International Journal of Biological Macromolecules xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Investigating the site selective binding of busulfan to human serum albumin: Biophysical and molecular docking approaches

Mohammad Siddiqi[a], Saima Nusrat[a], Parvez Alam[a], Sadia Malik[a], Sumit Kumar Chaturvedi[a], Mohammad Rehan Ajmal[a], Ali Saber Abdelhameed[b], Rizwan Hasan Khan[a,*]

[a] Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, 202002, Uttar Pradesh, India
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 11 July 2017
Received in revised form
28 September 2017
Accepted 2 October 2017
Available online xxx

Keywords:
Serum albumin
Binding
Fluorescence quenching
Molecular docking

ABSTRACT

We have studied the binding of busulfan (BN) to human serum albumin (HSA) at physiological pH 7.4 by using fluorescence, UV–vis and circular dichroism (CD) spectroscopic tools, as well as dynamic light scattering (DLS) measurements and molecular simulation approaches. HSA fluorescence quenching experiments showed that BN reduces the HSA native fluorescence intensity through the static mechanism. In addition, a single binding site on the HSA is occupied by BN with a binding constant at 298 K of $1.84 \times 10^3\ M^{-1}$. The enthalpy change (ΔH) and entropy change (ΔS) of BN-HSA interaction were calculated as -1.40 kcal mol^{-1} and +10.14 cal mol^{-1} K^{-1} respectively, which suggest the possible interaction mode as hydrophobic and hydrogen bonding. Moreover, the secondary structure alteration of HSA following its complexation with BN was studied and showed that α-helical content of HSA gets increased on interacting with BN. Ligand binding site to HSA was further investigated by site-specific markers in fluorescence measurements as well molecular modeling approach which indicated that BN bind to the nearby sudlow site II of HSA through hydrophobic as well as hydrogen bonding interaction. The present study will be helpful for understanding the binding mechanism of BN to human serum albumin.

1. Introduction

The study of ligand-protein interaction is significantly important to understand the nature of their action inside our bodies. The association of the bioactive compounds with the plasma proteins affects their bioavailability, distribution & metabolism, hence, their pharmacokinetic and pharmacodynamics properties [1,2]. For instance, the interaction of a bioactive entity with a protein improves this compound's plasma solubility, reduces its toxicity, prevents its oxidation and extends its in-vivo half-life. However, these interactions can otherwise lead to conformational alterations in the protein hence disturbing its functional characteristics. Plasma protein binding has long been considered as the most important physicochemical characteristic of drugs. Protein drug interaction also conferred solubilization of hydrophobic drugs that then helps drug delivery to cells in-vivo & in-vitro [3–5].

Human Serum Albumin (HSA) is the prime extracellular protein in the mammalian circulatory system. It has several physiological and pharmacological functions. It corresponds to almost 60% of the total plasma proteins with a concentration of 42 mg/ml and is thus the most abundant plasma protein [6,7]. In addition, it is a major contributor of about 80% to the colloidal osmotic pressure of blood [8]. HSA functions primarily as a carrier protein and acts as a chief depot competent to bind, transport and deliver an extremely diversified range of endobiotics and xenobiotics in the blood stream to their target organs. It carries metals, steroids, fatty acids, bile salts, thyroid hormones and play an important role in stabilizing extracellular fluid volume. Thus this model transport protein is well known for its structural adaptableness to an astonishingly wide range of ligands and plays an instrumental role in drug deposition [2,9].

X-ray crystallographic analysis of HSA revealed that this globular protein consists of a single polypeptide chain of 585 amino acid residues [10] and comprises three homologous domains–(I,II,III) each of which includes 10 helices that are further divided into six-helix and four-helix subdomains (A & B) and is stabilized by 17 disulphide bond. The two major drug/small molecule binding sites

* Corresponding author.
E-mail addresses: rizwanhkhan1@gmail.com, rizwanhkhan1@yahoo.com (R.H. Khan).

https://doi.org/10.1016/j.ijbiomac.2017.10.006
0141-8130/

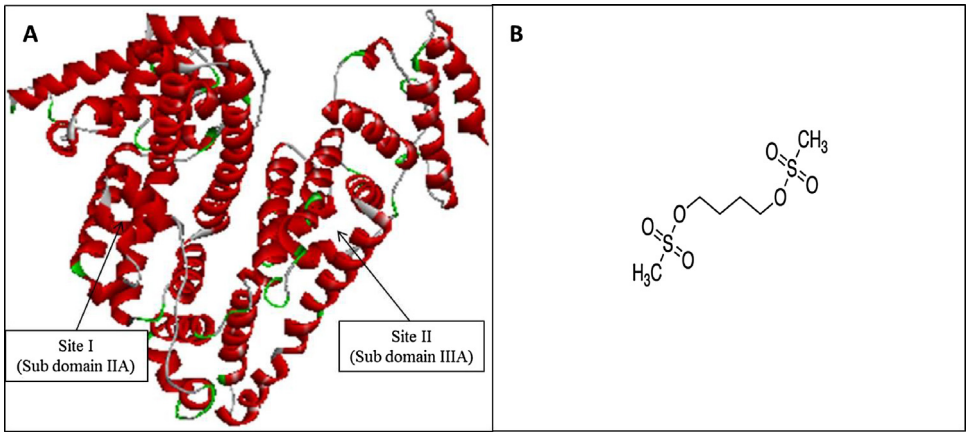

Fig. 1. (A) Crystal structure of HSA showing different binding sites (B) Molecular structure of BN.

on HSA are located in the hydrophobic cavities in subdomains II-A and III-A which are designated as Sudlow site I and II respectively (Fig. 1A). HSA contains only one Tryptophan residue at 214 in the subdomain II-A. Sudlow site I bind bulky heterocyclic compounds such as coumarin, sulphonates and salicylates. Sudlow's site II binds aromatic carboxylic acids and profens [11,12].

Busulfan (Myleran, Busulfex), an antineoplastic alkylsulfonate agent, designated as butane-1,4-diyl dimethane sulfonate (Fig. 1B) has been used for the treatment of myeloproliferative neoplasms (MPNs). It has been used since 1959, and it is a cell cycle non-specific alkylating agent. Busulfan is approved by the Food and Drug Administration and was commonly used for the treatment of chronic phase chronic myelogenous. Busulfan has also shown significant activity in BCR-/ABL1-negative MPNs, as shown in a number of studies. Busulfan is currently used by many hematologists and oncologists as second-line treatment in patients with BCR-/ABL1-negative MPNs that are intolerant to or developed side effects from hydroxyurea [13,14]. The mechanism of action corresponds to that of an alkylating agent, preventing from DNA replication due to the crosslinks taking place between guanine-adenine and guanine-guanine. Nowadays Busulfan also represents the cornerstone of many commonly used regimes in hematopoietic stem cell transplantation [15,16].

Drug-HSA interaction can provide information on drug storage, control of drug delivery to tissue receptors and prevention of drug from being rapidly metabolized. A large number of studies on the interaction of small molecules and drugs to HSA have been recently undertaken particularly on the structural aspects. The effectiveness of these small molecules on therapeutic agents may depend on their binding ability. Therefore the insights into the architecture and specificity of small drugs binding to HSA is essential. Herein, we have investigated the interaction of Busulfan (BN) with HSA by using fluorescence spectroscopy, UV–vis spectroscopy, circular dichroism spectroscopy, fluorescence resonance energy transfer method, dynamic light scattering technique and molecular docking study.

2. Materials and methods

Human serum albumin and Busulfan were purchased from Sigma Aldrich, India. All of the other reagents were of analytical grade.

2.1. Sample preparation

HSA (100 μM) stock solution was prepared in 20 mM phosphate buffer (pH 7.4). Protein concentration was determined spectrophotometrically using $E^{\%}_{1\,cm}$ of 5.30 at 280 nm by using Perkin-Elmer Lambda double beam UV–vis spectrophotometer [17,18]. BN stock solution (2 mg/ml) was prepared in 5% Dimethyl Sulfoxide (DMSO) and diluted to various concentrations in the same buffer. For each experiment respective blanks were taken.

2.2. pH determination

pH measurements were carried out on Mettler Toledo pH meter (Seven Easy S20–K) using Expert "Pro3 in 1" type electrode. The least count of the pH meter was 0.01 pH unit.

2.3. Steady state fluorescence quenching measurement

Fluorescence measurements were performed on a Shimadzu fluorescence spectrophotometer, model RF-5301 using a quartz cuvette of 1 cm path length. Excitation and emission slits were set at 3 and 5 nm respectively. Intrinsic fluorescence was measured by exciting the protein solution at 295 nm and emission spectra were observed between 300 and 500 nm. The titration of BN (0–160 μM) to HSA (5 μM) solution executed at 3 temperatures, viz. 288, 298, 310 K. The recorded fluorescence intensity at 339 nm was utilized to estimate K_b (binding constant). The fluorescence quenching data were analyzed by Stern-Volmer equation [19]:

$$F_0/F = K_{sv}[Q] + 1 = kq\tau_0[Q] + 1 \quad (1)$$

Where, F_0 and F are the intensities of the steady state fluorescence without and with the quencher, respectively, K_{SV} is the Stern-Volmer quenching constant and Q is the concentration of quencher, kq is the bimolecular quenching constant and τ_0 is the lifetime of tryptophan without the quencher. Lifetime of tryptophan for HSA (τ_0) is 5.6 ns [20].

Moreover, K_b and number of binding sites (n) were determined from the modified Stern- Volmer plot through Eq. (2):

$$Log(F_0/F - 1) = logKb + nlog[Q] \quad (2)$$

The enthalpy and entropy alterations at the varied temperatures were evaluated using the van't Hoff equation as shown below:

$$lnK_b = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

Where the enthalpy change is represented by ΔH, ΔS is entropy change, R (1.987 cal mol^{-1} K^{-1}) is the gas constant and T is the absolute temperature (K).