Asian Journal of Chemistry; Vol. 27, No. 4 (2015), 1283-1286

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17603

Fluorescence Spectrometry of Interaction of Acyclovir and Bovine Serum Albumin in Presence of Carbon Nanotubes

XIAOXIA WANG[1,*], ZHENGDE WANG[1], JINYAN LIU[1], BIN HOU[1], YUNYING LIU[1], HUI YAN[1] and GAOWA AODENG[2]

[1]School of Chemistry and Chemical Engineering, Inner Mongolia University of Science and Technology, Baotou 014010, P.R. China
[2]School of Chemistry and Chemical Engineering, Inner Mongolia University, Hohhot 010021, P.R. China

*Corresponding author: E-mail: wxx572369@163.com

Received: 14 March 2014; *Accepted*: 27 May 2014; *Published online*: 4 February 2015; AJC-16776

The interaction between acyclovir and bovine serum albumin in presence of carbon nanotubes was investigated by fluorescence spectrometry. Testing the fluorescence value of three material mixture by fluorescence spectrophotometer, scanning the synchronous fluorescence spectrum of acyclovir and bovine serum albumin and analyzing the quenching effect. The experimental results showed that fluorescence quenching of acyclovir for bovine serum albumin is mainly static quenching, acyclovir has a main fluorescence quenching effect on the tryptophan of bovine serum albumin and has no impact on the basic conformation of bovine serum albumin. The addition of carbon nanotubes in the binary mixture, the fluorescence quenching effect was more apparent and would become more obvious with the increasing amount of carbon nanotubes.

Keywords: Acyclovir, Bovine serum albumin, Carbon nanotubes, Synchronous fluorescence, Fluorescence quenching.

INTRODUCTION

Acyclovir is a kind of synthetic purine nucleoside analogues and chemical name is 9-(2-hydroxyl methyl ethyl oxygen) guanine. It has remarkable curative effect on some infections which caused by herpes virus, cytomegalo virus and Epstein-Barr virus infections. It is a kind of efficient broad-spectrum antiviral drug and has been included in the national essential drugs[1,2].

Multi-walled carbon nanotubes (MWCNTs) is a kind of nano materials which have excellent performance. Carbon nanotubes have special tubular structure, small volume, as a high efficient mass transfer unit, it can through the cell wall more easily. The specific surface area of carbon nanotubes is large, so it is easy to adsorption of organic molecules. Recently, the research results show that carbon nanotubes can be used for conducting organic molecules, such as amino acid, protein and nucleic acid. It can realize the control of drug or slow release as a drug carrier nanometer, reducing the side effects of drugs, improving the curative effect. It opens the way for the application of the drug delivery system[3,4].

This paper used the method of fluorescence quenching to study the fluorescence quenching effect of carbon nanotube on interaction of acyclovir and bovine serum albumin and the fluorescence spectral characteristics influence on a mixture of acyclovir and bovine serum albumin. Synchronous fluorescence method was used to study the interaction of carbon nanotube and acyclovir and bovine serum albumin (tyrossine, Tyr) tyrosine and tryptophan residues (tryptopan, Trp). This plays and important role in evaluating drug quantitatively and understanding the nonspecific effects of carbon nanotubes protein.

EXPERIMENTAL

F-2500 Fluorescence spectrophotometer(Japan's Hitachi Ltd.), thermostat water bath (GuoHua SHA-B): Acyclovir (identification of pharmaceutical and biological products of China), bovine serum albumin (BSA, Sigma company), carbon nanotubes (aqueous dispersion, Beijing DK nano technology Co. Ltd.), the medicine were analytically pure, the water is secondary distilled deionized water, no fluorescent impurities.

Bovine serum albumin solution: Using *tris*-HCl which concentration is 0.1 mol L^{-1}, pH is 7.4 to perpare solution, the concentration of the bovine serum albumin solution is 1×10^{-5} mol L^{-1} and use the solution of NaCl (0.5 mol L^{-1}) to maintain ionic strength.

Acyclovir solution: Using the double-distilled water to perpare solution, the concentration is 1×10^{-4} mol L^{-1}.

Carbon nanotubes solution: Diluting the carbon nanotubes water dispersion form 10 to 1 g/L.

Spectral measurements: (1) Adding bovine serum albumin solution and acyclovir solution with different concentrations to 10 mL colorimetric tubes, using the secondary distilled deionized water to constant volume and placed them at room temperature for 1 h, to determine the fluorescence spectrum. The experiment measured the excitation wavelength of acyclovir (Ex) is 280 nm, emission wavelength is 342 nm, slit width is 5 nm. In 310-550 nm range scanning emission spectrum, at the same time, fixing the wavelength differential of fluorescence emission and excitation wavelength $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm, scanning the synchronous fluorescence spectrum.

(2) Taking two samples of the same bovine serum albumin solutions to colorimetric tubes, adding quantitative acyclovir solution to one of the bovine serum albumin solution, both of them should be constant volume by the secondary distilled deionized water, shaking well. Putting them at 37, 25 and 44 °C in constant temperature water bath for 1 h, using the same parameters to scan the fluorescence spectrum under different temperature.

(3) Adding bovine serum albumin solution and acyclovir solution with different concentrations to 10 mL colorimetric tubes. Adding 0.6 mL solution which 1 g/L solution of carbon nanotubes to the systems. Shaking well and use the secondary distilled deionized water to constant volume at room temperature for 1 h. Using the same test conditions like (1) to scan its fluorescence spectrum.

RESULTS AND DISCUSSION

Fluorescence quenching effect of acyclovir and bovine serum albumin: Fig. 1 displays that the fluorescence intensity of bovine serum albumin reduce as the increase of the amount of acyclovir, there were a pronounced quenching. Emission peak position and peak shape remain unchanged basically, so there is an interaction.

According to the well-know Stern-Volmer equation[5]:

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

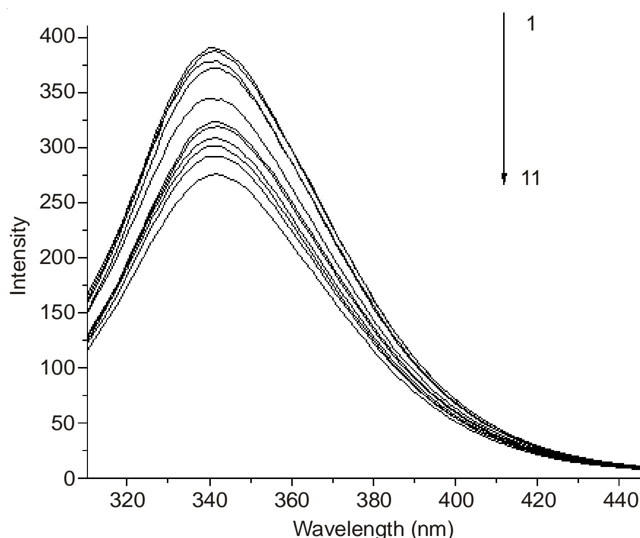

Fig. 1. Effect of acyclovir to bovine serum albumin on fluorescence spectrum $C_{(ACV)}/(\mu mol\ L^{-1})$: 1-11:0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50; C_{BSA} = 10 µmol L^{-1}

F_0 and F represent the fluorescence intensities in the absence of acyclovir and presence, k_q is the quenching rate constant of the biomolecule, $\tau_0(10^{-8}s)$ is the average life expectancy of biological macromolecules without any quencher, K_{SV} is the quenching constant of Stern-Volmer and [Q] is the concentration of the quencher.

In static quenching, relationship between fluorescence intensity and the amount of quencher can be elicited by binding constant expression about fluorescence quenching agent molecules[6].

$$\log[(F_0 - F)/F] = \log K_A + n\log[Q] \quad (2)$$

In the equation, F_0 and F represent the fluorescence intensities of bovine serum albumin in the absence and in presence of acyclovir, n is number of biding sites, K_A is the dinding constant, [Q] is the concentration of the quencher.

After linear fitting, we can know that the quenching constant (K_{SV}) is 1.179 × 10^4 L mol^{-1} and calculated binding sites (n) is 1.0603, the quenching constant (K_q) is 1.179 × 10^{12} L mol^{-1} s^{-1}. According to other literatures, we can know that the biggest spread impact quenching rate constant about all kinds of quenching agent to biological macromolecules is 2 × 10^{10} L mol^{-1} s^{-1}, but the fluorescence quenching constant of acyclovir and bovine serum albumin is larger than it. So we can know that quenching of acyclovir to bovine serum albumin is static quenching used by the molecular collision.

Study on the effect of acyclovir on fluorescence spectrum of bovine serum albumin by synchronous fluorescence: There are two tryptophan residue (Trp) (Trp 134 and Trp 212) in protein molecule. Trp 134 is near the bovine serum albumin molecular surface, Trp 212 is internal, attending the interfacial formation of IIA and IIIA hydrophobic cavity, hydrophobic cavity plays a major role in the process of combination drug. Generally, the tyrosine (Tyr) residues is in the molecule inside. So in the most cases, the fluorescence of protein is revealed almost by tryptophan. The small molecules of acyclovir can insert into the protein molecules inside, not only acting on the Try residues, but also on the Trp residues, so the binding constant is more great than carbon nanotubes. Using the method of synchronous fluorescence, adopting fixed wavelength and synchronous scanning, this can clearly distinguish the fluorescence of tyrosine and tryptophan residues[7,8].

For the synchronous fluorescence of protein, $\Delta\lambda$ = 15 nm just shows the fluorescence of tyrosine residues, $\Delta\lambda$ = 60 nm only shows the fluorescence of tryptophan residues. Because the maximum emission wavelength of protein amino acid residues is related to the polarity and hydrophobic property of environment where protein amino acid residues present. So we can judge the change of protein conformation by the change of the emission wavelength[9]. Fig. 2a and b show that the synchronous fluorescence quenching spectrum of the series solutions and the spectrum is scaned under $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm. According to the Figure, the maximum emission wavelength position of synchronous fluorescence spectrum do not change basically in Fig. 2a, the quenching phenomenon is not obvious; the maximum emission wavelength position of synchronous fluorescence spectrum appears the red shift in Fig. 2b, from 281 to 383 nm, the quenching effect is more

obvious. Thus, the interaction of acyclovir and bovine serum albumin molecules play major role in Trp residues, affecting the conformation of bovine serum albumin and affects the micro environment of Trp residues been in, making the polarity and hydrophobic property of bovine serum albumin's hydrophobic environment reduced and the internal hydrophobic structure of bovine serum albumin been collapsed, hydrophilic property increases, further make the extension of the peptide chain increased[10].

According to Stern-Volmer equation, we can get the K_{SV} under the $\Delta\lambda$ = 15 nm is 1.3686 × 104 L mol^{-1} s^{-1}, the K_{SV} under $\Delta\lambda$ = 60 nm is 1.9584 × 105 L mol^{-1} s^{-1}. So we can judge that the interaction of acyclovir and bovine serum albumin is major role in bovine serum albumin Trp residues and impact weakly on Tyr residues. The results which get by the method of fluorescence quenching show that, fluorescence quenching effect of acyclovir to bovine serum albumin is caused by Tyr and Trp residues, which is major role in Trp residues.

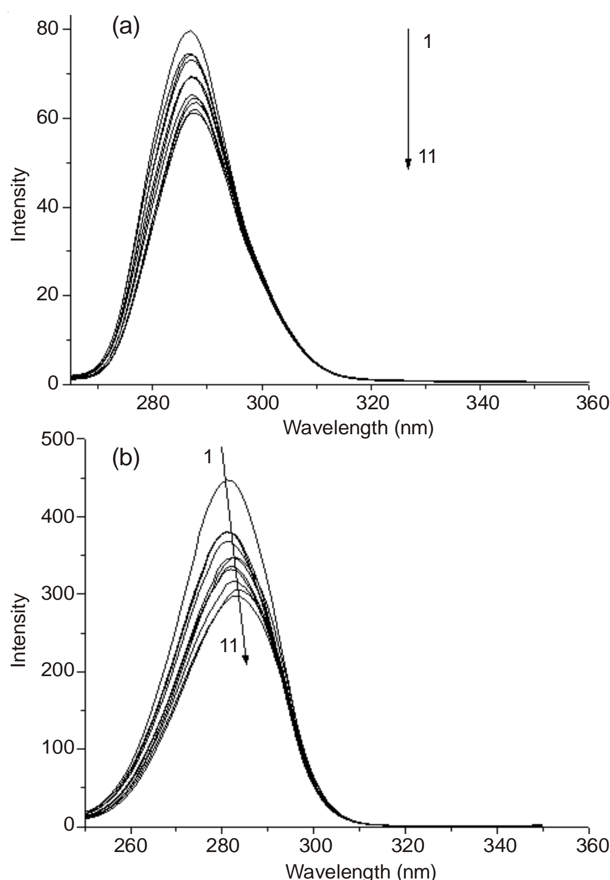

Fig. 2. Synchronous fluorescence spectra of acyclovir and bovine serum albumin (a) $\Delta\lambda$ =15 nm; (b)$\Delta\lambda$ = 60 nm $C_{(ACV)}$/(μmol L^{-1}): 1-11:0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50; C_{BSA} = 10 μmol L^{-1}

Effect of temperature on acyclovir and bovine serum albumin: If the temperature does not vary significantly, the enthalpy change (ΔH) of binding reaction can be regarded as constants. According to the binding constant at different temperatures and following equation, we can calculate the reaction enthalpy, Gibbs free energy and entropy change[11].

$$\ln\frac{K_{A,2}}{K_{A,1}} = \frac{\Delta H}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad (3)$$

$$\Delta G = -RT\ln K_A \quad (4)$$

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

ΔG is the Gibbs free energy, ΔH is enthalpy change of binding reaction, ΔS is the entropy change, R is the gas constant 8.314, T is the thermodynamic temperature, K is the binding constant.

TABLE-1
THERMODYNAMIC BINDING PARAMETERS
FOR ACYCLOVIR AND BOVINE SERUM ALBUMIN

T/K	ΔG/(kJ mol^{-1})	ΔH/(kJ mol^{-1})	ΔS/(J K^{-1} mol^{-1})
298.15	-11.44		40.79
310.15	-10.94	-23.60	40.80
317.15	-10.85		40.20

In Table-1, by calculating, $\Delta G < 0$, showing that the combination of acyclovir and bovine serum albumin is a spontaneous process; $\Delta H < 0$, $\Delta S > 0$ shows that effect of acyclovir and bovine serum albumin molecular is electrostatic forces.

Fluorescence quenching effect of carbon nanotubes for bovine serum albumin: Fluorescence intensity of bovine serum albumin decreased as the increase of the carbon nanotubes's concentration. There was no change of emission peak position and peak shape, it suggested that there is an interaction of each other. Carbon nanotubes make a significant fluorescence quenching effect on bovine serum albumin by nonspecific adsorption. According to the method of synchronous fluorescence, carbon nanotubes make a weak effect on bovine serum albumin tyrosine residues and it majorly affect on tryptophan residues. The result is consistent with the literature[12].

Fluorescence spectrum effect of multi-walled carbon nanotubes (MWCNTs) to acyclovir and bovine serum albumin: Adding carbon nanotubes solution with different concentration to mixed solutions of acyclovir and bovine serum albumin and measuring the change of fluorescence intensity (Fig. 3). Within a certain range, the inclusion effect of bovine serum albumin for acyclovir has an obvious influence with

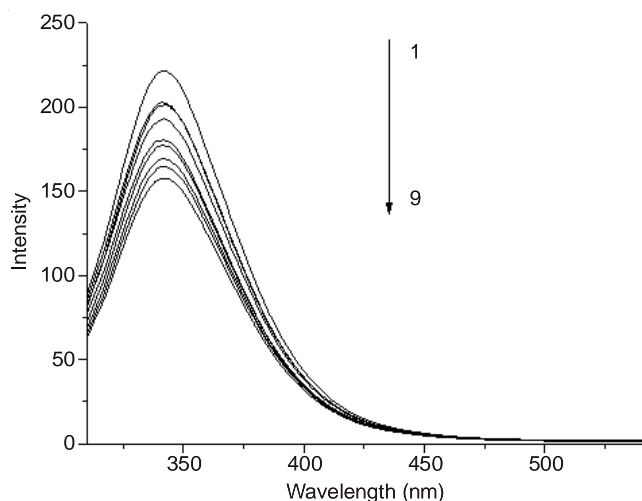

Fig. 3. Fluorescence spectra of 0.6 mL carbon nanotubes in the acyclovir and bovine serum albumin $C_{(ACV)}$/(μmol L^{-1}): 1-9:0, 5, 10, 15, 20, 25, 30, 35, 40; $C_{(CNTs)}$ = 0.06 g/L; $C_{(BSA)}$ = 10 μmol L^{-1}

the number of carbon nanotubes increasing, this can make the fluorescence intensity of bovine serum albumin decreased significantly. According to Stern-Volmer equation, we can get the binding constant and binding points of mixed solutions, which have been added by carbon nanotubes (Table-2).

TABLE-2
BINDING CONSTANTS AND NUMBER OF BINDING SITES OF ACYCLOVIR AND BOVINE SERUM ALBUMIN

C_{MWCNTs}	Linear equation	n	K_{SV}
0.0	log [(F_0-F)/F] = 1.0603 log [Q]-4.0716	1.0603	1.179×10^4
0.6	log [(F_0-F)/F] = 1.2805 log [Q]-5.5969	1.2805	3.953×10^5

Table-2 showed that the combination between bovine serum albumin and acyclovir was attenuated as the concentration of carbon nanotubes increasing. The size of bovine serum albumin is similar to the carbon nanotubes. Therefore, carbon nanotubes can combine with Trp residues on the surface of the protein molecules and could not combine with Tyr residues by entering into protein molecules. Thus, it can not affect the fluorescence of Tyr residues. At the same time, carbon nanotubes combine with the amino acid residues of bovine serum albumin, there is a competition on binding sites between carbon nanotubes and acyclovir, so that the point of acyclovir for bovine serum albumin molecules decreases, therefore, after joining carbon nanotubes, the combination of acyclovir and bovine serum albumin binding constant decreases.

Conclusion

The experimental results show that the interaction of acyclovir and bovine serum albumin is a static quenching which caused by molecular collision. By the research of synchronous fluorescence we know that acyclovir strongly quenched the Trp residues of bovine serum albumin, but the quenching effect on Tyr residues is weak and this affects the conformation of bovine serum albumin and micro environment of Trp residues. It is also concluded that the combination of acyclovir and bovine serum albumin is an spontaneous process and the forces are electrostatic interactions. After adding carbon nanotubes to the solutions, carbon nanotubes mainly act on Trp residues of bovine serum albumin, the binding constant of acyclovir and bovine serum albumin molecules decreases, two kinds of materials make the fluorescence quenching of bovine serum albumin increased.

ACKNOWLEDGEMENTS

This work was financially supported by the Ministry of Education "chunhui plan" funded projects (No. 2009-1-01040), College Scientific Research Fund Project in Inner Mongolia Department of Education (No. NJZY11145) and Inner Mongolia University of Science and Technology Innovation Fund Project (No. 2012NCL034).

REFERENCES

1. J.J. Li, J.J. Ju, T. Pu, J.J. Guo and C.M. Yu, *Chem. Prod. Technol.*, **19**, 9 (2012).
2. M. Yang, Y. Zou, S.J. Yu and X.H. Li, *Anal. Chem.*, **32**, 1237 (2004).
3. Q.X. Zhou, *Chem. Ind. Eng. Progr.*, **25**, 750 (2006).
4. S.S. Li, *Progr. Chem.*, **20**, 1798 (2008).
5. M.D. Meti, S.D. Gunagi, S.T. Nandibewoor and S.A. Chimatadar, *Monatsh. Chem.*, **144**, 1253 (2013).
6. S.A. Markarian and M.G. Aznauryan, *Mol. Biol. Rep.*, **39**, 7559 (2012).
7. B.S. Liu and X.N. Yan, *Chin. J. Lumin.*, **33**, 1018 (2012).
8. L.J. Li, *Spectrosc. Spectral Anal.*, **26**, 81 (2006).
9. G.W. Aodeng and Y.-C. Jin, *Chin. J. Lumin.*, **32**, 404 (2011).
10. B.S. Liu and C.L. Xue, *Chin. J. Lumin.*, **31**, 285 (2010).
11. B.-S. Liu, J. Wang, C.-L. Xue, C. Yang and Y.-K. Lv, *Monatsh. Chem.*, **143**, 401 (2012).
12. S.S. Li, *Spectrosc. Spectral Anal.*, **30**, 2689 (2010).