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Investigation on the interactions of silymarin to bovine serum albumin and lysozyme by fluorescence and absorbance

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ABSTRACT

The interactions of silymarin with bovine serum albumin (BSA) and lysozyme (LYS) were investigated in physiological buffer (pH = 7.4) by fluorescence spectroscopy and UV–vis absorption spectroscopy. The mechanism study indicated that silymarin could strongly quench the intrinsic fluorescence of BSA and LYS through static quenching procedures. At 291 K, the values of the binding constant K_A were 4.20×10^4 and 4.71×10^4 L mol^{-1} for silymarin–BSA and silymarin–LYS, respectively. Using thermodynamic equations, the conclusion that hydrophobic and electrostatic forces played an important role in stabilizing complex of silymarin–BSA or silymarin–LYS was obtained. The effects of Cu^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, and Fe^{3+} on the binding were also studied at 291 K. According to Förster's nonradiative energy transfer theory, the distances r_0 between donor and acceptor were calculated to be 3.36 and 2.71 nm for silymarin–BSA and silymarin–LYS, respectively. Synchronous fluorescence spectra showed that the conformation of BSA and LYS were changed by silymarin.

1. Introduction

Bovine serum albumin (BSA) is a plasma protein and has many physiological functions. From biopharmaceutical point of view, one of the most important functions of BSA is as a carrier in many drugs [1]. BSA has intrinsic fluorescence arose from two tryptophanyl residues, Trp−134 and Trp−212 [2]. Enzymes play an important role in many biologically relevant processes and are some attractive targets in the therapy and pharmaceutical research. Lysozyme (LYS) is a small monomeric globular protein consisting of 129 amino acids. Its composition is characterized by high contents of Trp residues, which contains 6 Trp residues at positions 28, 62, 63, 108, 111, and 123 [3,4]. Trp−62 and Trp−108 are the most dominant fluorophores of LYS, both being located at the substrate binding sites [5]. Therefore, the two proteins are well suited to these initial studies by molecular fluorescence technique. A lot of reports were published on the interactions of small molecules with BSA or LYS, such as cephalosporin analogs [6], farrerol [7], surfactant [8,9], dyes [10,11], and flavonoids [12,13], but the study of silymarin binding BSA or LYS has not been revealed.

Silymarin is a flavonoid extracted from the fruit and seeds of milk thistle (Silybum marianum L. Gaertn), consists of a mixture of 7 flavonolignans and polyphenols [14,15]. Its structure is shown in Fig. 1. It is well known that silymarin has hepatoprotective effect, and it has various pharmacological properties, such as being an antioxidant, antiinflammatory [16], antifibrotic [17], and inhibiting the generation of oxidized lowdensity lipoproteins [18]. Though we have reported many flavonoids including quercetin, rutin, hyperin, baicalin, naringenin, hesperetin, and apigenin binding to serum albumin [19,20], up to now little is known about the interactions of silymarin with BSA and LYS to the best of our knowledge. Especially LYS as the other protein in this work will give new insights on the understanding how this protein is involved in the interaction with silymarin. As we know, two forms for the drug exist in blood plasma: unbound drug and bound drug. The amount of drug binds to protein affect efficiency of drug. Unbound drug is free to traverse cell membranes or diffuse, reaching action site while the bound drug is kept in the blood stream and loses the pharmacological activity. But the binding of the drug with protein is often reversible. This means that the bound drug can be effective to control the release of the drug to the receptor. Therefore, studying the interaction between proteins and silymarin can give us a better understanding of its biological action in vivo.

2. Experimental details

2.1. Reagents

BSA and LYS were purchased from Changchun Dingguo Biotechnology Company, Sigma packaging. Silymarin was purchased from Chinese Drug Biological Products Qualifying Institute. Tris–HCl buffer (0.05 mol L^{-1}, pH 7.40) containing 0.1 mol L^{-1}

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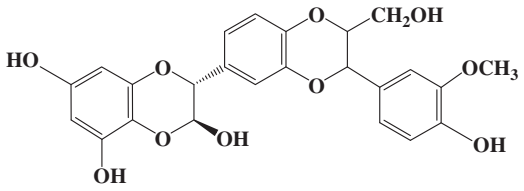

Fig. 1. Structure of silymarin.

NaCl was employed. $FeCl_3$, $FeCl_2$, $CuCl_2$, $MgCl_2$, and $CaCl_2$ were used to study the effects of ions on the interactions. All other chemicals used were of analytical reagent grade and double distilled water was used throughout.

2.2. Apparatus

All fluorescence spectra were recorded on a RF-5301PC spectrofluorometer (Shi madzu, Japan) equipped with a xenon lamp source, a $1.0 \times 1.0 \times 4.0$ cm quartz cell and a thermostatic controller. The UV–vis spectra were recorded on TU-1901 UV-Spectrometer (Beijing Purkinje General Instrument Co., Ltd.).

2.3. Procedures

A fixed volume of BSA or LYS with various concentrations of silymarin was added to each mark tube, respectively. The total volume was fixed to 5 mL with Tris–HCl buffer (0.05 mol L^{-1}, pH 7.4). All solutions were mixed thoroughly. The fluorescence spectra were recorded in the range of 290–500 nm by keeping excitation wavelength at 280 nm at different temperatures. Synchronous fluorescence spectra of the two proteins in the absence and presence of silymarin were recorded with $\Delta\lambda = 15$ and 60 nm.

The measurements of UV spectra of the silymarin were performed under the conditions: the scan rate is middle (8 nm/s), scan range is 290–500 nm.

3. Results and discussion

3.1. Binding characteristics

BSA (5×10^{-6} mol L^{-1}) and LYS (1×10^{-6} mol L^{-1}) solution were exited at 280 nm, respectively, the maximum fluorescence peak at 343 and 340 nm occurred. When various concentrations (from low to high) of silymarin were added to BSA or LYS solution successively, the emission intensity dropped regularly with the increasing the concentration of silymarin and the peak position was shifted to varying degrees. For BSA, the peak shifted from 343 to 348 nm; for LYS, the peak shifted from 340 to 348 nm. The results are shown in Fig. 2. Most proteins have the three fluorophores—Trp, tyrosine (Tyr), and phenylalanine (Phe). Among them, Trp and Tyr are the dominant intrinsic fluorophores. Generally, when proteins are excited at 280 nm, the fluorescence at about 340 nm mainly arises from Trp. In BSA, the fluorescence at 340 nm originated from Trp – 134 and Trp – 212 [2]. While in LYS, Trp – 62 and Trp – 108 play important roles [5]. The reduction of fluorescence intensity indicated that the silymarin as a quencher quenched the fluorescence of BSA or LYS, revealing that the interaction between the silymarin and protein occurred. This interaction induced the nonfluorescent complex to form, which is silymarin-BSA or silymarin-LYS. Moreover, the red shifts suggested that environment around Trps changed and was brought to a more hydrophilic [21] because of the interaction of silymarin with BSA or LYS. This viewpoint was further confirmed by synchronous fluorescence spectra described below.

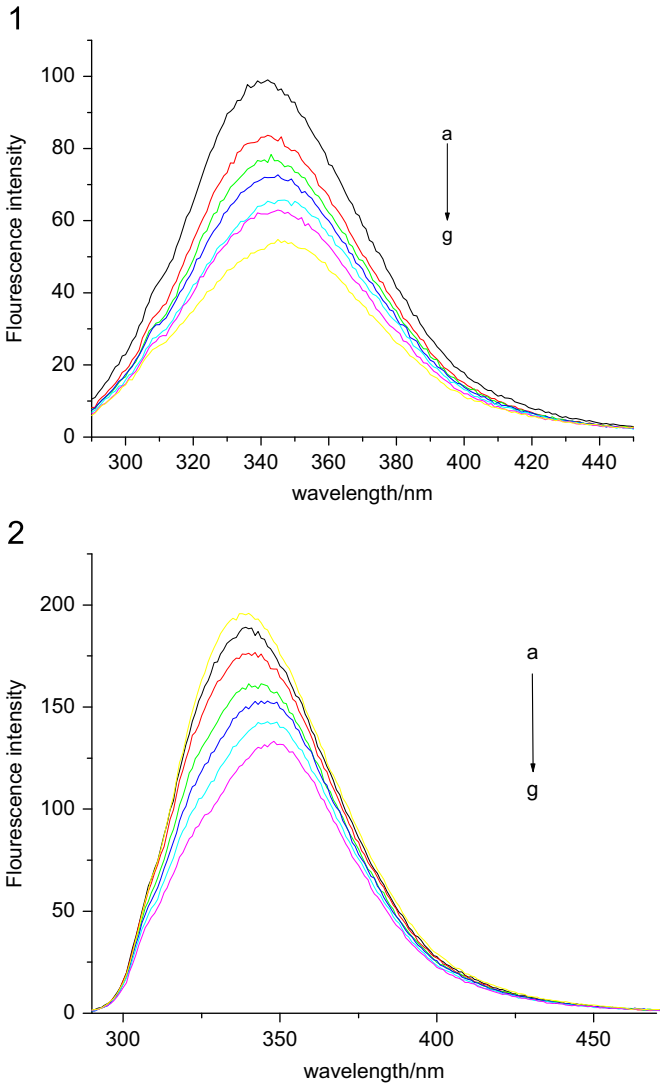

Fig. 2. The effects of silymarin on the fluorescence emission spectrum of (1) BSA and (2) LYS in Tris–HCl buffer of pH 7.4 at 291 K. The concentrations of BSA and LYS are 1.0×10^{-6} and 5.0×10^{-6} mol L^{-1}. The concentration of silymarin in BSA is: (a) 0.0, (b) 2.5, (c) 5.0, (d) 7.5, (e) 10.0, (f) 12.5, and (g) 15.0×10^{-6} mol L^{-1}. The concentration of silymarin in LYS is: (a) 0.0, (b) 2.28, (c) 4.56, (d) 6.84, (e) 9.12, (f) 11.40, and (g) 13.68×10^{-6} mol L^{-1}.

The fluorescence quenching may be dynamic, resulting from the diffusive encounter between quencher and fluorophore during the lifetime of the excited state, or static, resulting from the formation of a nonfluorescent ground-state complex (fluorophore–quencher) [22]. The quenching data was analyzed to clarify the quenching mechanism according to the Stern – Volmer equation [22,23]:

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

where F_0 and F denote the steady-state fluorescence emission intensities in the absence and in presence of quencher respectively. K_q is the biomolecule quenching rate constant, τ_0 is the average lifetime of molecules in the absence of quencher and its value is about 10^{-8} s [23], [Q] is the concentration of the silymarin, K_{sv} is the Stern–Volmer quenching constant. Using the Stern–Volmer equation shows the dependence of F_0/F on [Q] is linear (Fig. 3). The values of K_{sv} and K_q are listed in Table 1. From Table 1, the values of K_{sv} decreased with the increasing temperature. Higher temperatures result in faster diffusion and