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A fluorescence spectroscopic study of the interaction between Glipizide and bovine serum albumin and its analytical application

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ABSTRACT

The interaction between Glipizide and bovine serum albumin (BSA), as well as the effect of some metal ions (Zn^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, V^{5+}, Cr^{6+}, Mo^{6+}) on the BSA–Glipizide system were investigated at different temperatures by fluorescence spectroscopy. Results showed that Glipizide could quench the intrinsic fluorescence of BSA, and the quenching mechanism was a dynamic quenching process. The hydrophobic force played an important role on the conjugation reaction between BSA and Glipizide. The binding constants (K_a) were 1.45×10^4, 3.09×10^4, 4.51×10^4 L/mol at 293, 303 and 310 K, respectively, and the number of binding site (n) in the binary system was approximate to 1. The binding distance (r) was about 2.80 nm and the primary binding for Glipizide was located at the structure domain II A of BSA. The synchronous fluorescence spectra and CD spectra revealed that the microenvironment and the conformation of BSA were changed during the binding reaction. A new method of using BSA as probe to determine the content of Glipizide by fluorescence spectroscopy was established, and it was applied to analysis of Glipizide in tablets with a satisfying result.

1. Introduction

Serum albumin, the most abundant protein constituent in blood plasma, plays a fundamental role in the disposition and transportation of various molecules and can react with many different ligands in vivo and in vitro [1,2]. As the structure of protein determines its important biological functions, the resultant structural alternations due to its interaction with ligands can influence the transport, metabolism and availability of serum albumin for other ligands [3–5]. Glipizide is part of a group of diabetes medications called sulfonylureas (the structure showed in Fig. 1) [6]. Sulfonylureas help the pancreas to make more insulin and help the cells to respond better to insulin, it helps to lower blood sugar and keeps it under better control. Generally speaking, the protein–drug interaction may result in the formation of a stable protein–drug complex, which has an important effect on the distribution, free concentration and the metabolism of drug in the blood stream. Therefore, it is important and necessary to study the interaction between drug and serum albumins at the molecular level [7]. At present, the molecular interactions between BSA and many drugs have been investigated successfully in bio-medical domain [8]. However, the interaction between Glipizide and BSA has not been investigated, especially the effect of coexistent metal ion on the binding of drugs to BSA. In this report, we provided investigations on this interaction by fluorescence spectroscopy under physiological pH 7.40. This study is expected to provide important insight into the essence, potential toxicity between drugs and protein in real terms, and can also provide a useful clinical reference for future combination therapy. Therefore, investigating the interaction of drugs and serum albumins was significant to know the transport and distribution of drugs in body, and clarify the action mechanism and pharmaceutical dynamics.

2. Experimental

2.1. Apparatus

All fluorescence spectra were recorded with a Shimadzu RF-5301PC spectrofluorophotometer. Absorption was measured with an UV–vis recording spectrophotometer (UV-265 Shimadzu, Japan). CD spectra was recorded on a MOS-450/SFM300 circular dichroism spectrometer (Bio-Logic, France). All pH measurements were made with a pHS-3C precision acidity meter (Leici, Shanghai). All temperatures were controlled by a CS501 super-heated water bath (Nantong Science Instrument Factory).

2.2. Materials

Glipizide (CAS#, 29094-61-9) was obtained from Monitor of Chinese Veterinary Medicine (the purity grade inferior 99%). Bovine serum albumin was purchased from Sigma Company

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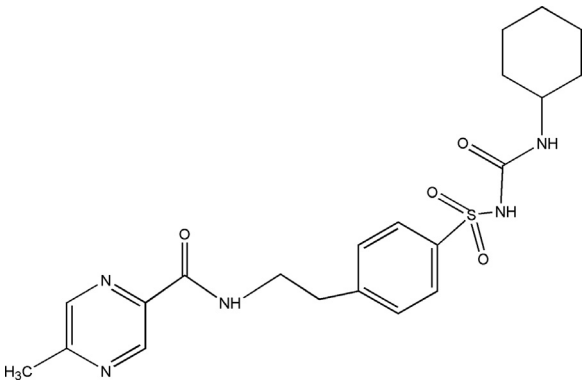

Fig. 1. Chemical structure of Glipizide.

(the purity grade inferior 99%). Stock solutions of BSA (1.0×10^{-5} mol/L), Glipizide (5.6×10^{-5} mol/L) and metal ions (1.0×10^{-3} mol/L) were prepared. And all the stock solutions were further diluted as working solutions prior to use. Tris-HCl buffer solution containing NaCl (0.15 mol/L) was used to keep the pH of the solution at 7.40, NaCl solution was used to maintain the ionic strength of the solution. All other reagents were analytical grade and all aqueous solutions were prepared with newly double-distilled water and stored at 277 K.

The fluorescence intensities were corrected for the absorption of excitation light and re-absorption of emitted light to decrease the inner filter using the following relationship [9]:

$$F_{cor} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad (1)$$

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensities respectively. A_{ex} and A_{em} are the absorbance values of Glipizide at excitation and emission wavelengths, respectively. The fluorescence intensity used in this paper was corrected.

2.3. Procedures

In a typical fluorescence measurement, 1.0 mL of pH=7.40 Tris-HCl, 0.3 mL of 1.0×10^{-5} mol/L BSA solution and different concentrations of Glipizide were added into a 10 mL colorimetric tube in sequence. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 30 min at different temperatures (293, 303 and 310 K). Excitation wavelength with excitation and emission slit at 5 nm for BSA was 280 nm (or 295 nm). The fluorescence spectra was then measured (emission wavelengths of 290~500 nm) at 293, 303 and 310 K. Besides, we recorded the fluorescence spectra when the value of $\Delta\lambda$ was between the excitation and emission wavelengths stabilized at 15 and 60 nm, respectively. CD spectra were performed between 200 and 300 nm using a 1.0 mm path length quartz cuvette at 293 K.

3. Results and discussion

3.1. Fluorescence quenching spectra of BSA–Glipizide system

Proteins were considered to have intrinsic fluorescence which were mainly tryptophan (Trp) and tyrosine (Tyr) due to the presence of amino acids. When the excitation wavelengths were at 280 and 295 nm, the emission peaks for BSA were both located at 340 nm. The fluorescence spectrum of BSA–Glipizide system was shown in Fig. 2. It is seen that the fluorescence intensity of BSA decreased regularly and there was no shift of the emission wavelength with the addition of Glipizide. This result showed that Glipizide could quench the intrinsic fluorescence of protein

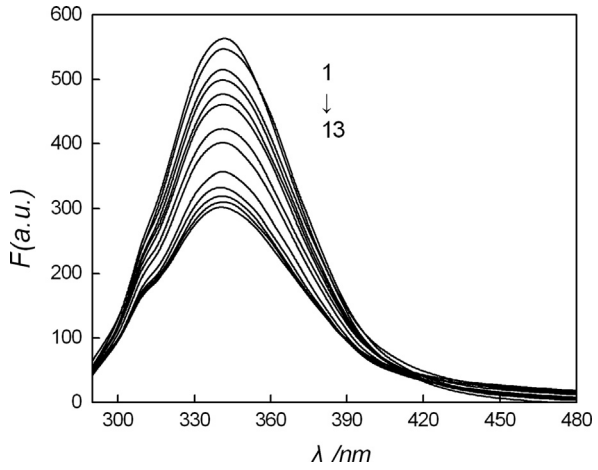

Fig.2. Fluorescence spectra of BSA–Glipizide system (T=293 K λ_{ex}=280 nm), $C_{BSA}=3.0\times10^{-7}$ mol/L, 1~13 $C_{Glipizide}$=(0, 0.028, 0.056, 0.28, 0.56, 1.12, 1.68, 2.24, 2.8, 3.36, 3.92, 4.48, 4.76)$\times10^{-5}$ mol/L.

strongly, and there was an interaction between Glipizide and BSA, while it did not lead to denaturation or unstabitily of BSA.

To interpret the data from fluorescence quenching studies, it is important to understand what kind of interaction has taken place between the fluorophore (BSA) and the quencher (Glipizide). If it is assumed that the fluorescence quenching of BSA induced by Glipizide is a dynamic quenching process, fluorescence quenching is described by the Stern–Volmer equation [10].

$$F_0/F = 1 + K_q\tau_0[L] = 1 + K_{sv}[L] \quad (2)$$

where F_0 and F were the fluorescence intensities of BSA before and after the addition of the Glipizide respectively. τ_0 was the average lifetime of fluorescence without ligand, which was about 10^{-8} s. K_{sv} was the Stern–Volmer quenching constant. K_q was the bimolecular quenching constant, and [L] was the concentration of the ligand. Based on the linear fit plot of F_0/F versus [L], the value of K_q could be obtained. The calculated results were shown in Table 1. Different mechanisms of quenching are usually classified as dynamic quenching and static quenching. Dynamic and static quenching can be distinguished by their different dependence on temperature. The quenching rate constants decrease with the rising temperature for the static quenching, but the reverse effect is observed for the dynamic quenching [11]. In Table 1, the Stern–Volmer quenching constants at different temperatures (293, 303 and 310 K) were presented. The results showed that K_q increased with the increase in temperature, which indicated that the quenching mechanism of BSA by Glipizide was a dynamic type. In addition, all the values of K_q were much greater than the maximum scatter collision quenching constant of various quenchers (2×10^{10} L/mol/s). This may be the effect of the ionic strength, which makes K_q too large [12].

The accessible fluorophore fraction (f) can be calculated by modified Stern–Volmer equation Eq. (3) [13]:

$$F_0/(F_0-F) = 1/(fK[L]) + 1/f \quad (3)$$

K was the Stern–Volmer quenching constant and it could be calculated from $F_0/F=K_{sv}[L]+1$, f was the fraction of accessible fluorophore to a polar quencher, which indicated the fractional fluorescence contribution of the total emission for an interaction with a hydrophobic quencher. The f values of interaction Glipizide to BSA were given in Table 1. When f was equal to 1, all the Trp residues were accessible to the quencher. Consequently, a change in the value of f (Table 1) signified that the fraction of fluorescent components accessible to the quencher became altered [14].