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Fluorescence spectroscopic and molecular docking studies of the binding interaction between the new anaplastic lymphoma kinase inhibitor crizotinib and bovine serum albumin

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ABSTRACT

Binding of the recently introduced anti-cancer drug, crizotinib (CRB) with the bovine serum albumin (BSA) was comprehensively studied with the aid of fluorescence and UV–Vis spectroscopic as well as molecular docking techniques. The collective results of the study under the simulated physiological conditions proposed a static type of binding occurring between the CRB and BSA with binding constants of 10^4 L mol^{-1}. BSA conformational changes were investigated using three dimensional (3D) and synchronous fluorescence measurements. Moreover, the results of site marker competitive experiments and molecular docking, it could be deduced that CRB was inserted into the subdomain IIA (site I) of BSA yielding a more stabilized system. This was further confirmed with the molecular docking results which revealed that CRB is located in the active site residues Try149, Glu152, Ser191, Arg194, Arg198, Trp213, Arg217, Arg256, His287, Ala290, Glu291, Ser343, Asp450 within a radius of 6 Å. Combining the molecular docking studies and the computed thermodynamic parameters, it can be inferred that hydrophobic and electrostatic interactions are the major binding forces involved in formation of the CRB-BSA complex.

1. Introduction

Lung cancer is the principal reason of mortality among cancer patients [1,2]. During the last few years, different discoveries have emerged that changed the way we describe lung cancer. The precise identification of the non-small cell lung cancer (NSCLC) subtypes has shown to be essential the standard choice of chemotherapy as well as the targeted therapies [2] including those newly developed tyrosine kinase inhibitors (TKIs). A recently introduced member of the TKIs family, is crizotinib (CRB, Fig. 1) which was granted the FDA accelerated approval in 2011 for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive non-small cell lung cancer (NSCLC) [3]. CRB is an orally bioavailable agent acts by competitively binding to the ATP-binding pocket of various kinases including ALK, MET, and ROS1 tyrosine kinases [3–6]. It is marketed under the trade name Xalkori® capsule containing 200 or 250 mg CRB (Pfizer Inc. NY, USA). Its peak plasma concentration is reached after 4 h of administration of single dose and its absorption is not affected by food. Hence, and with the growing progress in the development of new therapies to tackle the several types of tumors numerous potential interactions should be considered. Out of those possible interactions, binding of *anti*-cancer agents to the serum albumins is a fundamental requirement. Serum albumins represent the major carrier proteins in the blood of all mammals, they bind to a wide range of ligands including drug compounds [7]. They are essential determinants of the drug pharmacological behavior *i.e.* pharmacokinetic and pharmacodynamics profile. Their weak ligand binding result in decreased ligand lifetime and/or poor distribution whereas their strong binding can lead to lower concentrations of the free ligands circulating in the plasma [8]. They are also accountable for certain conformational dynamics and binding aggregation in solution [9]. Hence, studying the binding between the different ligands with serum albumins is essential to elaborate the detailed pharmacological behavior of drug compounds. BSA is structurally homologue to its human equivalent (HSA) with a well-studied structure [10–13], which in turn made the BSA a prime representative of the serum albumin family in the experimental studies of the ligand-protein binding [14–16], including BSA interaction to other tyrosine kinase inhibitors [17,18]. Therefore, this study is devoted to comprehensively investigate the interaction between the anticancer drug crizotinib (CRB) and BSA *via* studying the CRB-induced fluorescence quenching of BSA. We are also assessing the possible conformational changes of the protein upon its binding to CRB and studying the molecular docking of CRB on the BSA.

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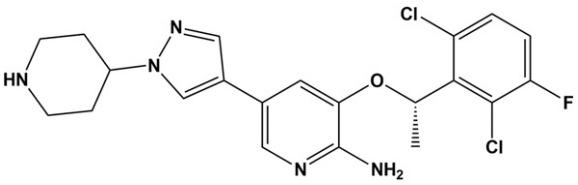

Fig. 1. Chemical structure of crizotinib.

2. Materials and Methods

2.1. Chemicals and Reagents

Crizotinib reference standard was purchased from Weihua Pharma Co. Ltd. (Zhejiang, China). Bovine serum albumin (BSA) was acquired from Techno Pharmchem (Haryana, India). Methanol (HPLC-grade) from BDH laboratory supplies (Poole, UK), and all other chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Ultrapure water of over 18.2 MΩ was produced from a Millipore Milli-Q® UF-Plus purification system (Millipore, MA, USA).

2.2. Sample Preparation

CRB standard was dissolved in methanol to yield a stock solution of 2.0 mM. Subsequent dilutions of the CRB solution took place using phosphate buffered saline (PBS buffer) pH .4 to finally yield a working solution of 40.0 μM (containing <5% of the dissolving organic solvent). Meanwhile, a concentration of 15 μM of BSA was prepared in the same buffer. Further dilutions of BSA solution were made in buffer to prepare a 1.5 μM solution. All solutions were prepared at ambient temperature, and were retained at −20 °C. All investigational solutions were prepared in 1× PBS buffer pH .4, the pH was determined with an Adwa AD1030 pH-meter (ADWA Instruments Inc., Romania).

2.3. Protein Concentration Determination

Protein concentration was determined from the specific extinction coefficient of $A^{1\%}_{280}$ ~ 6.7 for BSA using Nanodrop™ 2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.4. Steady State Fluorescence Measurements

Fluorescence spectra measurements were performed utilizing a Jasco FP-8200 (Jasco Int. Co. Ltd. Tokyo, Japan) with a 1-cm quartz cuvette. Fluorescence emission spectra were recorded over the wavelength range of 290–500 nm following excitation at 280 nm with slit widths of 5 nm for excitation and emission. The CRB quenching effect on BSA was investigated at three different temperatures of 288, 298 and 309 K. Individual mixture solutions of CRB and BSA were prepared with a constant BSA concentration of 1.5 μM with CRB concentrations varying over the range of 0–12 μM. Inner filter effect was diminished by correcting both intensities of the fluorescence originating from excitation and emission light absorption and re-absorption, respectively, using Eq. (1) [19,20]

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

In which the corrected and measured fluorescence intensities are F_{cor} and F_{obs}, respectively, with A_{ex} and A_{em} represent CRB absorbance values at the excitation and emission wavelengths, respectively.

2.5. UV–Vis Measurements

All UV–Vis absorbance spectral measurements were performed on a UV-1800 Schimazu™ double beam UV–Vis spectrophotometer (Shimadzu Corporation, Tokyo, Japan). UV–Vis absorption spectra were recorded over the wavelength range of 220–450 nm. BSA concentration was kept constant at 1.5 μM with CRB concentrations of 0, 15, 25 and 40 μM for the binding measurements and 10 μM of CRB was used for its reference measurement.

2.6. Synchronous and three Dimensional Fluorescence Measurements

Measurements of the synchronous fluorescence of CRB-BSA performed with the same concentrations of the mixture solutions used for the fluorescence quenching measurements only at ambient temperature. Spectra were recorded at two Δλ values namely at Δλ = 15 nm and 60 nm, which infer the tyrosine and tryptophan features of the BSA. Meanwhile, the three dimension (3D) fluorescence spectra of BSA 1.5 μM and CRB-BSA (CRB concentration of 15 μM) were recorded over the excitation and emission wavelength ranges of 210–350 and 240–610 nm, respectively.

2.7. Site Markers Competitive Binding

Binding displacement studies were carried out for the CRB-BSA interaction in the presence of the two site markers, phenylbutazone (PHB) and ibuprofen (IBP) as sites I and II markers, respectively. Concentrations of BSA and site markers were set at 1.5 μM, while the concentration of CRB gradually varied between 0 and 12 μM.

2.8. Molecular Docking Studies

The 3D crystal structure of bovine serum albumin with PDB code 4F5S [21] was downloaded from Brookhaven Protein Data and loaded to Molegro Virtual Docker (MVD) [22], all water molecules were removed. Simultaneously, ChemBio3D Ultra 10 [23] was used to draw the 3D structure of CRB, which was further pre-optimized using the free version of Marvinsketch 4.1.13 from Chemaxon with MM force field and saved in Tripos mol2 file format. MolDock score functions were used with a 0.3 Å grid resolution. Prior to the calculations of the subject compounds, the MVD software was benchmarked docking the BSA to PHB and IBP.

3. Results and Discussion

3.1. Fluorescence Measurements

Several spectroscopic techniques including fluorescence spectroscopy are considered efficient and reliable tools to investigate the binding

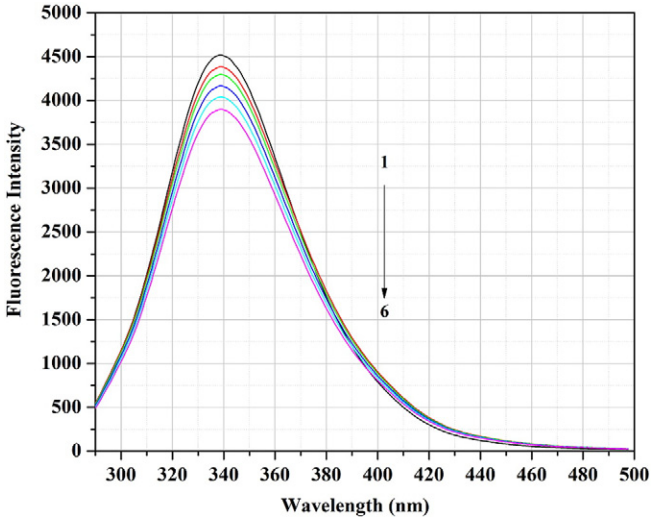

Fig. 2. Spectra of the BSA (1.5 μM) fluorescence quenching upon binding to CRB (1) 0 μM (2) 1.5 μM (3) 3.0 μM (4), 6.0 μM (5) 9.0 μM (6) 12.0 μM.