Effects of Temperature and Common Ions on the Interaction Between Puerarin and BSA

HUI CAO, QUAN LIU*
School of Chemistry and Chemical Engineering, Nantong University, Nantong, Jiangsu 226007, P. R. China

The effects of temperature and common ions on the interaction between puerarin and bovine serum albumin (BSA) were investigated. The apparent binding constants (K_a) between puerarin and BSA were 1.13 × 10^4 (20 °C), and 1.54 × 10^4 L mol^{-1} (30 °C), and the binding sites values (n) were 0.95 ± 0.02. But the higher temperature (40 °C) decreased the stability of the puerarin-BSA system, which resulted in lower binding constants (1.58 × 10^3 L mol^{-1}) and binding sites value (n=0.73) of the puerarin-BSA system. However, presence of Cu^{2+} and Fe^{3+} ions increased the binding constants and binding sites of puerarin-BSA complex.

Keywords: Bovine Serum Albumin, interaction, puerarin, temperature, ion

The interaction between bio-macromolecules and flavonoids has attracted great interest among researchers since several decades [1-2]. Among bio-macromolecules, serum albumins are the major soluble protein constituents of the circulatory system and have many physiological functions [3-6]. Bovine serum albumin (BSA) has been one of the most extensively studied of proteins particularly due to its structural homology with human serum albumin (HSA) [4]. The interaction between protein and drug molecules results in formation of a stable protein-drug complex, which may be considered as a model for gaining general fundamental insights into drug-protein binding [1-6].

Puerarin (fig. 1) the main isoflavone glycoside found in Chinese herb radix of *Pueraria lobata*, has been used for various medicinal purposes in traditional Chinese medicine for thousands of years [7]. Modern pharmacological research has demonstrated that puerarin exerts a protective effect against myocardial reperfusion injury [8] and ischemic retinopathy [9]. Puerarin possesses antioxidant properties [10] and protects the liver [11].

To gain some insights into the medicinal action of puerarin, the effects of temperature and ions on the interaction of puerarin and bovine serum albumin (BSA) were investigated.

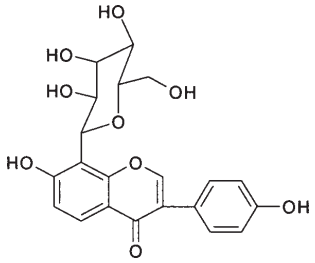

Fig. 1. Structure of puerarin

Experimental part

Apparatus

Fluorescence spectra were recorded on a JASCO FP-6500 spectrofluorometer equipped with a thermostated cell compartment using quartz cuvettes (1.0 cm) (Tokyo, Japan). The *p*H measurements were carried out on a PHS-3C Exact Digital pH meter equipped with Phonix Ag-AgCl reference electrode (Cole-Paemer Instrument Co.), which was calibrated with standard *p*H buffer solutions.

Reagents

Puerarin was obtained commercially from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). A working solution of puerarin (1.0 . 10^{-4} mol L^{-1}) was prepared by dissolving puerarin in methanol-water solution (1:1, v/v). Bovine serum albumin (fraction V) was purchased from Sigma Co. (St. Louis, MO, USA). The working solution of BSA (1.0 × 10^{-5} mol L^{-1}) in the doubly distilled water was prepared and stored in refrigerator prior to use. Tris–HCl buffer (0.20 mol L^{-1}, *p*H 7.4) containing 0.10 mol L^{-1} NaCl was selected to keep the *p*H value and maintain the ionic strength of the solution. All other reagents and solvents were of analytical reagent grade and used without further purification unless otherwise noted. All aqueous solutions were prepared using newly double-distilled water.

Fluorescence spectra

Appropriate quantities of 1.0 × 10^{-4} mol L^{-1} puerarin solution were transferred to a 10 mL flask, and then 0.5 mL of BSA solution was added and diluted to 10 mL with water. The resultant mixture was subsequently ultrasonicated for 5 min and incubated at 20°C, 30°C, and 40°C for 2h. The solution was scanned on the fluorophotometer with the range of 290-550 nm. The fluorescent intensity at 342.8 nm was determined under the excitation at wavelength of 280 nm. The dynamic quenching constant (K_{SV}) and the apparent binding constants (K_a) were attained according to reference [12].

Results and discussions

Characteristics of the fluorescence spectra

The interaction of puerarin with BSA was evaluated by monitoring the intrinsic fluorescence intensity changes of BSA upon addition of puerarin. Fluorescence quenching spectra of BSA at the presence of various concentrations of puerarin are shown in figure 2.

As illustrated in figure 2, the addition of puerarin led to a concentration-dependent quenching of BSA intrinsic fluorescence intensity along with a slightly red shift of maximum emission wavelength, implying that the binding of puerarin to BSA occurs and the microenvironment around chromophore of BSA is changed upon addition of puerarin. With the increasing concentration of puerarin, a new fluorescence peak (λ_{max}=477 nm) was observed. It is the same as the fluorescence emission spectra of puerarin.

* email: quanliu2009@yahoo.com

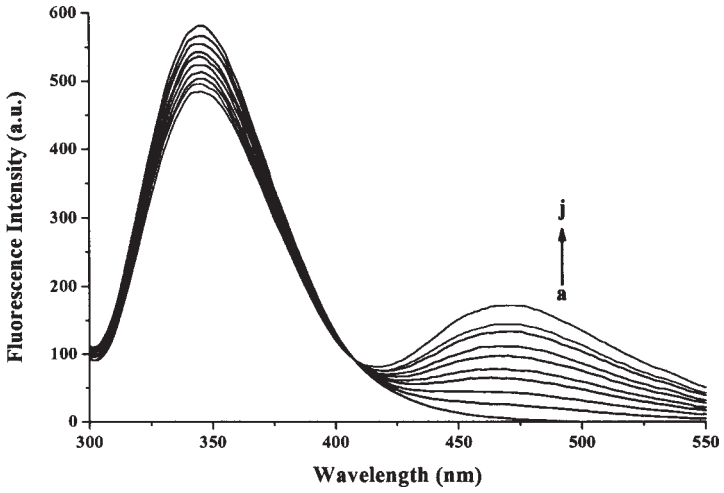

Fig. 2.Quenching effect of puerarin on BSA fluorescence intensity. λ_{ex} =280 nm, (a-j) BSA, 1.00 × 10^{-6} mol L^{-1}: 0.00, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 × 10^{-6} mol L^{-1} of puerarin

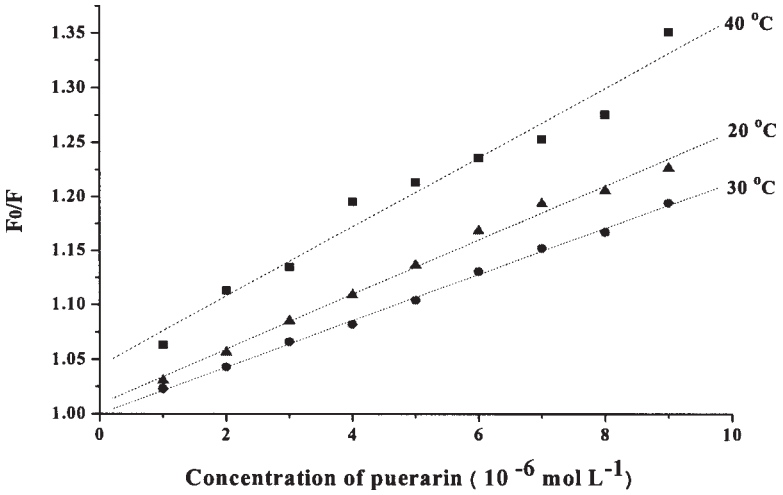

Fig. 3. Stem-Volmer curves of fluorescence quenching of BSA by puerarin at different temperatures

Quenching constants

Fluorescence quenching could proceed via different mechanisms, usually classified as dynamic quenching and static quenching. For the dynamic quenching, the mechanism can be described by the Stern–Volmer equation [12]:

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

where :

F_0 and F represent the fluorescence intensities in the absence and in the presence of quencher,

K_q is the quenching rate constant of the bimolecular,

K_{SV} is the dynamic quenching constant,

τ_0 is the average lifetime of the molecule without quencher and [Q] is the concentration of the quencher.

To clarify the fluorescence quenching mechanism of BSA by puerarin, it was first assumed that the interaction proceeds via a dynamic way. The temperature-dependent fluorescence quenching of BSA by puerarin was then carried out. The Stern-Volmer plots at different temperatures were shown in figure 3. From the experimental data, the corresponding dynamic quenching constants for the interaction between puerarin and BSA were K_{SV} = 2.52 × 10^4 (20 °C, R = 0.9966), K_{SV} = 2.14 × 10^4 (30 °C, R = 0.9989), and K_{SV} = 3.20 × 10^3 L mol^{-1} (40 °C, R = 0.9840), respectively. Because the fluorescence life time of the biopolymer is 10^{-8} s [20-22], the quenching constants K_q at 20°C and 30°C were calculated to be 2.52 × 10^{12}, 2.14 × 10^{12} and 3.20 × 10^{11} L mol^{-1} s^{-1}, respectively.

According to the literatures [1-6], for dynamic quenching, the maximum scatter collision quenching constant of various quenchers with the biopolymer is 2.0 × 10^{10} L mol^{-1} s^{-1}, and the K_{SV} increases with increasing temperature. Considering that in our experiment the rate constant of the BSA quenching procedure initiated by puerarin is much higher than 2.0 × 10^{10} L mol^{-1} s^{-1} and that the K_{SV} decreased with increasing temperature, it can be concluded that the quenching is not initiated by dynamic quenching, but probably by static quenching resulting from the formation of puerarin-BSA complex.

Binding constant and binding sites

For static quenching, the relationship between fluorescence quenching intensity and the concentration of quenchers can be described by the binding constant formula [12]:

$$\lg \frac{F_0 - F}{F} = \lg K_a + n \lg[Q] \quad (2)$$

where K_a is the binding constant, and n is the number of binding sites per BSA. After the fluorescence quenching intensities on BSA at 340 nm were measured, the double-logarithm algorithm was assessed by equation (2).

Figure 4 showed the double-logarithm curve and Table 1 gave the corresponding calculated results. The apparent binding constants (K_A) between puerarin and BSA were 1.13 × 10^4 (20 °C, R=0.9991), and 1.54 × 10^4 (30 °C, R=0.9991), and the binding sites values (n) were 0.95 ± 0.02. The correlation coefficients are larger than 0.9990, indicating that the interaction between puerarin and BSA agrees well with the site binding model underlying the equation (2). The data clearly showed that there was the binding site on BSA for puerarin independent of temperature from 20°C to 30°C. The binding constant increased with the increasing temperature in range of 20-30°C. However, the higher temperature (40°C) obviously decreased binding constant of the puerarin-BSA system (1.58 × 10^3 L mol^{-1},

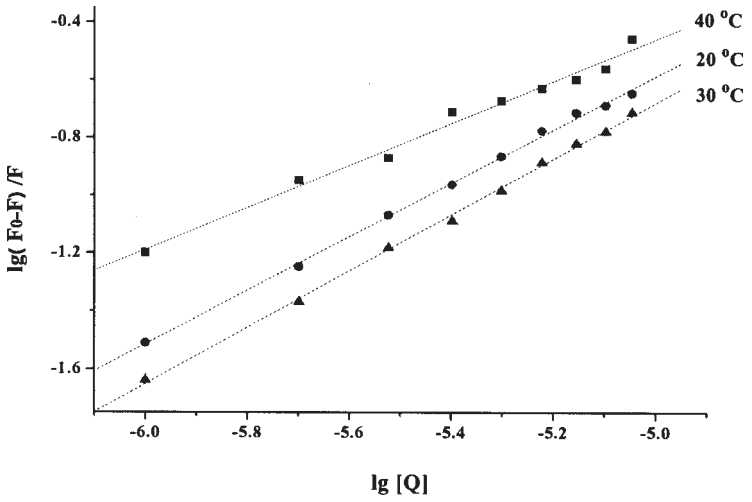

Fig. 4. Double-log plot of puerarin quenching effect on BSA fluorescence at different temperatures (20, 30 and 40°C)

Table 1
BINDING PARAMETERS FOR THE SYSTEM OF PUERARIN-BSA

Temp./°C	Binding constant/L mol^{-1}	Binding site	R
20 °C	1.13 × 10^4	0.93	0.9991
30 °C	1.54× 10^4	0.97	0.9991
40 °C	1.58× 10^3	0.73	0.9929

Table 2
BINDING CONSTANTS *Ka* AND BINDING SITE *n* OF PUERARIN-BSA COMPLEX IN THE PRESENCE OF VARIOUS METAL IONS AT 30 °C

	Ka (M^{-1})	*n*	R^2
Puerarin-BSA	1.54× 10^4	0.97	0.9991
Puerarin-BSA-Cu^{2+}	6.44× 10^5	1.30	1
Puerarin-BSA-Fe^{3+}	2.44× 10^5	1.24	1

$C_{BSA}=C_{Cu^{2+}}=C_{Fe^{3+}}=1.0\times 10^{-6}$ M.

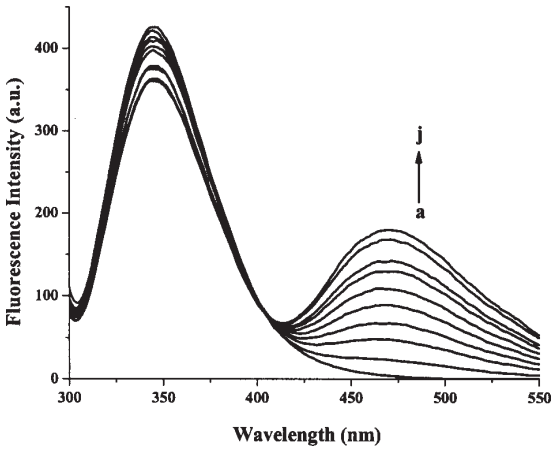

Fig. 5. The fluorescence spectra of puerarin-BSA-Cu^{2+} systems. λ_{ex} =280 nm, (a-j) BSA, 1.00 × 10^{-6} mol L^{-1} and Fe^{3+}, 1.0× 10^{-6} mol L^{-1}: 0.00, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 × 10^{-6} mol L^{-1} of puerarin (30 °C)

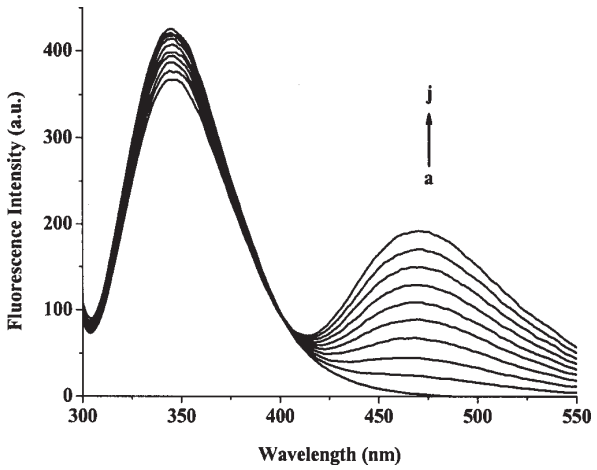

Fig. 6. The fluorescence spectra of puerarin-BSA-Fe^{3+} systems. λ_{ex} =280 nm, (a-j) BSA, 1.00 × 10^{-6} mol L^{-1} and Fe^{3+}, 1.0× 10^{-6} mol L^{-1}: 0.00, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 × 10^{-6} mol L^{-1} of puerarin (30 °C)

R=0.9929). The binding sites value of the puerarin-BSA system also decreased in the higher temperature (*n*=0.73). The temperature may affect the diffusion coefficient and stability of the puerarin-BSA system. The increasing temperature may result in the increasing diffusion coefficient, and also it leads to the lower stability of the puerarin-BSA system. The competition of the diffusion coefficient and stability of the puerarin-BSA system with increased temperature may induce the above results. The temperature also affects the stability of puerarin, which was easily oxidized and decomposed under high temperature. The by-products may have lower binding forces with the BSA.

Influences of common ions on binding constant

Metal ions, especially those of bivalent type, are vital to human body playing an essentially structural role in many proteins based on coordinate bonds. The presence of metal

ions in plasma may affect interaction of drugs with BSA. Effects of common bivalent metal ions (e.g. Cu^{2+} and Fe^{3+}) on binding constants of puerarin-BSA complex were investigated at 30°C. The Cu^{2+} and Fe^{3+} (1.0×10^{-6} M) could decrease the fluorescence intensity of puerarin-BSA systems, but these two metal ions do not affect the fluorescence intensity of puerarin (fig. 5 and 6). It possibly resulted from the formation of metal ion-BSA complexes, which also quench BSA fluorescence. Figure 7 showed the double-logarithm curve in presence of Cu^{2+} and Fe^{3+} ions. The values of binding constant *Ka* and binding site *n* acquired in the present of metal ions are listed in table 2.

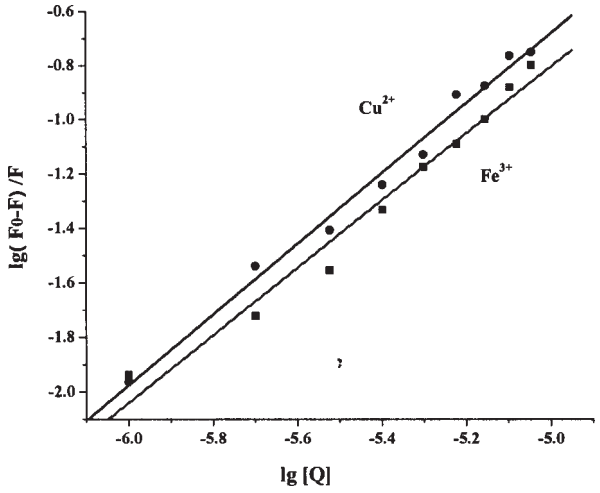

Fig. 7 Double-log plot of puerarin quenching effect on BSA in presence of Cu^{2+} and Fe^{3+}, respectively (30 °C).

It can be seen from table 2 that the presence of Cu^{2+} and Fe^{3+} ions increased the binding constants and binding sites of puerarin-BSA complex. The higher binding constant possibly results from the formation of metal ion-puerarin complexes via metal ion bridge. This may prolong storage period of puerarin in blood plasma and enhance its maximum effects.

References

1. XIAO, J., CHEN, X., ZHANG, L., TALBOT, S.G., LI, G.C., XU, M. Investigation the mechanism of enhanced effect of EGCG on huperzine A inhibiting acetylcholinesterase activity in rats by multi-spectroscopic method. J. Agr. Food Chem. 2008, 56, p. 910
2. SIRK, T.W.; BROWN, E.F.; SUM, A.K., Friedman, M. Molecular dynamics study on the biophysical interactions of seven green tea catechins with lipid bilayers of cell membranes. J. Agric. Food Chem. 2008, 56, p. 7750
3. XIAO, J. B.; CHEN, J.W.; CAO, H.; REN, F.L.; YANG, C.S.; CHEN, Y.; XU. M. Study of the Interaction between Baicalin and Bovine Serum Albumin by Multi-spectroscopic Method. J. Photochem. Photobiol. A, 2007, 191, p. 222
4. WANG, Y.Q.; ZHANG, H.M., ZHANG, G.C.; TAO, W.H.; FEI, Z.H.; LIU, Z.T. Interaction of the flavonoid hesperidin with bovine serum albumin: A fluorescence quenching study. J. Pharmaceut. Biomed. 2007, 43, p. 1869
5. XIAO, J.B.; SHI, J.; CAO, H.; WU, S.D.; REN, F.L.; XU, M. Analysis of binding interaction between puerarin and bovine serum albumin by multi-spectroscopic method. J. Pharmaceut. Biomed. 2007, 45, p. 609
6. XIAO, J.B., SUZUKI, M., JIANG, X.Y., CHEN, X.Q., YAMAMOTO, K., XU, M. Influence of B-ring hydroxylation on interactions of flavonols with bovine serum albumin. J. Agr. Food Chem. 2008, 56, 2350-2356.
7. FANG, Q., Some current study and research approaches relating to the use of plants in the traditional Chinese medicine. J. Ethnopharmacol., 2, 57-63 (1980)
8. FAN, L. L., SUN, L. H., LI, J., The protective effect of puerarin against myocardial reperfusion injury: study on cardiac function. Chin. Med. J., 105, 11-17 (1992)
9. XUAN, B., ZHOU, Y. H., YANG, R. L., Improvement of ocular blood flow and retinal functions with puerarin analogs. J. Ocular Pharmacol. Ther., 15, 207-216 (1999)
10. CERVELLATI, R., RENZULLI, C., GUERRA, M. C., SPERONI, E., Evaluation of antioxidant activity of some natural polyphenolic compounds using the Briggs-Rauscher reaction method. J. Agr. Food Chem., 50, 7504-7509 (2002)
11. HWANG, Y.P., CHOI1, C.Y., CHUNG, Y.C., JEON, S.S., JEONG, H.G., Protective Effects of Puerarin on Carbon Tetrachloride-Induced Hepatotoxicity. Arch. Pharm. Res. 2007, 30, p. 1309
12. LAKOWICZ, J.R., Principles of Fluorescence Spectroscopy, 2nd ed.; Kluwer Academic/Plenum Publishers: New York, 1999

Manuscript received: 20.01.2009