Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(12):1496-1499

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Investigation of the interaction of the synthesized chitosan-5-fluorouracil with bovine serum albumin using spectroscopy

Hong Lou[a], Xiaozhou Li[b*], Xingjia Guo[c], Tianyue Yang[b] and Siqi Li[d]

[a]*School of Life Science, Liaoning University, Shenyang, China*
[b]*School of Science, Shenyang Ligong University, Shenyang, China*
[c]*School of Chemistry, Liaoning University, Shenyang, China*
[d]*Integrated Life Sciences, Kent State University, Kent, America*

ABSTRACT

The interaction between chitosan macromolecular prodrug of 5-fluorouracil (CS-5-FU) and bovine serum albumin (BSA) was investigated by fluorescence quenching technique under the simulative physiological conditions. The quenching mechanism of fluorescence of BSA by CS-5-FU was discussed. The binding constants and the number of binding sites were measured. The values of thermodynamic parameters ΔG, ΔH, and ΔS were calculated at three different temperatures. Apparent binding constant and binding sites were obtained. The results showed that the interaction between CS-5-FU and BSA agrees well with the site-binding model.

Keywords: chitosan-5-fluorouracil, bovine serum albumin, fluorescence quenching

---

INTRODUCTION

5-fluorouracil (5-FU) as antitumor agents is widely used for gastrointestinal, pancreas, breast, ovary and colorectal cancer for several decades[1]. Unfortunately, 5-FU exhibited serious toxicity and aroused many side effects[2]. In addition, it has a short plasma half-life period of 10−20 min in vivo. In order to prolong its half-life period and then reinforce antitumor curative effects as well as mitigate the side effects[3]. Macromolecule prodrugs of 5-FU have been studied to overcoming these problematic side effects[4–6]. Chitosan (CS) is one of the widely used molecules in drug carriers for its biodegradability, biocompatibility and nontoxic properties[7].

Serum albumins are the most abundant proteins in blood. Bovine and human serum albumins display approximately 76% sequence homology, and the 3D structure of BSA is believed to be similar to that of HSA[8]. Besides, because of its medical importance, low cost, ready availability, unusual ligand-binding properties, and the results of all the studies are consistent with the fact that human and bovine serum albumins are homologous proteins. So it is often selected as protein model in the related research work[9].

In the present paper, the CS-5-FU conjugates were synthesized according to previously reported method[10]. And then the interaction between CS-5-FU and BSA was investigated using fluorescence approaches. The binding characteristics between CS-5-FU and BSA were described and the conformational changes of BSA were explored. The aim is expected to provide important insight into the interaction of the physiologically important biomolecules of CS-5-FU and BSA.

EXPERIMENTAL SECTION

All fluorescence measurements were carried out through a Cary Eclipse 300 FL spectrophotometer (Varian

Company, USA). For the fluorescence measurement, 3.0 mL of 4.0 $\times 10^{-6}$ mol·L^{-1} BSA solution and various amount of CS-5-FU were added to a 1.0 cm quartz cell with a micro-injector. The concentrations of CS-5-FU were ranged from 0 to 9.6×10^{-5} mol·L^{-1}. And the total accumulated volume of CS-5-FU was no greater than 100 μL. The corresponding fluorescence emission spectra were then recorded at 288, 298 and 308K in the range of 300–500 nm upon excitation at 290 nm in each case.

Bovine serum albumin (purity > 98%) was purchased from Sino-American Biotechnology Company (China) and used without further purification. 5-fluorouracil (purity: 99%) was purchased from Shandong Xi Bo C., Ltd. (China). Chitosan (deacetylation degree > 90%, relative molecular mass 1.0×10^{6}) was purchased from Golden-Shell pharmaceutical Co., Ltd. (China). The compound CS-5-FU was prepared in our laboratory according to the literature[10].

RESULTS AND DISCUSSION

Analysis of fluorescence quenching of BSA by CS-5-FU

The fluorescence spectra of BSA with varying concentrations of CS-5-FU are shown in Figure 1. The fluorescence of BSA regularly decreased with the increasing concentration of CS-5-FU, indicating that CS-5-FU interacted with BSA and quenched its intrinsic fluorescence. But no significant shift of the emission maximum wavelength was observed.

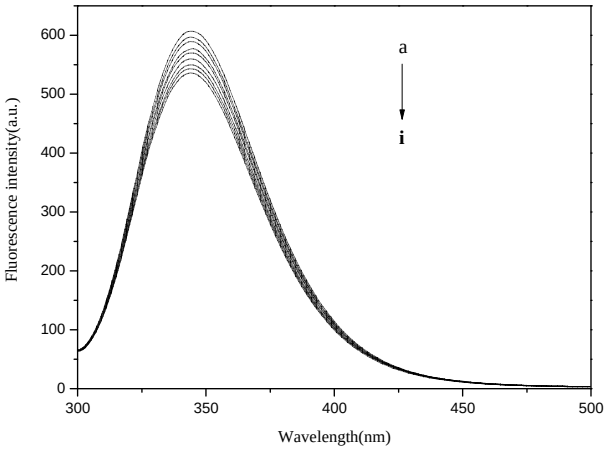

Figure 1 Fluorescence spectra of BSA in the presence of various concentrations of CS-5-FU at 290K, C_{BSA} = 4.0 μM. a→i: $C_{CS\text{-}5\text{-}FU}$ = 0, 12, 24, 36, 48, 60, 72, 84, 96 μM

In order to confirm the type of BSA fluorescence quenching, the fluorescence intensity data was then analyzed according to the Stern–Volmer equation[11]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \qquad (1)$$

where F_0 and F are the fluorescence intensities at 346 nm in the absence and presence of quencher, respectively. [Q] is the concentration of quencher, and K_{sv} is the Stern–Volmer quenching constant. The plots of F_0 /F against [CS-5-FU] were drawn (shown in Figure 2) at 290, 300, and 310 K, and the slope of each plot corresponds to the Stern–Volmer quenching constant K_{SV} at different temperature.

Two quenching mechanisms, namely dynamic quenching and static quenching, can be differentiated by their dependence on temperature and viscosity as well as by lifetime measurements[12]. Dynamic quenching depends upon diffusion: higher temperatures result in larger diffusion coefficients. As a result, the bimolecular quenching constants are expected to increase with temperature rising. In contrast, increased temperature is likely to result in decreasing stability of complexes, and thus lower values of the static quenching constants. It is apparent in Figure 2 that each plot exhibits a good linear relationship (correlation coefficient R^2 > 0.99) and the slope of the Stern–Volmer plots decrease with the temperature rising, indicating that the main quenching mechanism of fluorescence of BSA by CS-5-FU should be a static quenching process by forming a BSA-CS-5-FU complex.

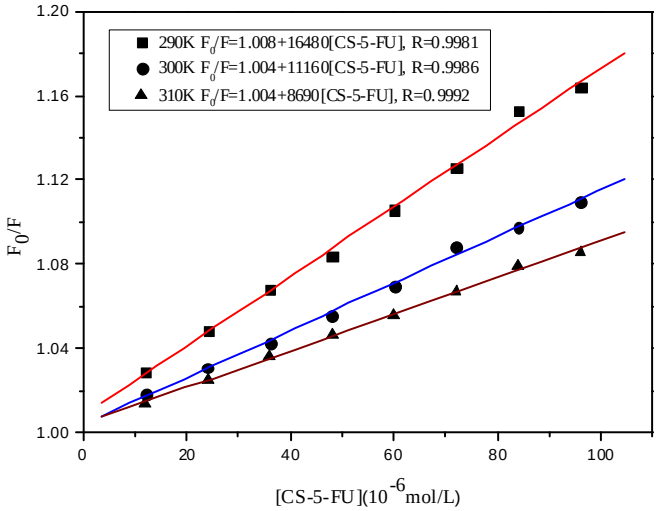

Figure 2 The Stern–Volmer Plots for the fluorescence quenching of BSA by CS-5-FU at three different temperatures. CBSA = 4.0 μM

For a static fluorescence quenching, the fluorescence data at different temperatures was further examined using the Lineweaver–Burk equation[13]:

$$\frac{1}{F_0 - F} = \frac{1}{F_0} + \frac{1}{K_{LB} F_0 [Q]} \tag{2}$$

where F_0 and F are the fluorescence intensities at 346 nm in the absence and presence of quencher, respectively. [Q] is the concentration of quencher, and K_{LB} is the static quenching constant. The values of K_{LB} and R^2 were calculated and listed in Table 1, indicating that the Lineweaver–Burk curve is linear and the stability of CS-5-FU–BSA complex is slightly reduced with increasing temperature. All these results demonstrate characters of static quenching.

Table 1 Binding constants K_a, binding sites n, and thermodynamic parameters of BSA–CS-5-FU system at three temperatures

T (K)	$K_{LB}\times10^{-3}$ (L mol^{-1})	n	R^{2a}	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
290	9.522	1.097	0.9964	−16.57	19.23	−22.14
300	8,141	1.015	0.9934			−22.33
310	6.094	1.009	0.9984			−22.53

[a]*R2 is the correlation coefficient.*

Thermodynamic parameters and nature of the binding forces

The interaction forces between quencher and biomolecules may include hydrophobic force, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc[14]. In order to elucidate the interaction of CS-5-FU with BSA, the thermodynamic parameters were calculated. If the enthalpy change (ΔH) does not vary significantly over the temperature range studied, then its value and that of ΔS can be determined from the van't Hoff equation:

$$\lg K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{3}$$

where K is the Lineweaver-Burk static quenching constant at corresponding temperature and R is the gas constant in this study[15]. The enthalpy change (ΔH) and the entropy change (ΔS) were obtained from the slope and intercept of the fitted curve of ln K_{LB} against 1/T, respectively. Then the free energy change (ΔG) was estimated from the following relationship:

$$\Delta G = \Delta H - T\,\Delta S \tag{4}$$

The values of ΔH, ΔS and ΔG are summarized in Table 1. As seen in Table1, ΔH is a small negative value, whereas ΔS is a positive value, and the main source of ΔG value is derived from a large contribution of ΔS term with little contribution from the ΔH factor. So the binding of CS-5-FU to BSA is an entropy driven process. The negative sign for free energy (ΔG) means that 5-FuAc binding to BSA is spontaneous.

As the aqueous solution in the complex formation of amaranth with BSA, the positive value of ΔS is regularly regarded as an evidence of hydrophobic interaction, because the water molecules that are arranged in an orderly way around the ligand and protein acquire a more random configuration[11]. Besides, the negative ΔH value observed can be mainly attributed to hydrogen bonding, but not to electrostatic interaction[16]. So the binding of ΔH to BSA was driven mainly by hydrophobic force and hydrogen bonds.

Apparent binding constant and binding sites

When small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the following equation[17]:

$$\lg\frac{F_0 - F}{F} = \lg K_a + n\lg[Q] \tag{5}$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, [Q] is the concentration of CS-5-FU, K_a is the apparent binding constant and n is the number of binding sites for drug–BSA interaction. Thus, the intercept and slope values of the plot of log (F_0-F)/F versus log [Q] give the K_a and n values. The values of K_a and n at 290 K were obtained to be 7.552×10^3 L mol^{-1} and 0.9987, respectively. The correlation coefficients are larger than 0.99 indicating that the interaction between CS-5-FU and BSA agrees well with the site-binding model underlined in the above equation. The value of n roughly equal to 1 indicates the existence of just one association site.

CONCLUSION

In this paper, the interaction of CS-5-FU with BSA has been investigated by spectroscopic techniques. The results of fluorescence quenching show that the probable quenching mechanism of fluorescence of BSA initiated by CS-5-FU is a static quenching process by a complex formation at 1:1 mole ratio. The values of thermodynamic parameters suggest that hydrophobic forces and hydrogen bonds play a major role in stabilizing the complex. This study is expected to provide some significant clues to clinical research and the theoretical basis for pharmacology.

REFERENCES

[1] G Bounous; R Pageau; D Regoli, *Int J Clin Pharmacol Biopharm*, **1978**, 16(6), 265–267.
[2] G Francini; R Petrioli; A Aquino; S Gonnelli, *Cancer Chemother. Pharmacol.*, **1993**, 32(5), 359–364.
[3] H-P Li; H Li; Z-D Wang; J-J Zhang; M-F Deng; S-L Chen, *Journal of the Korean Chemical Society*, **2013**, 57(1), 88–93.
[4] P Arbós; MA Campanero; JM Irache, *Journal of Pharmaceutical and Biomedical Analysis*, **2002**, 28(5), 857–866.
[5] Z Liu; S Rimmer, *Journal of Controlled Release*, **2002**, 81(1–2), 91–99.
[6] CW Li; G Li; JC Zuo, *Advanced Materials Research*, **2011**, 287-290, 1509–1512.
[7] MP Patel; RR Patel; JK Patel, *Journal of Pharmacy & Pharmaceutical Sciences*, **2010**, 13(4), 536–557.
[8] C Leslie; PJ Scott; FI Caird, *Med Lab Sci*, **1992**, 49(4), 319–325.
[9] V Anbazhagan; R Renganathan, *Journal of Luminescence*, **2008**, 128(9), 1454–1458.
[10] L Heping; R Jianming; C Hongge; P Tong, *Journal of Changsha University of Science and Technology (Natural Science)*, **2005**, 2(4), 84–88.
[11] JR Lakowicz. Principles of Fluorescence Spectroscopy, 2nd Edition, Kluwer Acacemic Publishers, New York, **1999**.
[12] Y Hu; S Xu; X Zhu; A Gong, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2009**, 74(2), 526–531.
[13] PD Ross; S Subramanian, *Biochemistry*, **1981**, 20(11), 3096–3102.
[14] G Sudlow; DJ Birkett; DN Wade, *Mol. Pharmacol.*, **1976**, 12(6), 1052–1061.
[15] H Bian; M Li; Q Yu; Z Chen; J Tian; H Liang, *Int. J. Biol. Macromol.*, **2006**, 39(4-5), 291–297.
[16] G Zhang; A Wang; T Jiang; J Guo, *Journal of Molecular Structure*, **2008**, 891(1–3), 93–97.
[17] AR Timerbaev; CG Hartinger; SS Aleksenko; BK Keppler, *Chem. Rev.*, **2006**, 106(6), 2224–2248.