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Deciphering the complexation process of a fluoroquinolone antibiotic, levofloxacin, with bovine serum albumin in the presence of additives

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ABSTRACT

The current work aims to explore the thermodynamic and conformational aspects for the binding of fluoroquinolone antibacterial drug, levofloxacin (LFC), with bovine serum albumin (BSA) using calorimetric, spectroscopic (UV–visible, fluorescence, circular dichroism, and ^{1}H NMR), dynamic light scattering (DLS) and computational methods (molecular docking). The binding of LFC with BSA at two sequential sites with higher affinity (~10^3 M^{-1}) at the first site has been explored by calorimetry whereas the binding at a single site with affinity of the order of ~10^4 M^{-1} has been observed from fluorescence spectroscopy. The calorimetric study in the presence of additives along with docking analysis reveals the significant role of electrostatic, hydrogen bonding, and hydrophobic interactions in the association process. The slight conformational changes in protein as well as the changes in the water network structure around the binding cavity of protein have been observed from spectroscopic and DLS measurements. The LFC induced quenching of BSA fluorescence was observed to be initiated mainly through the static quenching process and this suggests the formation of ground state LFC-BSA association complex. The stronger interactions of LFC in the cavity of Sudlow site I (subdomain IIA) of protein have been explored from site marker calorimetric and molecular docking study.

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1. Introduction

Recently, in the area of biophysics, the binding as well as the effect of various exogeneous ligands on whole plasma and isolated plasma proteins have been extensively studied [1]. Such type of binding can modulate the function of protein during blood transportation process as it may induce structural changes in protein. From the last several decades, the use of the drugs in the area of biological research has been widely expanded. The important data obtained from the study of drug-plasma protein binding enables to better understand the mechanism of their interaction. The profound effect induced by such binding on drug distribution and its free concentration in the body provides the fundamental information regarding the pharmacological and toxicological actions, biotransformation, and biodistribution of drugs [2,3]. Thus, in pharmacology and pharmacodynamics, the biological and therapeutic effects of drugs can be easily understood by taking help of such drug-protein binding data. Bovine serum albumin (BSA), a major constitute of plasma protein, has been widely used as a model protein to study its interaction with variety of ligands [4]. The majority of transportation, distribution, and metabolism processes were carried out by serum albumins in the blood [5,6]. Due to its low cost, easy availability, and 76% sequence conservation with human serum albumin (HSA), it is generally used as a substitute for HSA in binding studies [7,8]. Usually the binding of drugs takes place reversibly at two major drug binding sites of *BSA i.e.* Sudlow site I and Sudlow site II [9] *via* means of various weak interactions like ionic, van der Waals, hydrophobic, and hydrogen bonding among them [10]. Therefore, it is imperative to study the drug-serum albumin binding which also finds importance in the field of life sciences, chemistry, and clinical medicine in these days [11,12]. Much effort has been made in the area of drug-protein interactional study as it is considered as a second step in the rational drug design process [13–18].

Fluoroquinolones, a family of antibacterial agents, are potentially active against wide range of gram-positive and gram-negative bacteria [19]. They show their activity by inhibiting various kinds of enzymes like homologous type II topoisomerase, DNA gyrase, DNA topoisomerase IV which are required to control DNA replication, chromosome functions, and DNA topology [20,21]. Among the family of fluoroquinolones, levofloxacin (LFC, Fig. 1), a bacteriologically active third generation quinolone antimicrobial agent, is a pure (−)-(*S*)-enantiomer of the racemic drug ofloxacin [22,23]. It is widely used in the treatment of bacterial infections of respiratory, abdominal, gastrointestinal, and urinary tracts [24]. Although some reports [20,21,25–28] on the spectroscopic and non-spectroscopic studies on the binding of this class of antibiotics with serum albumins are available in literature, but the reports describing the detailed binding energetics, nature and location of binding sites,

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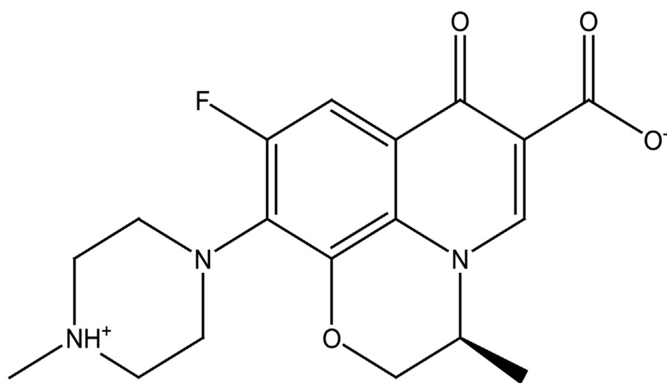

Fig. 1. The Zwitterionic structure of the drug levofloxacin (LFC) at pH 7.4.

and the effect on the conformation of BSA upon LFC binding are not much available. Moreover, the spectroscopic techniques provide vital information regarding the functional groups and chromophores/ fluorophores rather than direct energetics of the binding process whereas the isothermal titration calorimetry (ITC) has been widely used by researchers in recent days to get complete and accurate thermodynamic profile for the association process [29–31]. This is because ITC is the only technique that directly measures the heat evolved during interaction. In the present report, we have tried to cover many aspects (qualitative as well as quantitative) of the binding of antibacterial quinolone LFC with model transport protein, BSA, using spectroscopic (absorption, fluorescence, circular dichroism (CD), ^{1}H NMR), calorimetric, dynamic light scattering (DLS), and molecular docking techniques. The effect of temperature in conjugation with the effect of additives (salt, tetrabutylammonium bromide (TBAB), sucrose and surfactants), and site markers on LFC-BSA binding has also been studied using calorimetry. The structural as well as the topological changes have been monitored using CD and DLS measurements. The information about the mechanism of quenching, binding parameters and the effect on the microenvironment of amino acids has been obtained from spectroscopic measurements. The theoretical studies explore the type of interactions as well as the most probable site of binding on protein. Due to the prospective biological properties of fluoroquinolones, the knowledge of LFC-BSA interactional scenario has significant importance in the safe-engineered drug delivery field and in further discovery and development of drugs.

2. Experimental

2.1. Materials

Bovine serum albumin (Catalog no. B-4287, purity: ≥98%), levofloxacin (purity: ≥99%), cefotaxime sodium (purity: ≥98%), and ibuprofen (purity: ≥98%), procured from Sigma-Aldrich Chemical Company, have been used in the current study. All the experiments were performed in 0.01 M phosphate buffer of pH 7.4 by using Mettler Toledo AB 265-S weighing balance (resolution of 0.01 mg) for mass measurements. Phosphate buffer has been prepared in Milli-Q water (resistivity 18.2 MΩcm). The stock solution of protein was further purified by overnight dialysis at 4 °C against appropriate buffer. The concentration of BSA solution was estimated spectrophotometrically using molar absorption coefficient $^{1\%}A_{1cm} = 6.8$ at 280 nm [32]. Any error in the concentration of protein and ligand has direct effect on the values of the binding parameters. An error of 5% in extinction coefficient value results in the uncertainty in values of binding parameters within 10%. All other analytical grade reagents used in this study were dried in vacuum desiccator before use.

2.2. Methods

2.2.1. Isothermal Titration Calorimetry (ITC)

The calorimetric experiments were conducted on MicroCal iTC_{200} equipped with a 40 μl Hamilton syringe. After the baseline stability had been achieved, the titrations of 10 mM LFC (filled in 40 μl syringe) into 200 μl sample chamber containing 0.1 mM BSA solution were carried out automatically with continuous stirring at 500 rpm by maintaining a time interval of 120 s between successive injections. Reference cell was loaded with 0.01 M phosphate buffer of pH 7.4. Within similar experimental window, the control experiments (buffer into buffer, buffer into protein, and drug into buffer) were also carried out to correct the heat of LFC-BSA binding. The experimental data were analyzed using Origin 7 software provided with the instrument after applying dilution corrections.

2.2.2. Molecular Docking

Glide docking protocol in Schrodinger 2016 was used to perform the docking experiments while Jaguar was used for geometry optimization of ligands. The three dimensional crystalline structure of BSA (PDB ID: 4F5S) was fetched from Protein Data Bank (http://www.rcsb.org/pdb) at a resolution of 2.47 Å and was constructed with the help of Glide package to perform the protein preparation wizard [33] from which the water molecules were removed to avoid unnecessary interaction. Subsequently, to fulfil the inappropriate valency of protein atoms, polar hydrogens were incorporated which results in increase in the polarizability of the bonds. This increased polarizability was responsible for increase in the probability of inter ligand-protein interaction. Later, the processed protein structure was checked for stereochemical quality by residue-by-residue geometry as well as overall geometry. The preparation of ligands for docking was performed using LigPrep module [34]. The minimization and optimization of the prepared structures were done by the optimized potential liquid simulation (OPLS 20005) forcefield to acquire an energetically stable geometry. After the preparation of protein, the binding site prediction using sitemap was carried out as per Lamarckian Genetic Algorithm (LGA) docking. The grid size was determined using the sitemap points with a length of 10 Å. In order to visualize the obtained docked conformations, the Pymol and Chimera software package was used. For further analysis, the docked conformation having lowest binding free energy was selected as the binding mode. The binding energy estimation protocol, Qsite, and Glide docking modules were used to carry out MM-GBSA and QM/MM calculations.

2.2.3. Dynamic Light Scattering (DLS) Measurements

DLS measurements were conducted on Zetasizer NanoZS (Malvern, instruments, UK) light scattering apparatus at 298.15 K and at a scattering angle of 173° to the incident beam. The temperature of the instrument was controlled using built-in temperature controller equipped with the instrument having ±0.1 K accuracy. A He-Ne laser light (4 mW, 632.8 nm) was used in backscattering mode. For DLS measurements, all the solutions were purified by filtration through 0.22 μm pore sized filters after centrifugation. The titrations of LFC solution into BSA solution, taken in disposable polystyrene cuvette (1 cm path length), were carried out and a total of 6 measurements were taken for each titration. The following Stoke's-Einstein equation was used to determine the hydrodynamic diameter (d_h) of the particles:

$$d_h = k_B T / 3\pi\eta D_0 \qquad (1)$$

where k_B is Boltzmann constant, T is the absolute temperature, and η is the medium viscosity.

2.2.4. UV–visible Absorption Spectroscopy

The absorption spectra were recorded on Shimadzu-1800 UV–visible spectrophotometer using quartz cuvettes of 1 cm cell length. The concentration of BSA was maintained to 0.01 mM while recording the

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