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Exploring the interaction forces involved in the binding of the multiple myeloma drug lenalidomide to bovine serum albumin

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ABSTRACT

The binding characteristics of lenalidomide (LND) and bovine serum albumin (BSA) have been explored using spectroscopic techniques and molecular docking methods. Such interaction was shown to result in a static binding with a binding constant of 10^4 $Lmol^{-1}$ in a single binding site for LND on the BSA at the investigated temperatures. The thermodynamic characterization of the LND-BSA system revealed a spontaneous interaction with a $\Delta H°$ of -4.73 ± 1.3 kJ mol^{-1} and $\Delta S°$ of 67.88 ± 4.40 J mol^{-1} K^{-1} indicating the possible involvement of various binding forces in the LND-BSA binding. Competitive binding of LND to BSA using previously reported site markers showed that LND was bound to BSA within subdomain IIA (site I). Further confirmation was achieved through molecular docking studies of the LND-BSA binding, which established the binding site of LND with the most stable configuration of LND within the BSA. This LND conformer was shown to be situated within the active site residues Arg194, Leu197, Arg198, Ser201, Ala209, Trp213, Arg217, Val342, Ser343, Leu346, Ser453, Leu454, Asp450, Leu480, Val481, mainly forming three hydrogen bonds with Ser343, Trp.213 and Arg194 within a radius of 3.28 Å. Hence, the consistent results obtained in the current study suggested that hydrogen bonding and electrostatic forces dictate the binding of LND to BSA.

1. Introduction

Lenalidomide (LND; Fig. 1) a synthetic derivative of glutamic acid approved by the U.S. FDA as an immunomodulatory agent for the treatment of multiple myeloma [1]. LND can act via four general cellular mechanisms: immunomodulation [2], tumoricidal effects [3], tumor microenvironmental effects [4], and proerythropoiesis [5]. The mean protein binding of LND in plasma from myeloma patients and healthy volunteers is 23 and 29%, respectively [6]. LND is not a substrate inhibitor or inducer for CYP group of enzymes. Clinically relevant pharmacokinetic drug–drug interactions are unlikely to occur between LND and co-administered CYP substrates or inhibitors [7]. The excretion of LND is mainly via the renal pathway, with the overall urinary recovery of unchanged drug being approximately 85% of the administered single oral dose [8]. After intravenous administration, the majority of the compound is excreted in urine, while after oral administration equal amounts were found in urine and feces. Pharmacokinetics analyses in patients with impaired renal function indicate that as renal function decreases (<50 mL/min), the total drug clearance decreases proportionally resulting in an increase in AUC [3]. In general, the drug activity hinges upon its protein binding which shapes the cascade of functions in the body.

As the main transporting proteins in the blood, serum albumins (SA) can bind to a varied array of ligands including small molecule drugs [9–11]. The affinity and extent of the different drugs binding to SA play a crucial role in affecting their pharmacokinetics and pharmacodynamics [12]. SA are also responsible for some of the conformational dynamics and binding aggregation in solution [13]. Therefore, comprehensive investigation of the interaction between the diverse ligands and SA is indispensable, to explore the thorough pharmacological profile of those ligands. In the same context, the present study is designed to thoroughly examine the binding of LND to bovine serum albumin (BSA) as a representative of the SA family, as it stands as a homologous protein to its human counterpart (HSA) [14–17]. This study is mainly utilizing the LND-induced fluorescence quenching of BSA along with the UV–Vis spectral measurements to explore the mechanism of LND-BSA interaction and the various binding parameters. Additionally, as the ligands-protein binding may result in a variety of conformational changes in the protein, hence this study endeavors to model the interaction between LND and BSA, to allow the characterization of the LND behavior in BSA-binding site as well as determining its binding affinity.

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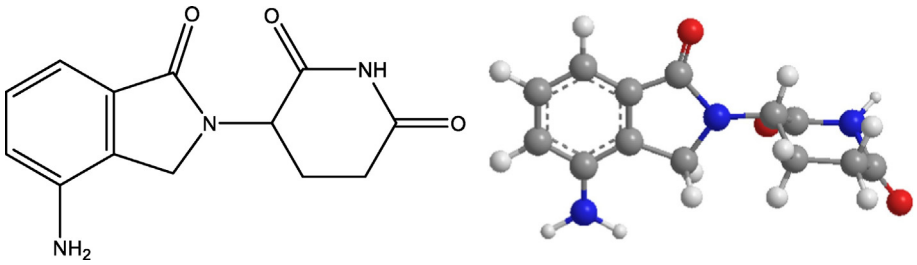

Fig. 1. 2D and 3D Chemical structures of lenalidomide (LND).

2. Materials and methods

2.1. Chemicals and reagents

Lenalidomide standard was procured from LC Laboratories (Woburn, MA, USA). Bovine serum albumin (BSA) was purchased from Techno Pharmchem (Haryana, India). HPLC grade Methanol (MeOH) obtained from BDH laboratory supplies (Poole, UK), other chemicals were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). Ultrapure water used throughout the study obtained via a Millipore Milli-Q® UF-Plus purification system (Millipore, MA, USA).

2.2. Sample preparation

LND powder was weighed and solubilized in MeOH producing a 2.0 mM solution. Further dilutions of the latter solution were made in a 1× phosphate buffered saline (PBS buffer) pH 7.4 preparing a solution of 40.0 μM which was used to prepare the different working solutions of LND. A solution of 15 μM BSA was made in PBS, and further diluted to a 1.5 μM working solution. All sample preparation procedure was achieved at normal laboratory temperature, solutions were then stored at −20 °C.

2.3. Spectrofluorimetric studies

Spectrofluorimetric investigation of the LND-BSA interaction was accomplished using a Jasco FP-8200 (Jasco Int. Co. Ltd. Tokyo, Japan) with the solutions measured in a 1 cm quartz cuvette. A wavelength range of 290–500 was specified for the emission spectra recordings after exciting the analyte solutions at 280 nm with slit widths of 5 nm for both excitation and emission. Three temperatures (288, 298 and 309 K) were identified for investigating the LND quenching behavior of the BSA intrinsic fluorescence. Five solutions of LND covering the concentration range of 1.5–7.7 μM were mixed with an equivolume of BSA 1.5 μM, fluorescence measurements of the solutions were performed 30 min after the mixing. Inner filter effect was reduced by correcting both intensities of the fluorescence originating from excitation and emission light absorption and re-absorption, respectively, through the following equation [18,19]

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

where F_{cor} and F_{obs} are the corrected and measured fluorescence intensities, respectively. While, A_{ex} and A_{em} are the LND absorbance readings at λ excitation and emission, respectively.

2.4. Synchronous and three dimensional fluorescence measurements

Synchronous fluorescence of the Compound **3**-BSA system was investigated at Δλ 15 nm and 60 nm to demonstrate the tyrosine and tryptophan characteristics of the protein utilizing the same solutions used for the fluorescence emission measurements. Concomitantly, three dimensional fluorescence (3D) spectra were recorded for a BSA-only solution of 1.5 μM and an LND-BSA (LND 1.6 μM) solution with λ excitation range set to 210–350 nm and λ emission set to 240–610 nm.

2.5. Competitive binding using site markers

Fluorescence intensity measurements were used study the binding displacement on the BSA between LND and phenylbutazone (PHB)/ibuprofen (IBP) as two known site markers for the BSA sites I and II, respectively. BSA and site markers solutions were prepared at 1.5 μM concentration, while LND concentration was steadily varied between 0 and 7.7 μM.

2.6. UV–Vis studies

UV-spectrophotometric studies of the LND-BSA binding were performed on a UV-1800 Schimazu™ double beam UV–Vis spectrophotometer (Schimazu Corporation, Tokyo, Japan). Absorption spectra were determined over a wavelength range of 220–450 nm. Binding studies were carried out using the same LND-BSA mixture solutions as described in spectrofluorimetric studies Section 2.3, while a 7.7 μM solution of LND was used for the drug reference measurements.

2.7. Molecular docking

The crystal structure (3D) of BSA was downloaded from the Protein Data Bank (PDB code 4OR0) [20] and loaded into Molecular Operating Environment software package (MOE® 2014) for pre-optimization through removal of water molecules and heteroatoms and addition of the hydrogen atoms. The 3D structure of LND was produced by ChemDraw® Ultra 14.0, minimized energy structure and geometries of LND were obtained by MOE® 2014 software package in the compatible file format. Binding pocket on the BSA was determined, London dG scoring function and the rescoring function GBVI/WSA dG in MOE® were set to rank the docked poses of the LND. The most suitable configuration of LND with BSA was designated based on the score and Root-Mean-Square Deviation (RMSD) values.

3. Results and discussion

3.1. Fluorescence measurements

The induced fluorescence quenching of a protein through its interaction with the various ligands can permit the interpretation of several binding parameters such as binding mechanism, strength and thermodynamic features as well as conformational changes in the protein itself [21–23]. Such quenching can be either dynamic and/or static [24–26], as dynamic type arises from molecules diffusion in solution, and static type is a result of a ground state complex formation. The two quenching pathways exhibit dissimilar dependency on temperature changes, viz., high temperatures boost binding constants for dynamic quenching and the opposite is always correct for static quenching. In the current study, the intrinsic fluorescence of BSA was quenched via the incremental addition of LND (Fig. 2) with no alteration of the emission wavelength and/peak shape. This quenching was observed at the investigated 3 different temperatures (288 K, 298 K and 309 K), with the temperature change affecting the degree of quenching in a way