International Journal of Advanced Research in Chemical Science (IJARCS)
Volume 5, Issue 7, 2018, PP 12-18
ISSN No. (Online) 2349-0403
DOI: http://dx.doi.org/10.20431/2349-0403.0507003
www.arcjournals.org

Fluorescence Spectroscopic Study of the Interaction between the Baltic Amber Extract and BSA

Kexin Yin, Kaihong Xie, Zhenzhen Liu, Sufang Sun*

College of Chemistry and Environmental Science, Hebei University, Baoding, China

Corresponding Author:** ***Sufang Sun, *College of Chemistry and Environmental Science, Hebei University, Baoding, China*

Abstract: *The Baltic amber extract was prepared and it was firstly used to react with BSA in order to develop the medicinal value of the amber. Fluorescence quenching spectroscopy found that the fluorescence intensity of BSA gradually decreased with the increase of the amber extract and the peak of the maximum excitation light of BSA was blue-shifted. Meanwhile, the quenching constants obtained decreased with increasing temperature, which indicated the quenching mechanism was static. The result from Rayleigh Scattering (RLS) Spectroscopy also showed the same quenching mechanism, Finally Synchronous fluorescence investigated that the binding site of amber extract with BSA was closer to tryptophan.*

Keywords: *Baltic amber, Fluorescence spectroscopic, Bovine serum albumin, Rayleigh Scattering*

1. INTRODUCTION

Serum albumin is the most abundant protein in vertebrate blood plasma. It has the characteristics of good elasticity, small molecular weight, high water solubility, good stability and easy purification and preparation[1-2]. Serum albumin consists of Three similar structures, which are α-helical domains I, α-helical domain II and α-helix domain III. Each domain has two subdomains A and B. A and B form a cylindrical structure in such a way that the notches face each other to form a hydrophobic chamber. Because of its unique structure, serum albumin binds and transports both endogenous and exogenous substances [3], allowing it to reversibly bind to many drugs. It has pharmacokinetics and biological functions pharmacokinetic and biological functions. Therefore, in recent decades, serum albumin has become an ideal protein for scientists to study the binding of proteins to small molecules. Serum albumin often uses bovine serum albumin (BSA) and human serum albumin (HSA) to study their interactions with various drugs. Because their tertiary structure has 76% similarity [4], and BSA is more easily available and cheaper, so this article chooses BSA for its combination with drugs.

Amber is not only a beautiful ornament, but also has a certain medicinal value. It is an important traditional Chinese medicine in ancient China, such as the effect of calming the nerves, revitalizing blood circulation, and diuresis-passing et al [5-6] .Therefore, the study of medicinal value of amber is very meaningful and promising. However, since amber is not completely soluble in any of the solvents, the interaction between amber and the human body has not been further studied so far.

In this article, the ethyl alcohol was used to extract the soluble substance in the Baltic amber, and the products obtained were used to interact firstly with the BSA under simulative human physiological conditions in order to provide a theoretical basis for the intensive study of amber as a drug.

2. MATERIALS AND METHODS

2.1. Reagents and Apparatus

Reagents: Baltic amber powder (from Ukraine amber), BSA (≥98%, 68000 Da, Biotopped), absolute ethyl alcohol (≥99.7%,Tianjin Fengchuan chemical reagent technology Co. Ltd.) All materials except Baltic amber were of analytical grade and used without any further purification.

Apparatus: Magnetic Hotplate-Stirrer with timer (MS-H-ProT), Ultraviolet visible spectrophotometer (TU-1950), Electronic balance (CP224C), Table model high speed centrifuge (TG16-WS), Electro-thermostatic blast oven (DHG-9035A) and Fluorescence spectrometer (F-7000) were used.

2.2. Preparation of Experimental Materials

The BSA stock solution was prepared with 0.10 mol/L PBS buffer solution (pH 7.4) and diluted to a concentration of 1.0×10^{-4} mol/L. The prepared BSA stock solution was stored at 4°C in refrigerator

The baltic amber extract was obtained according to the following process: 100g Baltic amber powder was weighed and soaked in 1000mL absolute ethyl alcohol for two days and nights, after that the mixture was centrifuged, the supernatant was collected and steamed at 40^0C in rotary evaporator, the extract was obtained and stored for the next use at room temperature.

Different concentrations (0.0g/L, 2.0×10^{-3} g/L, 3.0×10^{-3}g/L, 4.0×10^{-3} g/L, 5.0×10^{-3} g/L, 6.0×10^{-3}g/L, 7.0×10^{-3} g/L, and 8.0×10^{-3} g/L) of Baltic extract was prepared with phosphate buffer (PH 7.4) containing 2% alcohol.

2.3. Determination of Fluorescence Spectroscopy

Samples preparation: 0.1 mL of 1.0×10^{-4} mol/L BSA solution was taken and added into 10mL Baltic extract solution with different concentration varying from 0.0 to 8.0×10^{-3} g/L prepared in section 2.2, 20 minutes after reaction at the specified temperature, the fluorescence qualities were determined using circulating water throughout the experiment to keep the temperature of the sample constant.

Fluorescence quenching spectroscopy: the samples were subjected to fluorescence quenching at three temperatures of 298K, 304K, and 310K with the excitation wavelength 280 nm, the emission wavelength 290-500 nm, the excitation and emission slit width 5 nm. The voltage used in this experiment was set to 500 V and the scan rate was 2400 nm/min.

Synchronous fluorescence spectroscopy: the synchronous fluorescence data was collected at 298K, 304K, and 310K temperatures with the emission wavelength 250-320 nm, $\Delta\lambda$=15 nm and 60 nm, and the scan rate 1200 nm/min. The other scanning parameters were the same as those of the fluorescence quenching spectrum.

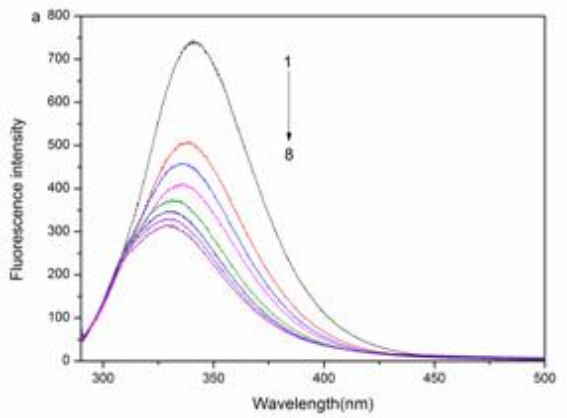
Rayleigh Scattering (RLS) Spectroscopy: the experiments were done at 298K with the excitation wavelength 250 nm, the emission wavelength 250-600 nm, and the scan rate 1200 nm/min. The other scanning parameters were the same as the fluorescence quenching spectrometry.

3. Results and Discussion

3.1. Fluorescence Quenching Spectroscopy

The fluorescence in the albumin protein was mainly tyrosine residues and tryptophan residues[7], and the change of their surrounding environment could directly affect the fluorescence intensity and the change of the peak position of the maximum emission peak. Therefore, changes in fluorescence intensity and changes in the peak position of the maximum emission peak could be used to explore the interaction between drugs and proteins [8].

Fluorescence quenching experiments were performed at three temperatures of 298K, 304K, and 310K, the data obtained was list a, b, and c in Fig.1. From the Figure, it could be seen that the fluorescence intensity of BSA reaction solution gradually decreased as the concentration of the added amber extract increased, and blue shift of the maximum emission peak occurred. At 298 K, the maximum emission peak shifted from 340.6 nm to 330.6 nm. At 304 K, the maximum emission peak shifted from 340.4 nm to 330.8 nm. And at 310 K, the maximum emission peak shifted from 341 nm to 330.8 nm. These phenomena indicated that the addition of amber powder extract increased the hydrophobicity of the microenvironment around BSA and decreased its polarity. Fig. 1 also showed that the degree of quenching was getting smaller and smaller with the concentration of the added amber getting bigger and bigger, which meant that the binding sites of the amber extract were gradually taken up in BSA and slowly reached saturation [9].



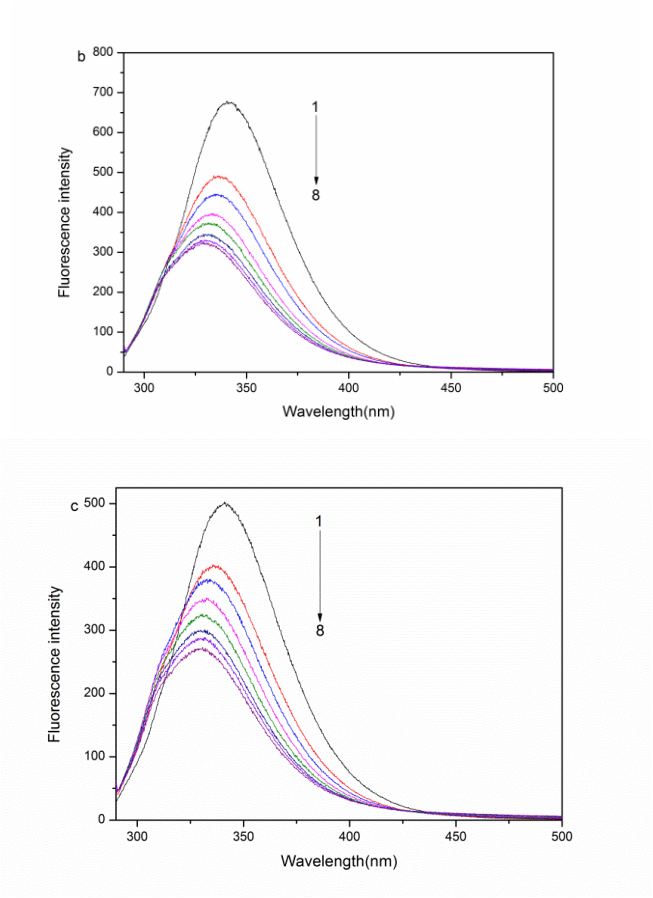


Fig1. *Fluorescence quenching spectra of BSA in different amber extract concentration at 298K(a)、304K(b), 310K(c) (λ_{ex}=280nm);c(BSA)=1.0×10^{-6}mol/L; c(amber extract)(1-8): 0.0, 2.0×10^{-3}, 3.0×10^{-3}, 4.0×10^{-3}, 5.0×10^{-3}, 6.0 ×10^{-3}, 7.0×10^{-3}, 8.0×10^{-3}g/L*

3.2. Fluorescence Quenching Mechanism and Quenching Constant

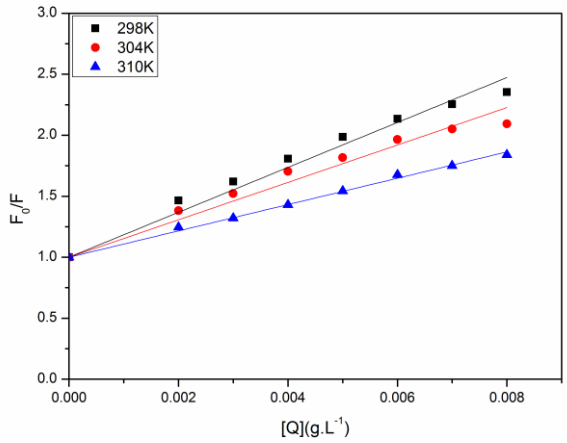


Fig2. *Stern-Volmer plots for the quenching of BSA in different amber extract concentration at 298K, 304K, 310K*

Table1. *The parameters of Stern-Volmer plots at 298K, 304K, 310K*

T(K)	Stern-Volmer linear equation	R	K_{SV}(L.g^{-1})	Kq(×10^{-8}L.g^{-1})
298	Y=1.0000+184.1103X	0.9984	184.1103	184.1103
304	Y=1.0000+153.3384X	0.9981	153.3384	153.3384
310	Y=1.0000+107.9222X	0.9999	107.9222	107.9222

R is the correlation coefficient for the Stern-Volmerplot respectively.

The fluorescence data obtained in Fig.1 was put into the following Stern-Volmer equation[10],

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

Here, F_0 was the fluorescence intensity in the absence of amber extract, F was the fluorescence intensity in the presence of the Baltic amber extract; K_q was the fluorescence quenching rate constant, τ_0 was the fluorescence lifetime of BSA, which was about 10^{-8}s [11], K_{SV} was the Stern-Volmer quenching constant, and $[Q]$ was the concentration of amber extract added, the unit is $g.L^{-1}$.

The results from the Stern-Volmer were plotted in Fig.2, which showed a F_0/F -to-$[Q]$ Linear fitting graph at three temperatures (298K, 304K, and 310K). According to Fig.2, the K_q and K_{SV} values were determined and were listed in Table 1, from the results it was found that the value of K_{SV} decreased with the increase of temperature, indicating that the quenching effect of amber extract on BSA was static.

3.3. Rayleigh Scattering (RLS) Spectroscopy

Fig.3 showed the Rayleigh scattering spectrum of the amber extract-BSA reaction system. It could be seen from the Fig.3 that the RLS signal of pure BSA was very weak in the wavelength ranging from 250nm to 600nm, As the concentration of Baltic amber extract increased the RLS signal gradually increased which meant the particle size in the solution increased or different sizes of particle existed. From this, it could be concluded that the Baltic amber extraction reacted with BSA and formed complexes. It also demonstrated that the quenching mechanism between BSA and amber powder extracts was static.

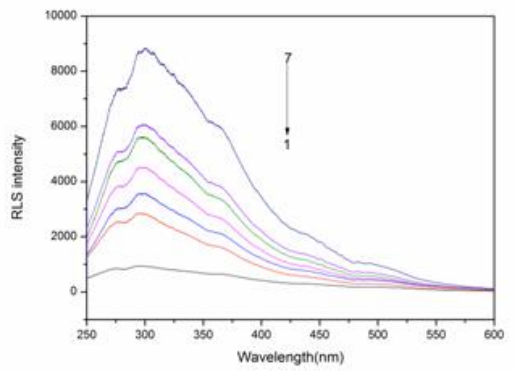
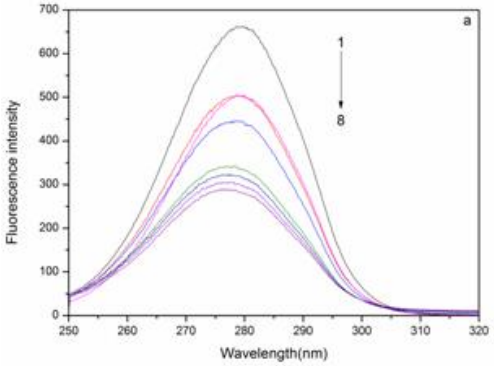


Fig3. *RLS spectra of amber extract-BSAsystem. c(amber extract)(1-7): 0.0, 2.0×10^{-3}, 3.0×10^{-3}, 4.0×10^{-3}, 5.0×10^{-3}, 6.0×10^{-3}, 7.0×10^{-3}g/L; c(BSA)=1.0×10^{-6}mol/L; T=298K*

3.4. Synchronous Fluorescence

Synchronous fluorescence spectroscopy was more selective than conventional fluorescence, so it was often used to explore the effect of drugs on protein conformation. If the difference between the emission wavelength and the excitation wavelength was 15nm, the synchronous fluorescence spectra only reflected the spectral characteristics of tyrosine residues; if Δ λ=60 nm, only the spectral characteristics of tryptophan residues were reflected [12]. The change of the corresponding residue environment was judged by the change of the maximum excitation wavelength peak position.



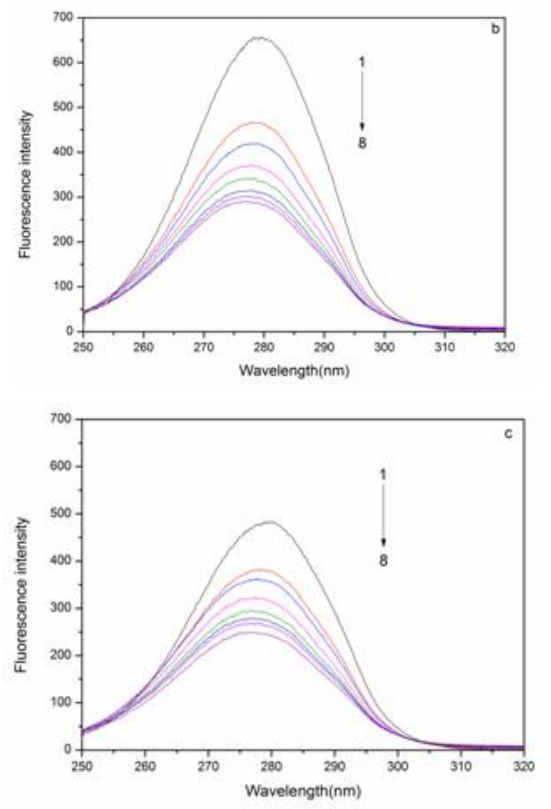
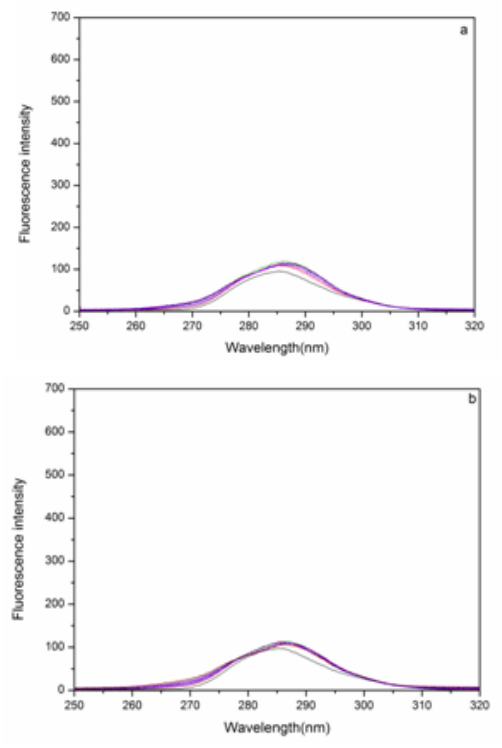


Fig4. *Synchronous fluorescence spectra of BSA by the amber extract at 298K(a), 304K(b), 310K(c) (Δλ=60nm);c(BSA)=1.0×10^{-6}mol/L; c(amber extract)(1-8): 0.0, 2.0×10^{-3}, 3.0×10^{-3}, 4.0×10^{-3}, 5.0×10^{-3}, 6.0 ×10^{-3}, 7.0×10^{-3}, 8.0×10^{-3}g/L*

As seen in Fig.4, when Δλ = 60nm, the fluorescence intensity of BSA gradually decreased with the increase of the concentration of Baltic amber extract at three temperatures of 298K, 304K and 310K, and 3nm blue shift occurred at the peak position at each temperature. The above phenomenon proved that the environment near the tryptophan residue was affected by the addition of the amber extract, which made the polarity of the external micro-environment reduce and the hydrophobicity increase, indicating the conformation of BSA was changed.



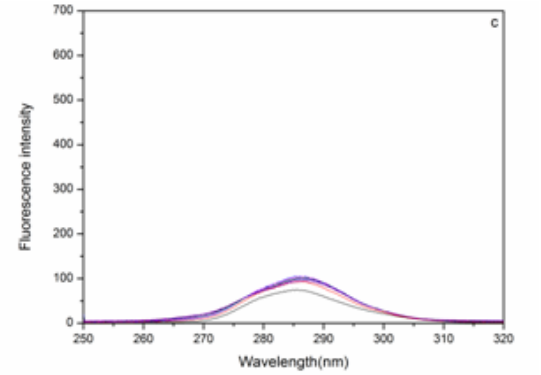


Fig5. *Synchronous fluorescence spectra of BSA by the amber extract at 298K(a), 304K(b), 310K(c) (Δλ=15nm); c(BSA)=1.0×10^{-6}mol/L; c(amber extract)(1-8): 0.0, 2.0×10^{-3}, 3.0×10^{-3}, 4.0×10^{-3}, 5.0×10^{-3}, 6.0 ×10^{-3}, 7.0×10^{-3}, 8.0×10^{-3}g/L*

As seen in Fig.5, when Δλ = 15 nm, the fluorescence intensity of BSA hardly changed, and the peak position of the maximum emission peak was hardly changed, indicating that there was nearly no change in the conformation of tyrosine residues.

In summary, the addition of amber extract had a great effect on tryptophan, nearly no influence on tyrosine residues, which meant that the binding site of the interaction between the amber extract and BSA was closer to the tryptophan residue.

4. CONCLUSION

In this paper, the extract substance was obtained from Baltic amber and used to react with BSA firstly, and the fluorescence spectroscopy was done to study the interaction between them. From fluorescence quenching spectroscopy, it could be seen that the fluorescence intensity gradually decreased with the increase of the amber extract, indicating that BSA was quenched to form a non-fluorescent complex. The peak of the maximum excitation light of BSA was blue-shifted, which meant that the addition of the amber extract reduced the polarity of the external microenvironment of fluorescent substance in BSA. The quenching constants calculated gradually decreased with the increase of the temperature, proving that the quenching effect of amber powder extract on BSA was static. Rayleigh scattering (RLS) spectrum showed that the particle size in the solution increased or different sizes of particle existed, which also demonstrated that the static quenching mechanism described above. Finally, synchronous fluorescence spectroscopy investigated the binding site of amber extract with BSA was closer to tryptophan. All in all, the above results will lay a theoretical foundation for further developing the medicinal value of amber.

REFERENCES

[1] Cheng F Q, Wang Y P, Li Z P, et al. Fluorescence study on the interaction of human serum albumin with bromsulphalein [J]. Spectrochim Acta A Mol Biomol Spectrosc, 2006, 65(5):1144-1147.

[2] Shaikh S M T, Seetharamappa J, Kandagal P B, et al. Binding of the bioactive component isothipendyl hydrochloride with bovine serum albumin[J]. Journal of Molecular Structure, 2006, 786(1): 46-52.

[3] Liu Yuan, Qin Chuan, Hou Feier, et al. Study on the Interaction Mechanism of Betaine Flavonoids with Different Isomers of Human Serum Albumin [J]. Chinese Journal of Chemical Chemistry, 2009, 67(7): 629-636.

[4] Qi Wei, He Zhimin. Homologous modeling and structural analysis of bovine serum albumin[J]. Computers and Applied Chemistry, 2006, 23(9): 821-824.

[5] Yang Jianfang, Dong Xiaoping, Guo Li et al. Progress in chemical research of amber[J]. Beijing Zhong Yi Yao, 2002, 21(4):245-248.

[6] Li Chunbo, Shao Bin. Vitamin E and its succinate and calcium succinate in health food applications [J]. Journal of China Pharmaceutical University, 2003, 34(2): 190-192.

[7] Yi Li. Study on the interaction between p-nitrophenol, Sudan Red III and bovine serum albumin by spectroscopy [D]. Liaoning University, 2013.

[8] Naveenraj S, Anandan S. Binding of serum albumins with bioactive substances – Nanoparticles to drugs[J]. Journal of Photochemistry & Photobiology C Photochemistry Reviews, 2013, 14(14):53-71.

[9] Jain M K. Principles of Fluorescence Spectroscopy: Joseph R. Lakowicz Plenum Press, New York, 1983, 496 pp. $32.50 [J]. Journal of Biochemical & Biophysical Methods, 1984, 9(2):181-181.

[10] Zhang L N, Wu F Y, Liu A H. Study of the interaction between 2,5-di-[2-(4-hydroxy-phenyl)ethylene]-terephthalonitril and bovine serum albumin by fluorescence spectroscopy[J]. Spectrochimica Acta Part A Molecular & Biomolecular Spectroscopy, 2011, 79(1):97.

[11] Lakowicz J R, Weber G. Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules. [J]. Biochemistry, 1973, 12(21): 4161-70.

[12] Guo M, Lü W J, Li M H, et al. Study on the binding interaction between carnitine optical isomer and bovine serum albumin [J]. European Journal of Medicinal Chemistry, 2008, 43(10):2140-2148.

Citation: *Kexin Yin et al., "Fluorescence Spectroscopic Study of the Interaction between the Baltic Amber Extract and BSA", International Journal of Advanced Research in Chemical Science (IJARCS), vol. 5, no. 7, pp. 12-18, 2018. http://dx.doi.org/10.20431/2349-0403.0507003*