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Study on the binding of luteolin to bovine serum albumin

Yumin Yang [a], Qiuluan Hu [a,*], Yanli Fan [a], Hongshuai Shen [b]

[a] *Life and Science Department of Luoyang Teachers' College, Luoyang 471022, PR China*
[b] *College of Earth and Environmental Sciences, Lanzhou University, China*

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Abstract

Binding of luteolin (LU) to bovine serum albumin (BSA) was investigated at 298, 308 and 318 K at pH 7.4 using spectrophotometric techniques such as fluorescence emission, circular dichroism (CD). The data obtained from fluorescence quenching experiments showed that LU was bound to BSA and binding constants and the number of binding sites ($n \approx 1$) were obtained. The thermodynamic parameters ΔH^0, ΔS^0, ΔG^0 at different temperatures were calculated. They indicated that both hydrophobic forces and hydrogen bonds are the major interactions between LU and BSA. A value of 3.12 nm for the average distance r between LU (acceptor) and tryptophan residue (Trp) of BSA (donor) was derived from the fluorescence resonance energy transfer. The effects of some common metal ions on the binding are also considered. Besides, the interaction of BSA with LU led to a change in the conformation of BSA.

Keywords: Luteolin; Bovine serum albumin; Fluorescence quenching; Binding parameters

1. Introduction

Serum albumin is the most abundant protein in animal's including human circulatory system. It is in charge of the transport of a variety of endogenous and exogenous substances in body and plays an important role in the distribution and deposition of these substances [1]. When drugs are absorbed, they enter into the circulatory system and extensively and reversibly bind to serum albumin [2]. Drug–protein interaction has significance in pharmacology. It can affect the biological activity [3,4] and toxicity [5–7] of drug. The binding parameters are helpful in the study of pharmacokinetics and the design of dosage forms [8,9].

Luteolin (3′,4′,5,7-tetrahydroxyflavone or LU) is a hydroxylated flavone derivative (Scheme 1) possessing a strong anti-oxidant and radical scavenge properties, which reportedly inhibits the development of a series of solid tumor, ascites, and leukemic cell lines [10]. Flavonoids possess pro-oxidant and anti-oxidant action. Anti-oxidant effects of flavonoid [11] and LU were reported by several authors [12]. LU has been shown to have anti-oxidant [13], anti-tumorigenic [14], anti-inflammatory/anti-allergic [15] activities, as well as inhibits protein kinase C [16] and lipoxygenase [15]. Thus, the nature and magnitude of the interactions between drug and BSA have important pharmacokinetic and pharmacodynamic implications. Yet, few works have been published for the mechanism of the interactions and detailed physicochemical characterizations of LU binding to BSA.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA, fatty acid free <0.05%), purchased from Sino-American Biotechnology Company, was used without further purification. Luteolin (LU) was of analytical grade and purchased from the National Institute for Control of Pharmaceutical and Bioproducts, China. NaCl (0.5 mol L^{-1}) solution was used to maintain the ionic strength. Buffer (pH 7.40) consists of Tris (0.1 mol L^{-1}) and the pH was adjusted to 7.40 by adding HCl (0.1 mol L^{-1}) monitored by pH-meter. Solutions of common metal ions (5×10^{-3} mol L^{-1}) were prepared by $MgCl_2$, $AlCl_3$, $ZnSO_4$ and $CuSO_4$, respectively. The final of concentration common metal ions fluorescence titration were 1×10^{-5} mol L^{-1}. All reagents are of analytical grade. Water is doubly distilled water.

* Corresponding author. Tel.: +86 931 8912890.

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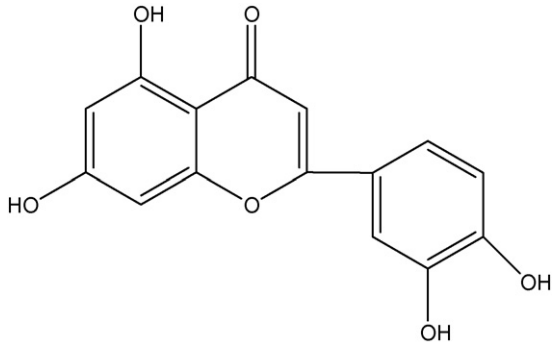

Scheme 1. The chemical structure of luteolin.

2.2. Apparatus and methods

All fluorescent measurements were carried out on an F-4500 fluorescence spectrophotometer (Hitachi, Kyoto, Japan) equipped with a xenon lamp source and 1.0 cm cell. The fluorescence emission spectra were recorded in the wavelength of 290–425 nm upon excitation wavelength at 295 nm and both excitation and emission bandwidths were 10 nm. The fluorescence quenching experiments were performed by keeping the BSA concentration constant while varying the concentration of LU. The synchronous fluorescence spectra were obtained by scanning simultaneously the excitation and emission monochromator. The synchronous fluorescence spectra were recorded in the wavelength of 250–320 nm. The quantitative analysis of the interaction between BSA and LU was performed by a fluorometric titration as follows: 3 mL of a 3×10^{-6} mol L^{-1} solution of BSA was titrated by successive addition of LU solution to reach a final concentration of 7.5×10^{-6} mol L^{-1} LU.

Circular dichroism measurements were run on a JASCO-J-715 automatic recording photospectrometer using a cell with 1 mm optical length at 298 K, with three scans averaged for each CD spectra at room temperature (298 K). The spectrophotometric results were expressed as $[\theta]_{MRW}$ obtained.

3. Results and discussion

3.1. Studies on fluorescence quenching of BSA

Fig. 1 showed the fluorescence emission spectra of BSA in the absence and presence of LU. The emission of BSA is characterized by a broad emission band at 350 nm. It could be seen that the fluorescence intensity of BSA dropped regularly with the increase in LU concentration. Otherwise, a slight blue shift occurred. This observation implies that the fluorescence quenching process may be mainly controlled by a static quenching mechanism rather than a dynamic quenching mechanism.

Assumed the procedure to be dynamic quenching. By Stem–Volmer [17] quenching equation:

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

where F_0 and F are the fluorescence intensity in the absence and presence of the quencher, respectively, Q the quencher concentration, and k_q the quenching rate constant for a biomolecular reaction, K_{sv} the Stern–Volmer quenching constant and τ_0 the average lifetime for fluorophore in the absence of quencher evaluated at 5 ns [18]. Fig. 2 shows the plots of F_0/F for BSA versus $[Q]$ of LU ranging from 0.75 to 7.5 μM of LU, respectively, at 298, 308, and 318 K. Plots of F_0/F versus $[Q]$ in Fig. 2 show positive deviation, being concave towards the y-axis at higher $[Q]$. This observation may suggest that a combined quenching (static and dynamic) process occurs at higher concentrations of LU [19,20].

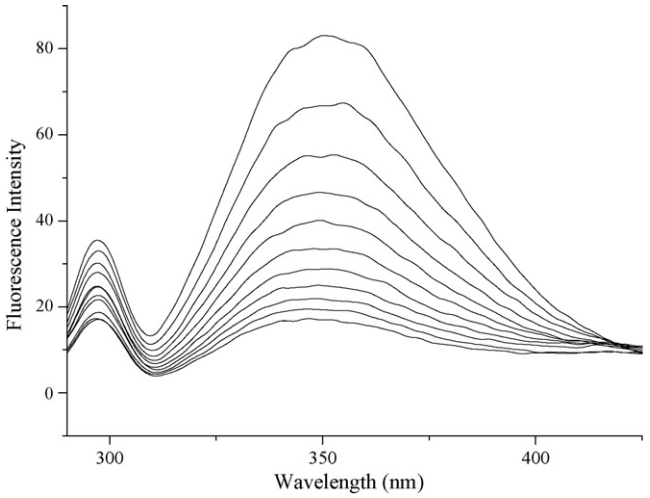

Fig. 1. The fluorescence spectra of LU-BSA system (A). [BSA] = 3 μM; [LU] = 0 (1), 0.75 μM (2), 1.5 μM (3), 2.25 μM (4), 3 μM (5), 3.75 μM (6), 4.5 μM (7), 5.25 μM (8), 6.0 μM (9), 6.75 μM (10) and 7.5 μM (11), respectively. λ_{ex} = 295 nm, T = 298 K, pH 7.40.

As is known, a linear Stern–Volmer plot represents a single quenching mechanism, either static or dynamic [19]. Thereinto, in a static quenching process, generally, a linear Stern–Volmer plot indicates either only one drug binding site in the proximity of fluorophore exists, or more than one binding site all equally

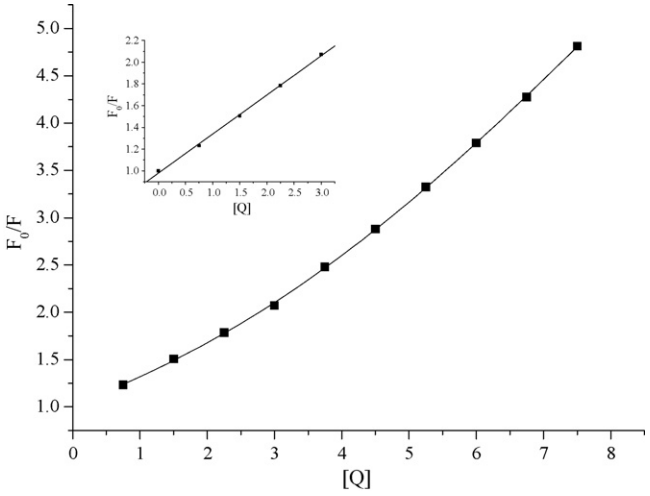

Fig. 2. Plots of F_0/F for BSA against $[Q]$ of LU ranging from 0.75 to 7.5 μM at: (■) 298 K. [BSA] = 3 μM, λ_{ex} = 295 nm, λ_{em} = 350 nm, and pH 7.40. Straight lines in the inset are plots of F_0/F for BSA against $[Q]$ of AB ranging from 1 to 3 μM.