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Study on the interaction between amphiphilic drug and bovine serum albumin: A thermodynamic and spectroscopic description

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ABSTRACT

Herein we report the interaction of amphiphilic drug clomipramine hydrochloride (CLP—a tricyclic antidepressant) with bovine serum albumin (BSA) studied by fluorescence, UV–vis, and circular dichroism (CD) spectroscopic techniques. Clomipramine hydrochloride is used to treat a variety of mental health problems. The quenching rate constant (k_q) values, calculated according to the fluorescence data, decrease with increase in temperature indicating the static quenching procedure for the CLP–BSA interaction. The association binding constants (K_A), evaluated at different conditions, and the thermodynamic parameters (free energy, enthalpy and entropy changes) indicate that the hydrophobic forces play a major role in the binding interaction of drug. The interaction of BSA with CLP was further confirmed by UV absorption spectra. Blue shift of position was detected due to the complex formation between the BSA–CLP. The molecular distance, r_0, between donor (BSA) and acceptor (CLP) was estimated by fluorescence resonance energy transfer (FRET) whose value (4.47 nm) suggests high probability of static quenching interaction. The CD results prove the conformational changes in the BSA on binding with the drug. Thus, the results supply qualitative and quantitative understanding of the binding of BSA to CLP, which is important in understanding their effect as therapeutic agents.

1. Introduction

The interaction between proteins and various kinds of pharmaceuticals is imperative for wide range of pharmacological, biological, and clinical applications. Understanding the mechanism and related parameters of this kind of interaction, such as number and location of binding sites and binding constant(s), is essential for gaining insight regarding the pharmacodynamics and pharmacokinetics of a drug [1–3]. This includes providing information concerning the influence of binding to proteins on the absorption, elimination, distribution, and metabolic pathway of a drug. It is noteworthy mentioning here that this influence appears chiefly during transporting the drug in blood plasma to the targeted tissues via carrier proteins, such as serum albumins [4,5], whose structures are vulnerable to pH, temperature, ionic strength, etc.

Many drugs, particularly those with local anesthetic, tranquillizer, antidepressant, and antibiotic, exercise their action by interactions with biological membranes. These compounds must be carried to their site of action and, usually, this function is achieved by globular protein serum albumins (blood carrier proteins) at which they bind/attach with different affinities. As such, strong binding vis-à-vis weak binding plays a crucial role: the former can lessen the concentration of free drug in plasma, while the latter can lead to a low circulation time or poor distribution. In addition, binding processes modify the fragile equilibrium of the marginal stability of the native protein conformation [6], which is a delicate balance of various interactions such as van der Waals, electrostatic, hydrogen bonds, hydrophobic, and disulfide bridges. There is, therefore, noteworthy need to further research and have a full knowledge of the extent and intensity of the interactions between plasma proteins and amphiphilic drugs in order to resolve the optimal dose of administration of these compounds and also to avoid irreversible structural changes in protein molecules, which can lead to a deprivation of their biological activity and to side effects as well [7–9].

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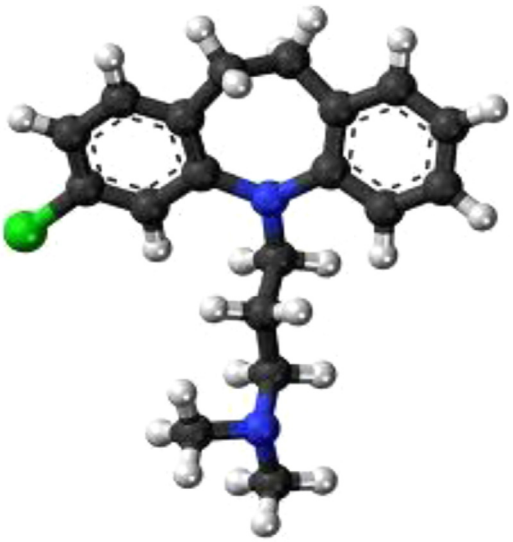
Various studies on serum albumins involving binding of small molecules, in particular fatty acids and amphiphiles/drugs, based on different techniques (fluorescence spectroscopy, UV–vis absorption spectroscopy, FTIR, Raman spectroscopy, CD spectroscopy, electrochemistry, NMR, etc.) have been described [10–13]; when these molecules bind to a serum albumin, the intramolecular forces creditworthy for sustaining the secondary structure can be altered, developing conformational changes in the protein [14].

Herein, we focus on studying the biophysical interactions of clomipramine hydrochloride (CLP) with bovine serum albumin (BSA) using the fluorescence, UV absorption and circular dichroism (CD) techniques. Clomipramine hydrochloride (3-chloro-5-[3-(dimethylamino)propyl]-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine monohydrochloride) (Scheme 1) is an antiobsessional drug that belongs to the class dibenzazepine of pharmacologic agents known as tricyclic antidepressants. The bovine serum albumin (BSA) presents 76% sequence resemblance with the human serum albumin (HSA) [15,16]. One of the main differences within the two proteins is that BSA has two tryptophan residues (Trp-134 and Trp-212) whereas HSA has only one (Trp-212). The additional tryptophan residue in BSA is buried in a hydrophobic sack, which lies near the surface of the albumin molecule in the second α-helix of the first domain [15]. Like others, BSA possesses a wide range of physiological functions associated with the binding, transport and distribution of biologically active compounds.

Drug interactions at protein binding level notably affect important factors such as drug availability, drug efficacy, drug transport, elimination rate, etc. Hence, the studies on this aspect can furnish information of the structural features that influence the therapeutic effectiveness of drug, and have been an interesting research field in life sciences, chemistry and clinical medicine [17]. In particular, our investigation is focused on quenching of BSA by CLP, determination of the binding constant and number of binding sites of the CLP–BSA system, energy transfer and binding distance between BSA and CLP, thermodynamic analysis and conformation changes of BSA upon binding to CLP.



Scheme 1. Molecular model of clomipramine hydrochloride (CLP).

2. Experimental

2.1. Materials

All starting materials were of analytical grade and double distilled water was used throughout. Clomipramine hydrochloride (CLP) with purity ≥98% and bovine serum albumin (BSA, fatty acid free) were purchased from Sigma and used without further purification. A 20 mM Tris-hydrochloride buffer (pH=7.40) was prepared by dissolving the required amount of Tris in water and adjusting pH with hydrochloric acid.

2.2. Apparatus

Fluorescence measurements were carried out with Shimadzu-RFPC5301 spectrofluorimeter equipped with a computer. The fluorescence spectra were deliberated using a 1 cm path length cell and a thermostatically controlled cell holder attached to Neslab's RTE—110 water bath with an accuracy of ± 0.1 K.

The UV absorption measurements were carried out with a double-beam Perkin Elmer Lambda 25 spectrophotometer using a cuvette of 1 cm path length.

A Jasco spectropolarimeter, model J-720, equipped with a microcomputer was used for recording the circular dichroism (CD) spectra in the Far UV-region.

2.3. Procedures

2.3.1. CLP–BSA interactions

The steady-state fluorescence experiments were performed at a constant concentration of BSA (2 μM) wherein the CLP concentration was varied from 0 to 18 μM. The fluorescence spectra were recorded at three temperatures (298, 310 and 318 K) in the range of 300–400 nm upon excitations at 280 and 295 nm.

2.3.2. UV measurements

The UV spectroscopic measurements of BSA in the absence and presence of CLP were made in the range of 245–310 nm. BSA concentration was fixed at 10 μM while the drug concentration was varied from 20 to 135 μM.

2.3.3. Energy transfer between CLP and BSA

The absorption spectrum of CLP was recorded in the range of 300–450 nm. The emission spectrum of BSA was also recorded in the range of 300–450 nm. Then, the overlap of the UV absorption spectrum of CLP with the fluorescence emission spectrum of BSA was used to calculate the energy transfer.

2.3.4. Circular dichroism (CD) measurements

The CD measurements of BSA in presence and absence of CLP were made in the range of 200–250 nm. Using a stock solution of 5 μM BSA, solutions of molar ratios (BSA:CLP) 1:5, 1:10, 1:15 and 1:20 were prepared for recording the CD spectra. The instrument was calibrated with *d*-10-camphorsulfonic acid. All the CD measurements were carried out at 303 K with a thermostatically controlled cell holder attached to Neslab's RTE-110 water bath. The spectra were collected with scan speed of 20 nm/min and response time of 1 s.

All the experiments were performed in Tris-hydrochloride buffer of pH 7.4 (20 mM). The concentration of protein was determined spectrophotometrically using $\epsilon^{1\,cm}_{1\%}$ of 6.5/M/cm at 280 nm.