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Review Article

Fluorescence study on the interaction of human serum albumin with Butein in liposomes

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ABSTRACT

The interaction of Butein with human serum albumin in L-egg lecithin phosphatidycholine (PC) liposome has been investigated by fluorescence and absorption spectroscopy. The results of the fluorescence measurement indicated that Butein effectively quenched the intrinsic fluorescence of HSA via static quenching. The Stern–Volmer plots in all the liposome solutions showed a positive deviation from the linearity. According to the thermodynamic parameters, the hydrophobic interactions appeared be the major interaction forces between Butein and HSA. The effect of Butein on the conformation of HSA was also investigated by the synchronous fluorescence under the same experimental conditions. In addition, the partition coefficient of the Butein in the PC liposomes was also determined by using the fluorescence quenching process. The obtained results can be of biological significance in pharmacology and clinical medicine.

Contents

1. Introduction

Fluorescence quenching is frequently employed in order to investigate the interaction between molecules and proteins because it is sensitive, quick, and relatively easy to use. The fluorescence quenching process depends upon the accessibility of the quencher molecules to the fluorophores, and helps to provide clues in the understanding drug interactions with body proteins [1–4]. The Stern–Volmer constant describes the apparent complex formation constant of the drug to protein. Liposomes, spherical bilayer structures in water, which are made up of lipids, are an excellent model in order to investigate biological membrane processes, including transport, and a variety of metabolic pathways [5,6]. The pharmacological active compound distribution between the lipid and water phases is usually expressed by the liposome/water partition coefficient. This distribution determines their concentration in the lipid and water phase, and thereby governs the extent of their permeation into the cell membranes. This partition can provide information regarding their pharmacokinetics profile, including absorption, distribution, excretion and metabolism [7,8]. However, the

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lipid affinity of the pharmacological active molecules affects their levels of activity and toxicity. Therefore, the value of the partition coefficient is certainly an important parameter in the design, development, and working of the pharmacologically active compounds [6–10]. Human serum albumin is one of the most abundant carrier proteins in blood plasma (approximately 5 g/100 mL). It plays an important role in the transportation and deposition of various endogenous and exogenous molecules in blood, and contributes to 80% of osmotic blood pressure [11,12]. The distribution and metabolisms of drugs in the blood depends on the degree of binding on to the albumin [13–15]. Many drugs and other bioactive small molecules bind reversibly to plasma proteins such as HSA, and this in turn alters the distribution, free concentration, and the metabolism of compounds. In addition, this situation can prolong the movement time from being metabolized [16–18]. Moreover, Compound–Albumine complex blocks compounds from penetrating into the membrane walls [10,19]. Consequently, it is very important for us to understand the pharmacodynamics and pharmacokinetics of a pharmacological active compound during the development of new drugs at an early stage. Butein (3,4,2′,4′-tetrahydroxychalcone) is a chalcone compound that is a member of the flavonoid family of polyphenols [20]. Various reports have stated that Butein possesses extensive pharmacologically effects such as anti-fibrogenic [21], anti-inflammatory activity [22], and anticancer activities against various human cancer cells [23–30]. The pharmacological active compounds such as in Butein are especially suited for studies with natural and model membranes. However, there is little information concerning the transportation and metabolism of Butein on the human body. In addition, to date, the interaction between Butein and HSA at the molecular level in the liposome has not been reported. The present investigation focuses on understanding the association of Butein on human serum albumin (HSA), and the effects of liposomes on the binding of Butein to HSA. The type of quenching mechanism in the liposome media was discussed by using UV–vis spectroscopy and fluorescence spectroscopy. The main types of binding forces were identified. The conformation changes of HSA on the binding to Butein were discussed on the basis of the synchronous fluorescence spectra. It also determined the partition coefficient of Butein in liposomes.

2. Experimental details

2.1. Materials

L-egg lecithin phosphatidylcholine (purity 99%), HSA (purity 99%) and phosphate-buffered saline were obtained from Sigma-Aldrich Company (USA). Chloroform was purchased from Scharlau Company (Spain). Butein (purity 99%) was obtained from Merck. All the chemicals were used without further purification and double distilled water was used throughout the experiment. For all the experiments, the concentration of HSA was maintained at 2.0×10^{-6} M.

2.2. Preparation of liposome

Liposomes were prepared by the thin film hydration method [5,31], in which appropriate amounts of L-egg lecithin phosphatidylcholine (PC) solution in chloroform were evaporated to dryness under a gentle nitrogen stream. Finally, the dry lipid films were hydrated with phosphate-buffered (pH = 7.4,) and the liposomes were formed by vortexing the mixture. Appropriate amounts of Butein and HSA were added in the liposome suspensions and the mixtures were equilibrated at room temperature for 30 min before conducting experiments. Doubly distilled water was used to prepare liposome solutions.

2.3. Apparatus

The UV–Vis spectra were recorded with a Shimadzu UV-3101PC UV–VIS-NIR spectrophotometer using a 1-cm quartz cuvette. The absorption spectrum of the sample solution was measured against the reference solution (only liposome solution) by using a spectrophotometer. The fluorescence and synchronous fluorescence spectra were recorded with a Perkin–Elmer (Model LS 55) spectrofluorometer. The excitation wavelength was set to 270 nm and Fluorescence spectra were recorded from 290 nm to 440 nm. The width of the excitation and emission slit was set to 5.0 and 2.5 nm, respectively. The synchronous fluorescence spectra were recorded from 220 to 400 nm at ($\Delta\lambda = 15$ nm) and from 240 to 310 0 nm ($\Delta\lambda = 60$ nm) at room temperature.

3. Results and discussion

3.1. Fluorescence quenching spectra of HSA with Butein

The fluorescence spectra of HSA in the liposomes with varying concentration of the Butein at 303 K are shown in Fig. 1. It was found that the fluorescence intensity decreased gradually with the increase in the concentration of the Butein, implying that the interaction between the HSA and Butein occurred. The position of the maximum wavelength of the HSA shifted from 345 to 337 nm after the addition of Butein. It also indicated that the polarity around the chromophore of HSA in the liposome solution decreased, and that the hydrophobicity increased [1,3]. Fluorescence quenching refers to any process that decreases the intensity of the fluorescence of a fluorophore caused by a variety of molecular interactions with a quencher molecule. These include exciting-state reactions, molecular rearrangements, energy transfer, the ground-state complex formation, and the collisional quenching processes [3,17]. The quenching of the mechanism fluorescence can usually be classified into two categories: dynamic quenching and static quenching. The dynamic quenching process results from the diffusion of the fluorophore and quencher molecules in the medium, and does not change the absorption spectra because it only affects the excited state of the fluorophore. Static quenching is due to the formation of a dark complex between the fluorophore and the quencher molecules, and often leads to a change in the absorption spectra. The dynamic and static quenching can be differentiated by their differing dependence on temperature. In static quenching, the quenching rate constant reduces with an increase in temperature due to the reduced stability of the complexes. On the contrary, for dynamic quenching, the quenching rate constants are expected to increase with the increasing temperature as in this case, a higher temperature results in a faster diffusion of quencher, and therefore, a larger amount of collisional quenching. For

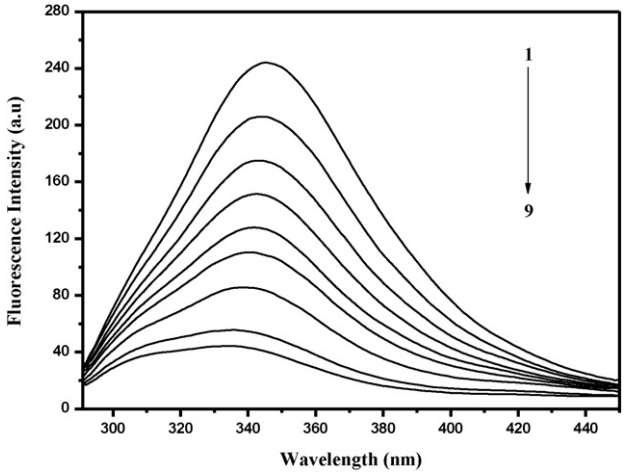

Fig. 1. Fluorescence spectra of HSA induced by different concentrations of Butein in 185 μM Liposome solution. Total concentrations of Butein: (1) 0 μM; (2) 2 μM; (3) 4 μM; (4) 6 μM (5) 8 μM; (6) 10 μM; (7) 14 μM; (8) 20 μM, (9) 24 μM.