodouridine and residues of HSA such as ARG (218), ARG (222), ASP (451) and LYS (195). The results of molecular modeling suggested that the interaction between HSA and 5-iodouridine was dominated by hydrophobic force, which was in agreement with the binding mode

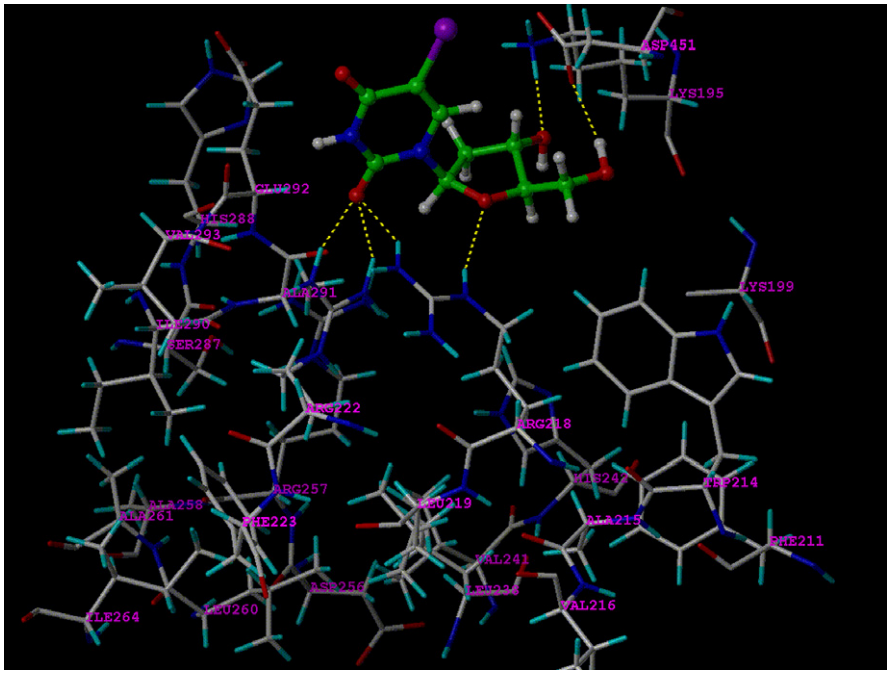

Fig. 7. The interaction model between HSA and 5-iodouridine. The residues of HSA and 5-iodouridine are represented using different tinctorial stick model. The hydrogen bond between the ligand and the protein is indicated by dashed line.

proposed in thermodynamic analysis and nature of the binding force. Furthermore, this phenomenon provided a good structural basis to explain the very efficient fluorescence quenching of HSA emission in the presence of 5-iodouridine.

3.8. Determination of HSA

Synchronous fluorescence spectroscopy (SFS) technique was introduced by Lloyd (Lloyd, 1971). Because of its advantages, SFS serves as a very simple, effective method of obtaining data for quantitative determination in a single measurement (Patra & Mishra, 2002). There are few reports on determination of protein by synchronous fluorescence method. In this study, the synchronous fluorescence technique was applied to determine protein in body fluids including serum, urine and saliva samples.

3.8.1. Characteristics of synchronous fluorescence spectra

The synchronous fluorescence spectra were obtained by scanning simultaneously by the excitation and emission monochromators. Thus, the synchronous fluorescence applied to the synchronous luminescence (Rubio, Gomez-Hens, & Valcarcel, 1986):

$$I_{SF} = kcdE_{ex}(\lambda_{em})E_{em}(\lambda_{ex} + \Delta\lambda) \quad (9)$$

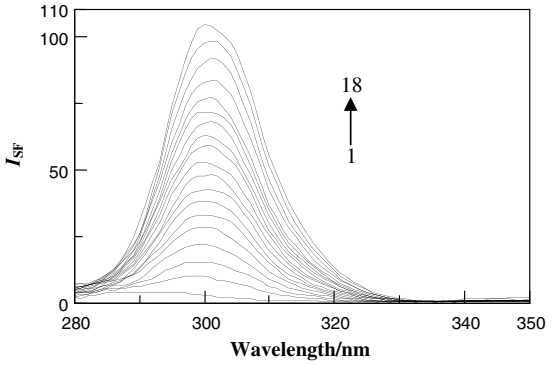

Fig. 8. The synchronous fluorescence spectra of 5-iodouridine with HSA under the optimum condition (2.0 mL Tris–HCl + 2.0 mL NaCl + 0.1 mL 5-iodouridine + XHSA). $X = 0$, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4, 4.8, 5.2, 5.6, 6.0, 6.4, 6.8, 7.2×10^{-6} mol/L from 1 to 18.

where I_{SF} is the relative intensity of synchronous fluorescence, E_{ex} is the excitation function at the given excitation wavelength, E_{em} is the normal emission function at the corresponding emission wavelength, c is the analytical concentration, d is the thickness of the sample cell, and k is the characteristic constant comprising of the "instrumental geometry factor" and related parameters. Since the relationship of the synchronous fluorescence intensity (I_{SF}) and the concentration of the HSA should follow the Eq. (7), I_{SF} should be in direct proportion to the concentration of HSA.

3.8.2. Optimization of determination conditions

Under the optimal conditions described above, the effects of as temperature and time on the determination were also tested. The results showed that the effect of temperature on I_{SF} was evident, so the experiment was tested at the room temperature and the synchronous fluorescence intensity remained stable for at least 5 h.

3.8.3. Precision, limits of detection and working curves

Under the optimum conditions described above, the synchronous spectra were scanned with increasing the concentration of HSA when the amount of 5-iodouridine was fixed. As shown in Fig. 8. The intensity of the synchronous fluorescence had a noticeable increase, and the enhancement intensity of synchronous fluorescence was proportion to the concentration of HSA. The linear range was 1.10–496.8 μg/mL, the linear regression equation was $I_{SF} = 5.3051 + 13.3408 \times 10^{-7} C_{HSA}$ (mol/L) with a correlation coefficient (R) of 0.9986. The detection limit for HSA as defined by IUPAC was determined to be 0.247 μg/mL (Irving & Freiser, 1987). The relative standard deviations (RSD) were 0.47–2.48% for HSA, as obtained from six replicate determinations.

3.8.4. The effect of coexisting substances

Under the optimum conditions, at the HSA concentration of 2.76 μg/mL and with a change of ±5% in synchronous fluorescence intensity as the criterion for interference, the effect of more than twenty coexisting substances such as mental ions and organic substances. Some metal ions were too exiguity in human body fluids including serum, urine

Table 5
The effect of common ions on the determination of protein

Substance	Interferential ratio	Change of I_{SF} (%)	Substance	Interferential ratio	Change of I_{SF} (%)
K^+	106	4.87	Starch	5	3.10
NH_4^+	50	5.15	Dextrine	18	5.24
NO_3^-	9	4.7	Maltose	27	−5.14
SO_4^{2-}	43	4.68	Sucrose	131	5.26
CO_3^{2-}	10	5.05	Glutamic acid	2	−5.07
PO_4^{3-}	18	5.02	Fructose	20	4.92
F^-	28	4.96	Serine	44	4.97
Zn^{2+}	11	−4.55	DL-Threonine	26	5.14
Ca^{2+}	90	5.01	Starch	5	3.29
Zn^{2+}	28	4.76	$C_2O_2^{2-}$	19	4.21

Table 6
Determination results of protein in samples ($n = 6$) in human body fluids including serum, urine and saliva

Samples	Added (μg)	Found (μg)	Recovery (%)	RSD (%)
Serum	0	85.66	100	0.98
	55.2	146.77	104.2	1.75
	110.4	199.39	101.7	0.93
	165.6	251.10	99.9	1.45
Urine	0	6.22	100	2.48
	55.2	63.05	102.6	1.77
	110.4	117.31	100.6	1.61
	165.6	171.59	99.9	0.47
Saliva	0	22.89	100	2.09
	55.2	78.52	100.6	1.05
	110.4	134.66	101.0	0.97
	165.6	187.18	99.3	1.12

and saliva, it can be eliminated by diluting with double distilled water. The results of interfering by organic substances were summarized in Table 5. Therefore, the present method has good selectivity and can be applied to the direct determination of biological samples.

3.8.5. Analysis of samples

The present method was applied to quantify total protein in human body fluids including serum, urine and saliva samples. Human serum samples were stored at 0–4 °C and diluted 100-fold with double distilled water just before the determination. Urine and saliva samples were also diluted appropriately. Table 6 summarized the results determined by a standard addition method with a recovery of 98.6–103.0%. The results showed that the method was reliable, sensitive and practical.

4. Summary and conclusion

5-iodouridine is an important compound of nucleosides. It was widely used to manufacture medicine of anticancer, antimicrobial and antiviral. In this work, fluorescence and UV absorption approaches were used to investigate the interaction of 5-iodouridine with HSA. The results showed that the fluorescence quenching of 5-iodouridine to HSA was static quenching and the binding mode was mainly based on the hydrophobic interaction under the simulative physiological conditions. The result of molecular modeling also confirmed this point. According to the overlap of UV absorption spectra of 5-iodouridine with the fluorescence emission spectra of HSA, the energy transfer of 5-iodouridine with HSA was calculated and the binding distance was 3.01 nm. The effect of other ions on the binding constants was also discussed at the room temperature (19 °C). In the presence work, synchronous fluorescence spectrum was investigated to determine the total protein in human body fluids including serum, urine and saliva samples. The results showed that the method is simple, stable, and reliable. This report has a great significance in pharmacology and clinical medicine as well as methodology.

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