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Investigation on the binding activities of citalopram with human and bovine serum albumins

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ABSTRACT

The binding interactions of citalopram (CIT), an efficient antidepressant, with human serum albumin (HSA) and bovine serum albumin (BSA) were investigated by a series of spectroscopic methods including fluorescence, UV–vis absorption, circular dichroism (CD) and ^{1}H nuclear magnetic resonance (^{1}H NMR). The fluorescence quenching and UV–vis absorption studies reveal that CIT could form complexes with both HSA and BSA. The CIT–BSA complex exhibits higher binding affinity than CIT–HSA complex. The thermodynamic study further suggests that the interactions between CIT and SAs are mainly driven by hydrophobic forces and hydrogen bonds. The ^{1}H NMR analysis indicates that the participation of different functional groups of CIT is unequal in the complexation of CIT–HSA and CIT–BSA. Site marker competitive experiments show that the interactions between CIT and SAs primarily locate at sub-domain II A (site I). The effects of CIT on the conformation of SAs are further analyzed via synchronous fluorescence, three-dimensional fluorescence and CD spectra techniques. The results prove that the presence of CIT decreases the α-helical content of both SAs and induces the slight unfolding of the polypeptides of protein. Additionally, the conformational change of BSA induced by CIT is larger than that of HSA.

1. Introduction

The interaction between drug and plasma protein in biological systems is a key process that can modulate a number of properties of the carried agents, such as solubility, toxicity, and half-life *in vivo* [1]. Therefore, studies on the formations of drug–plasma protein complexes are essential for the understanding of the binding activities between drugs and transport proteins and pharmacological effects of drugs in the body [2–5]. Serum albumin (SA) is the most abundant protein in plasma and has multiple functions such as maintaining the blood pressure and transporting of a large range of endogenous and exogenous compounds [1,6–9]. The pharmacokinetic properties of those compounds can be influenced by reversible binding to SA, which is thought to be one of the primary determinants of the pharmacokinetic properties of drugs. Due to the important roles of transport proteins in drug transportations and good spectroscopic performances, SAs have been widely used as model proteins to investigate the binding activities between drugs and SAs through different scientific methods in recent years [10–15].

The primary structures of human serum albumin (HSA) and bovine serum albumin (BSA) are single chains containing 585 and 582 amino acid residues, respectively [16,17]. Both SAs are similarly composed of three homologous domains (I, II and III) and each domain includes two subdomains (A and B) [6,16]. The binding sites of SAs are situated in these domains and the principal regions of these sites are often located in the hydrophobic cavities of subdomain IIA (site I) and IIIA (site II) [16,17]. Both SAs contain tryptophan residues and have intrinsic fluorescence. However, the obvious difference between these two SAs is that BSA has two tryptophan residues (Trp-134 and Trp-213), while HSA has only one (Trp-214) [1]. The Trp-213 of BSA is located in a highly similar microenvironment as the single Trp-214 of HSA is. The other tryptophan residue (Trp-134) of BSA is situated in a rather superficial site that is less hydrophobic [18]. BSA presents structural homology of 76% in the amino acids sequences with HSA [1], so the two SAs have almost the same conformations and binding sites. Despite of the structural and functional similarities of HSA and BSA, the binding activities are different [19–21].

Citalopram[(±)-1-[3-(dimethylamino)propyl]–1-(4-fluorophenyl)–1,3-dihydroisobenzofuran–5-carbonitrile] (CIT, structure in Scheme 1), is one of the most effective antidepressants functioning as selective serotonin reuptake inhibitors (SSRIs) [22], which has been widely used for its safety and well-tolerance since it was approved by the American Food and Drug Administration in 1998 [23,24]. The free concentration and pharmacologic activity of CIT *in vivo* is closely related to its binding capacity towards transport protein. In addition, research on the drug–protein interaction at

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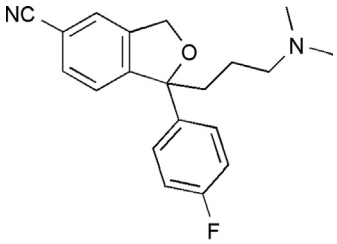

Citalopram (CIT)

Scheme 1. The structure of citalopram.

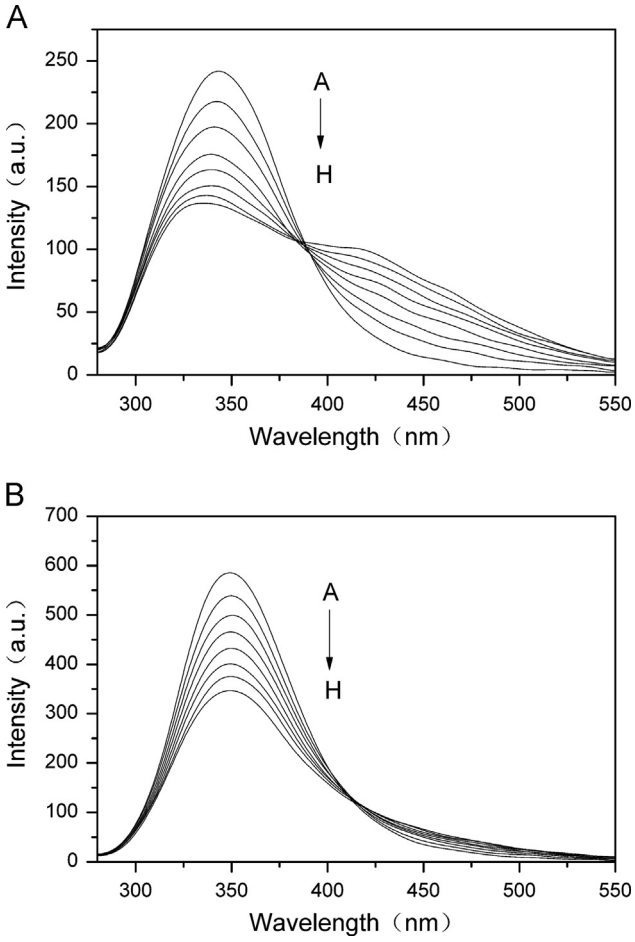

Fig. 1. Fluorescence quenching of HSA (A) and BSA (B) by increasing concentration of CIT 0–22.295 × 10^{-5} M (3.185 nM each increment) at 298 K. Concentration of SAs: 1.2 × 10^{-6} M. Excitation at 280 nm.

molecular level is significant to evaluate the reliability of extrapolating the experimental results from animals to humans [25,26]. Therefore, study on the binding activities of CIT to SA is of fundamental importance and will be helpful in understanding the pharmacology and pharmacokinetics of the antidepressant *in vivo* [27,28].

In this study, the interactions of CIT with HSA and BSA under simulative physiological conditions were investigated by a series of spectroscopic techniques including fluorescence, UV–vis absorption, circular dichroism (CD), and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopies. Based on the use of these methods in the drug-protein interaction, the binding mechanism of CIT-SAs complexation and the information about conformational change of protein induced by CIT could be comprehensively clarified.

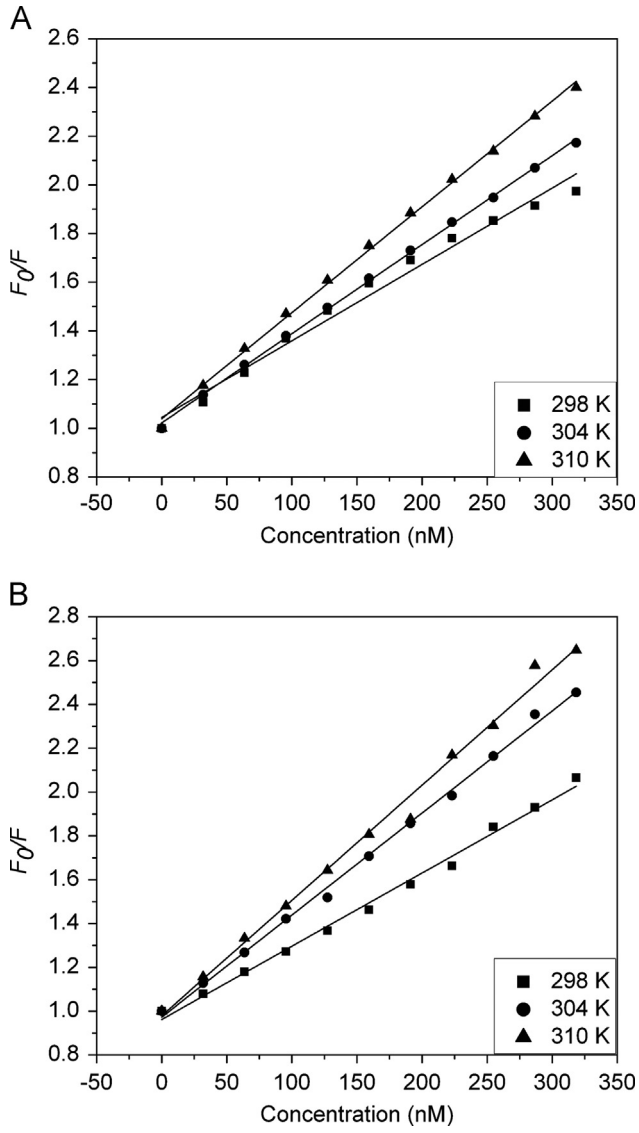

Fig. 2. Stern–Volmer plots for fluorescence quenching of HSA (A) and BSA (B) at 298 K, 304 K and 310 K.

2. Materials and methods

2.1. Materials

BSA and HSA were purchased from Sigma and used without further purification. Stock solutions of BSA and HSA (1.2 × 10^{-4} M) were prepared by dissolving the accurately weighed amounts of compounds in 0.01 M phosphate buffer solution (pH=7.4) and stored in dark at 277 K before use. The stock solution of CIT (6.37 × 10^{-2} M) was prepared in ethanol. All reagents were of analytical purity. Double distilled water was used throughout the experiments.

2.2. Apparatus

Fluorescence measurements were conducted on a Cary Eclipse Spectrofluorimeter (Varian corporate, America) equipped with a 10 mm path-length quartz cell. A circulating water bath was used to keep the temperature needed. The excitation and emission slit widths were set as small as possible when the signal intensity was satisfactory, and the widths were kept the same for BSA and HSA to guarantee the comparability. The excitation and emission filters