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Research Article

Binding Interaction Study of β-sitosterol and Luteolin-7-glucoside with Bovine Serum Albumin by Fluorescence Quenching

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ABSTRACT

The study on drug-protein interactions is an important field of interest because of the perspective of unraveling of drug action mechanisms and the possibility of designing novel medicines. Bovine serum albumin (BSA) has been studied extensively, because of its structural homology with human serum albumin (HSA). β-Sitosterol is a phytosterol compound that has a variety of pharmacological properties Luteolin-7- glucoside is a glycosyloxy flavones, which has a role as an antioxidant. By fluorescence spectroscopy, this study investigated the interaction between β-sitosterol and Luteolin-7-glucoside with bovine serum albumin (BSA) at physiological pH 7.4. The study revealed that the fluorescence quenching of BSA by molecules resulted from forming a molecule-BSA complex. Fluorescence quenching constants were determined using the Stern-volmer to measure the binding affinity between the molecules and BSA. Thermodynamic parameters ΔG, ΔH, and ΔS at different temperatures indicated that action was an endothermic and spontaneous process, and hydrophobic interaction played a major role in molecule-BSA association.

Introduction

Protein, one of the most important bioactive molecules, is related to alimentation, immunity and metabolism. Thus, interaction between bio-macromolecules and drugs has attracted great interest for several decades.[1-3] Most research has focused on two main questions about proteins: the critical factors that influence the protein structures and functions, and how a factor affects their biological activity.[4,5] In this regard, bovine serum albumin (BSA) has been studied extensively, because of its structural homology with human serum albumin (HSA).[6,7]

β-Sitosterol is a phytosterol or plant sterol compound, found in various fruits, vegetables, and seeds, with various pharmacological properties.[8] Luteolin-7-glucoside is a glycosyloxy flavone that is luteolin substituted by a beta-D-glucopyranosyl moiety at position 7 *via* a glycosidic linkage. It is an antioxidant and a plant metabolite.[9]

BSA is composed of three linearly arranged and structurally homologous sub-domains. The binding sites of BSA for endogenous and exogenous ligands may be in these domains and the principal regions of drugs binding sites of albumin are often located in hydrophobic cavities in sub-domains IIA and IIIA.[10,11]

Protein–drug interaction plays an important role in pharmacokinetics and pharmacodynamics. In a series of methods concerning the interaction of drugs and protein, fluorescence techniques are great aids in studying interactions between drugs and serum albumin because of their high sensitivity, rapidity, and ease of implementation.[12]

The present investigation aimed to study the affinity of β-sitosterol and Luteolin-7-glucoside for BSA using fluorescence spectroscopy to understand the carrier role of serum albumin for such compound in the blood under physiological conditions.

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Materials and Methods

Materials

Fatty acid free BSA was obtained from Sigma Chemical Company (USA). All chemicals used were of A. R. grade and milli-Q water was used throughout the experiments. β-sitosterol isolated from *Feronia limonia* and Luteolin-7-glucoside isolated from *Bougainvillea glabra* was used for fluorescence quenching studies.

Instrument

Steady-state fluorescence measurements were carried out using a Photo-Fluorimeter (Make-Systronics, Model-151). Balance AY-120 was used for weighing. Digital pH Meter (Make: Delux Sr. No: 0801010)

Methodology

Solution Preparation and Spectroscopic Measurement Conditions

β-sitosterol (βS) and Luteolin-7-glucoside (LUT) was dissolved in ethanol to obtain 1 × 10^{-3} M stock solution. A tris–HCl buffer (0.10 M, pH = 7.4) containing 0.10 M NaCl was selected to keep the pH value constant and maintain the solution's ionic strength. Fluorescence measurements were carried out, keeping the concentration of BSA fixed at 4.0 × 10^{-7} M for all experiments. Analyte concentration varied from 0 M to 45 ×10^{-6} M for β-sitosterol and 0 to 18 × 10^{-7} M for Luteolin-7-glucoside. The excitation wavelength was 280 nm and the intrinsic fluorescence emission spectra of BSA were recorded at two different temperatures (25°C, 30°C) (298, 303 K) at an emission wavelength of 340 nm

Results and Discussion

Fluorescence Quenching of BSA by the β-sitosterol[13-15]

Upon addition of the β-sitosterol and Luteolin-7-glucoside into BSA solution, the fluorescence intensity of BSA at around 340 nm regularly decreased in each titration curve, indicating that the β-sitosterol as well as Luteolin-7-glucoside interacts with BSA and that BSA binding site is getting gradually saturated with increase in the concentration of β-sitosterol (Table 1, Fig. 1).

To further confirm the possible quenching mechanism of the β-sitosterol and Luteolin-7-glucoside binding to BSA, the fluorescence quenching constants were usually analyzed by the stern–volmer equation [Eqn 1]. The data is presented in Table 2 and the results are listed in Table 3.

$$F_0/F = 1 + kq\tau_0 [Q] = 1 + KSV [Q] \quad \text{(Eqn. 1)}$$

Where, F and F_0 are the fluorescence intensities of BSA with and without molecule. kq is the quenching rate constant and τ_0 is the average lifetime of the molecule without the quencher (~10^{-8} s). Ksv represents stern-volmer quenching constant and Q is the concentration of the quencher. Slope obtained from the linear regression of the plot of F_0/F against [Q] gives the value of the quenching constant (Ksv) (Fig. 2). Calculated quenching parameters are mentioned in Table 3. The plot exhibited good linear relationship and the value of Ksv decreased with increasing temperature. As the temperature was increased, the stability constant of the complex decreased explaining static quenching mechanism. In Table 3, kq values obtained at two different temperatures are greater than 2×10^{10} L mol^{-1} s^{-1} which is

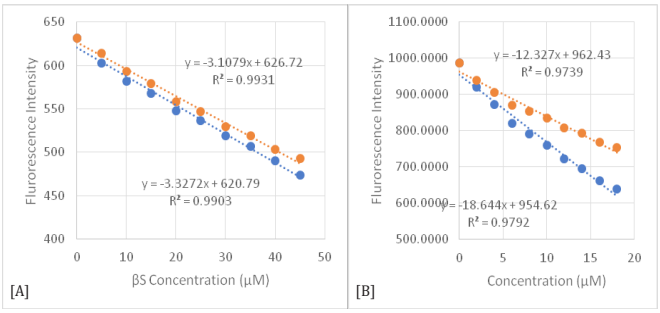

Fig. 1: Fluorescence intensity values of BSA (4×10-7 M) decreasing with [A] βS addition (0 M to 45 ×10^{-6} M) and [B] LUT addition (0 M to 18 ×10^{-7} M) at 298 K [Blue] and 303 K [Brown]

Table 1: Fluorescence intensity values of BSA (4×10^{-7} M) interacting with βS addition (0 M to 45 ×10^{-6} M) and LUT addition (0 M to 18 ×10-7 M) at 298 K and 303 K

	β-sitosterol (βS)			*Luteolin – 7- glucoside (LUT)*		
S no.	***Conc (μM)***	***298 K***	***303 K***	***Conc (μM)***	***298 K***	***303 K***
F0	0	631.796	631.796	0	987.1813	987.1813
F1	5	602.7433	614.4172	2	920.4964	938.9063
F2	10	582.0841	593.3579	4	871.851	906.725
F3	15	568.2191	579.0307	6	820.3302	869.55
F4	20	548.0319	558.6462	8	790.4456	853.6813
F5	25	536.3442	546.7321	10	759.5227	835.475
F6	30	519.1075	529.1616	12	721.9866	808.625
F7	35	506.9324	518.7894	14	695.4167	792.775
F8	40	490.5413	503.1002	16	662.7586	768.8
F9	45	473.4946	492.8589	18	638.2627	753.15

Table 2: Data for Stern-Volmer plot for βS interaction with BSA at different temperature

	βS conc 10^{-6} M	*F0/F 25°C*	*F0/F 30°C*	*βS conc 10^{-6} M*	*F0/F 25°C*	*F0/F 30°C*
F_0	0	1	1	0	1	1
F_0/F1	5	1.0482	1.0283	2	1.0724	1.0514
F_0/F2	10	1.0854	1.0648	4	1.1323	1.0887
F_0/F3	15	1.1119	1.0911	6	1.2034	1.1353
F_0/F4	20	1.1528	1.1309	8	1.2489	1.1564
F_0/F5	25	1.178	1.1556	10	1.2997	1.1816
F_0/F6	30	1.2171	1.194	12	1.3673	1.2208
F_0/F7	35	1.2463	1.2178	14	1.4196	1.2452
F_0/F8	40	1.288	1.2558	16	1.4895	1.2841
F_0/F9	45	1.3343	1.2819	18	1.5467	1.3107
Slope		0.0071	0.0063		0.0298	0.0166
Intercept		1.0071	0.9994		1.0102	1.0177
r		0.9983	0.9994		0.9992	0.9958

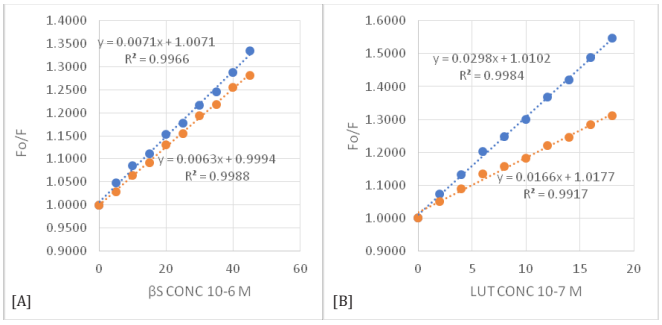

Fig. 2: Stern-Volmer plot for[A] βS and [B] LUT interaction with BSA at 298 K [Blue] and 303 K [Brown]

the limiting diffusion constant. This suggests that static quenching is involved in quenching of BSA by β-sitosterol molecule.

Binding Constant and Number of Binding Sites[16,17]

For static quenching, double logarithmic regression curve of $\log(F_0\text{-}F)/F$ vs log[Q] [BS] as well as $\log(F_0\text{-}F)/F$ vs log[Q] [LUT] plotted using fluorescence intensity data can provide the information related to binding constant and number of binding sites. Table 4 and 5 gives data for a modified Stern-Volmer plot at different temperature for BSA interaction with β-sitosterol and Luteolin-7-glucoside, respectively and Fig. 3 and 4 represents the modified Stern-Volmer plot (based on Eqn. 2)

$$\log (F_0\text{-}F)/F = \log Kb + n \log [Q] \qquad \text{(Eqn. 2)}$$

From the slope and intercept of the plot, n and Kb can be calculated. [Q] is the quencher concentration with constant BSA concentration. Kb and n values are presented in Table 4 and 5. The numbers of binding sites (n) are approximately equal to one at all temperatures, indicating that only a single binding site is possible for β-sitosterol and Luteolin-7-glucoside on BSA. β-sitosterol and Luteolin-7-glucoside could be stored and carried by BSA under physiological

Table 3: Stern-Volmer quenching constant and dynamic quenching constant of BSA- βS and BSA-LUT system at different temperatures

S no.	*Temp (K)*	*Ksv (L mol $^{-1}$)*	*Kq (L mol $^{-1}S^1$)*	*r^2*
	BSA- βS			
1	298	0.707×10^4	0.707×10^{12}	0.9966
2	303	0.634×10^4	0.634×10^{12}	0.9988
	BSA-LUT			
1	298	2.975×10^5	2.975×10^{13}	0.9984
2	303	1.663×10^5	1.663×10^{13}	0.9917

Table 4: Data for modified Stern-Volmer plot for βS interaction with BSA at different temperature

βS Conc (10^{-6} M)	*Log (Conc)*	*Log [F0-F (25)/F]*	*Log [F0-F (30)/F]*
0	0	0	0
5	-5.301	-1.3169	-1.5484
10	-5	-1.0685	-1.1886
15	-4.8239	-0.9512	-1.0404
20	-4.699	-0.8157	-0.8829
25	-4.6021	-0.7497	-0.808
30	-4.5229	-0.6634	-0.7123
35	-4.4559	-0.6085	-0.6619
40	-4.3979	-0.5407	-0.5921
45	-4.3468	-0.4758	-0.5499
Slope (n)		0.8692	1.0361
Intercept		3.2718	3.9653
r		0.9976	0.9988
Antilog of Intercept (Kb)		1869.821	9232.09
		(1.8698×10^4)	(9.2321×10^4)
InKb		7.5336	9.1304
1/T (K-1)		0.003355705	0.00330033

conditions via forming the mole ratio 1:1 complex. As per the binding constant (Kb) values, the moderate binding affinity was shown by molecules with BSA (order of Kb values in the range of 10^4 M^{-1}). As the binding constant depends on temperature, the thermodynamic processes are considered to be involved in the complex formation of biomacromolecule with a small molecule. Therefore, thermodynamic parameters such as enthalpy change (ΔH), entropy change (ΔS) and Gibbs free energy (ΔG) were calculated to exemplify the reaction between molecules and BSA using van't Hoff equation (Eqn. 3).

$$\ln Kb = (-\Delta H / RT) + (\Delta S / R) \quad \text{(Eqn. 3)}$$

Where Kb is the binding constant at the corresponding temperature (T) and R is the universal gas constant. The plot of ln Kb vs 1/T for the interaction of molecules with BSA is shown in Fig. 5. Slope and intercept obtained from the linear plot provided –ΔH/R and ΔS/R. Gibbs free energy can be calculated using the following Equation

$$\Delta G = \Delta H - T\Delta S \quad \text{(Eqn. 4)}$$

The results are presented in Table 6. The negative value of

Table 5: Data for modified Stern-Volmer plot for LUT interaction with BSA at different temperature

LUT Conc (10-7 M)	*Log (Conc)*	*Log [F0-F (25)/F]*	*Log [F0F(30)/F]*
0	0	0	0
2	-6.699	-1.14	-1.2889
4	-6.3979	-0.8785	-1.0519
6	-6.2218	-0.6917	-0.8688
8	-6.0969	-0.604	-0.8058
10	-6	-0.5233	-0.7409
12	-5.9208	-0.435	-0.656
14	-5.8539	-0.3772	-0.6104
16	-5.7959	-0.3102	-0.5466
18	-5.7447	-0.2623	-0.5076
Slope (n)		0.9152	0.8112
Intercept		4.9853	4.147
r		0.9992	0.9983
Antilog of Intercept (Kb)		96671.84 (9.6672×10^4)	14028.137 (1.4028×10^4)
InKb		11.4791	9.5488
1/T (K-1)		0.003355705	0.00330033

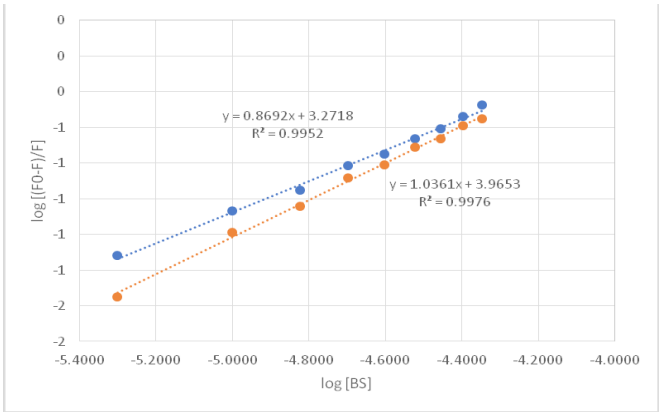

Fig 3: Modified Stern-Volmer plot for the BSA- βS system at 298 K [Blue] and 303 K [Brown]

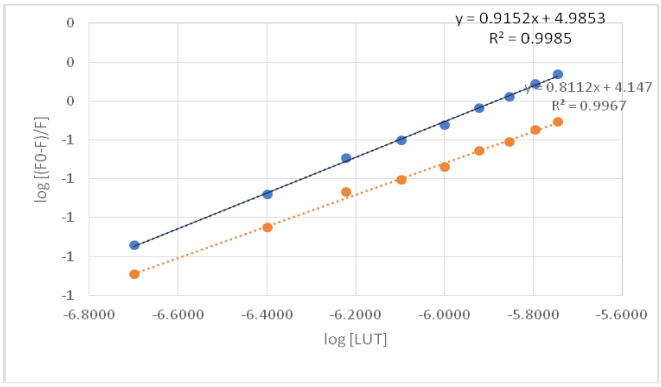

Fig. 4: Modified Stern-Volmer plot for the BSA-LUT system at 298 K [Blue] and 303 K [Brown]

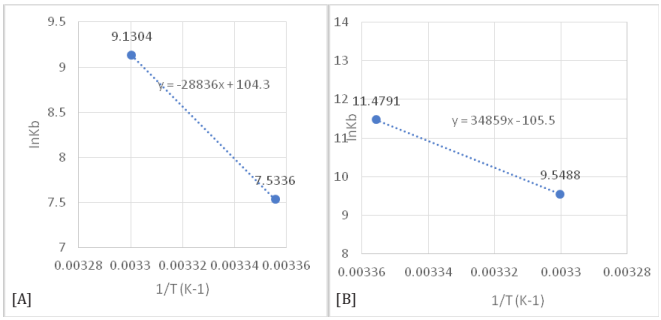

Fig. 5: Van't Hoff plot of the [A] BSA- βS and [B] BSA-LUT system

Table 6: Binding constant and thermodynamic parameters for βS -BSA and LUT-BSA system at different temperatures

Temp. (K)	*Kb (L mol^{-1})*	*n*	*ΔH (KJ/mol)*	*ΔS J/(mol K)*	*ΔG KJ/mol*
	βS -BSA				
298	1.8698×10^4	0.8692			-18.6683
303	9.2321×10^4	1.0361	239.743	867.15	-23.004
	LUT-BSA				
298	9.6672×10^4	0.9152			-28.4339
303	1.4028×10^4	0.8112	-289.818	-877.127	-24.0482

ΔG indicates that the interaction between β-sitosterol and Luteolin-7-glucoside and BSA is spontaneous. ΔH, ΔS values were found to be positive. The positive ΔH and ΔS values were frequently taken as the reaction is entropy driven and the binding process belonged to the endothermic process.

Conclusion

In this work, the interaction of β-sitosterol and Luteolin-7-glucoside with BSA was studied by fluorescence spectroscopy. We observed that these molecules most likely involve static quenching in the quenching mechanism of fluorescence of BSA by these molecules. The binding reaction was spontaneous, and hydrophobic interaction played a major role. The determination and understanding of drug interacting with serum albumin are important for the therapy and design of drug. Knowledge of the interaction and binding of BSA may open new avenues for the design of the most suitable pyrimidine derivatives.

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