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Spectroscopic studies on the interaction between chalcone and bovine serum albumin

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ABSTRACT

The interaction between chalcone and bovine serum albumin (BSA) has been studied by spectroscopic techniques under physiological condition. By the analysis of fluorescence spectrum and fluorescence intensity, it was observed that the chalcone has a strong ability to quench the intrinsic fluorescence with BSA through a static quenching procedure and non-radiation energy transfer were the main reasons for the fluorescence quenching. The association constants of chalcone with BSA were determined at different temperatures based on fluorescence quenching results. The positive entropy change and enthalpy change indicated that the interaction of chalcone and BSA was driven mainly by hydrophobic forces. The process of binding was a spontaneous process in which Gibbs free energy change was negative. The distance, *r*, between donor (BSA) and acceptor (chalcone) was obtained according to the Forster's theory of non-radiation energy transfer. The UV–vis, CD, FT-IR, synchronous and 3-D spectral results revealed the changes in the secondary structure of BSA upon interaction with chalcone. The effects of some common metal ions on binding of BSA–chalcone complex were also investigated.

1. Introduction

Serum albumins are one of the most abundant proteins in blood plasma, which are the major soluble protein constituents of the circulatory system. They play a dominant role in the transport and deposition of endogenous and exogenous ligands in blood, since serum albumins often increase the apparent solubility of hydrophobic drugs in plasma and modulate their delivery to cells in vivo and in vitro. Hence, it is important and necessary to study the interaction of drug with BSA (Fig. 1) at molecular level [1].

Investigating the interaction of drugs to serum albumins can elucidate the properties of drug–protein complex, as it may provide useful information of the structural features that determine the therapeutic effectiveness of drugs. Interaction with albumin could also be critical for understanding the drug toxicity and its distribution in the organism. It has been an interesting research field in life science, chemistry and clinical medicine.

Chalcone (1,3-Diphenyl-2-propen-1-one) (Fig. 2) is an aromatic ketone that forms the central core for a variety of important biological compounds, which are known collectively as chalconoids. They show antibacterial, antifungal, antitumor and anti-inflammatory properties [2]. Some chalcones demonstrated the ability to block voltage-dependent potassium channels [3]. They are also intermediates in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities. Chalcones are also intermediates in the Auwers synthesis of flavones. Due to ineffective drugs, cancer is the second most leading cause of death after heart attack. Therefore, the researchers have accelerated their efforts for the generation of new anticancer drugs with high therapeutic index. In this connection, a clinically effective antitumor derivative of chalcone (calicheamicin) has stimulated the investigators to concentrate their studies on chalcones and related compounds [4].

Drug interactions at protein binding level will in most cases significantly affect the apparent distribution volume of the drugs and also affect the elimination rate of drugs. Therefore, the studies on this aspect can provide information of the structural features that determine the therapeutic effectiveness of drug and have been an interesting research field in life sciences, chemistry and clinical medicine [5,6]. In order to understand the binding mechanism of BSA with chalcone under physiological condition, we planned to carry out detailed investigation of chalcone–BSA association using fluorescence spectroscopy. In addition, conformational change of BSA is discussed on the basis of UV–vis and IR spectroscopy. This study is expected to provide important insight into the interaction of protein BSA with chalcone, under physiological conditions.

The molecular interactions are often monitored by spectroscopic techniques because these methods are sensitive and relatively easy to use. They have advantages over conventional approaches such as

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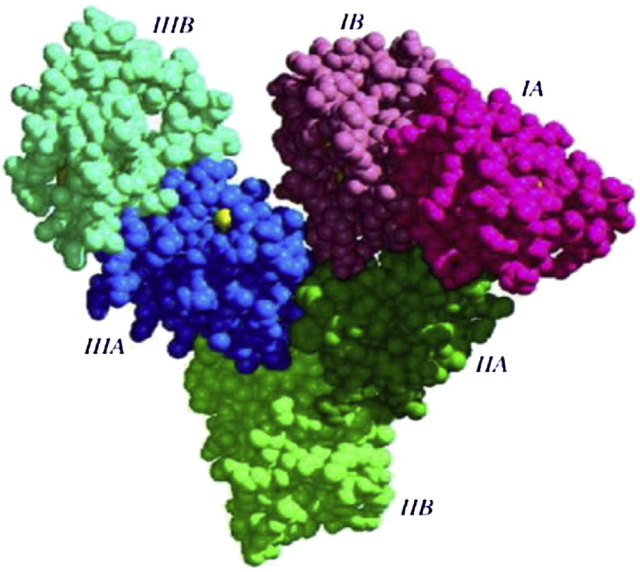

Fig. 1. Structure of BSA.

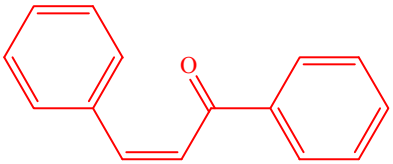

Fig. 2. Chemical structure of chalcone.

affinity and size exclusion chromatography, equilibrium dialysis, ultra filtration and ultracentrifugation, which suffer from lack of sensitivity, long analysis time or both and use of protein concentrations far in excess of the dissociation constant for the drug–protein complex [7,8] and for drug–protein interaction studies. In the present paper, we are reporting the mechanism of interaction of chalcone with BSA using three spectral methods for the first time.

2. Experimental section

2.1. Reagents and Chemicals

Bovine serum albumin (BSA) was purchased from Sigma Chemical Company, St. Louis, USA and used without purification. Chalcone was obtained from Sigma Aldrich. The solutions of chalcone and BSA were prepared in 0.1 M phosphate buffer of pH 7.4. BSA solution was prepared based on their molecular weight of 65,000. All other materials were of analytical reagent grade and millipore water was used throughout the work.

2.2. Instrumentation and analytical procedure

Fluorescence spectra were recorded using a RF-5301 PC Hitachi spectrofluorometer Model F-2000 (Tokyo, Japan) with a 150 W Xenon lamp, a 1 cm quartz cell and thermostatic cuvette holder. The excitation and emission bandwidths were both 5 nm. The temperature of the sample was maintained by recycling water throughout the experiment. The absorption spectra were recorded on a double beam CARY 50-BIO UV–vis. Spectrophotometer (Victoria, Australia), FT-IR Nicolet-5700; USA was used to record infrared spectra. All of the pH measurements were performed with an Elico LI120 pH meter (Elico Ltd., India).

2.3. Procedures

2.3.1. Chalcone–protein interaction study

An appropriate volume of the BSA solution (40 μM from 250 μM stock), chalcone solution (40 μM from 250 μM stock) were transferred into a 5 mL conical flask. The mixtures were diluted with phosphate buffer solution (pH 7.4) to make the total volume (2 mL) and then were shaken. On the basis of preliminary experiments, BSA concentration was fixed at 5 μM and drug concentration was varied from 5 μM to 45 μM. Fluorescence spectra were recorded at five different temperatures (288, 293, 298, 303 and 308 K) in the range 300–550 nm upon excitation at wavelength of 295 nm in each case.

2.3.2. UV measurements

The UV measurements of BSA in the presence and absence of chalcone was made in the range of 225–450 nm. BSA concentration was fixed at 5 μM while the drug concentration was varied from 5 μM to 65 μM in the presence of phosphate buffer as a solvent.

2.3.3. Circular dichroism (CD) measurements

The CD spectra of BSA in presence and absence of chalcone at 298 K were recorded in the range of 200–250 nm. The BSA was fixed at 5 μM while that of chalcone was varied from 10 μM to 30 μM in presence of phosphate buffer.

2.3.4. FT-IR measurements

The FT-IR spectra of BSA in presence and absence of chalcone at 298 K were recorded in the range of 1000–2000 cm^{-1}. Bovine serum albumin concentration was fixed at 5 μM while that of chalcone was 5 μM in presence of phosphate buffer.

2.3.5. Synchronous fluorescence measurements

The synchronous fluorescence characteristics of BSA–chalcone were noted down at different scanning intervals of $\Delta\lambda$ ($\Delta\lambda=\lambda_{em}-\lambda_{ex}$). When $\Delta\lambda=15$ nm, the spectrum characteristics of protein tyrosine residues were observed and when $\Delta\lambda=60$ nm, the spectrum characteristics of protein tryptophan residues were noticed.

2.3.6. 3-D fluorescence studies

3-D fluorescence spectrum was recorded under the following conditions: excitation wavelength range of 250–350 nm and emission wavelength range of 200–550 nm and an increment of 10 nm with other parameters were just the same as that of fluorescence quenching spectra. $C_{protein}=5$ μM and $C_{chalcone}=20$ μM.

2.3.7. Displacement studies

The displacement experiments were performed using different site probes viz., warfarin, ibuprofen and digitoxin for site I, II and III, respectively [9] by keeping the concentration of BSA and the probe constant (5 μM each). The fluorescence quenching titration was used as before to determine the binding constant of BSA–chalcone in presence of above site probes.

2.3.8. Effects of some common ions

The effects of some common ions viz., Co^{2+}, Cu^{2+}, Ni^{2+}, Ca^{2+} and Zn^{2+} were investigated on chalcone–BSA interactions. The fluorescence spectra of chalcone–BSA system were recorded in presence of above ions at 343 nm upon excitation at 295 nm. The overall concentration of BSA and that of the common ions was fixed at 5 μM.