Journal of Molecular Structure 1127 (2017) 283–288

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

A spectroscopic and molecular docking approach on the binding of tinzaparin sodium with human serum albumin

Saleh M.S. Abdullah [a, **, 1], Sana Fatma [b, 1], Gulam Rabbani [c], Jalaluddin M. Ashraf [a, *]

[a] Department of Biochemistry, Faculty of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia
[b] Analytical Division, Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi, 221005, India
[c] Department of Medical Biotechnology, Yeungnam University, Gyeongsan, Republic of Korea

ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form
25 July 2016
Accepted 27 July 2016
Available online 31 July 2016

Keywords:
Human serum albumin
Tinzaparin
Circular dichroism
Fluorescence spectroscopy
UV–Visible spectroscopy

ABSTRACT

Protein bound toxins are poorly removed by conventional extracorporeal therapies. Venous thromboembolism (VTE) is a major cause of morbidity and mortality in patients with cancer. The interaction between tinzaparin, an inhibitor of angiotensin converting enzyme and human serum albumin, a principal plasma protein in the liver has been investigated in vitro under a simulated physiological condition by UV–vis spectrophotometry and fluorescence spectrometry. The intrinsic fluorescence intensity of human serum albumin was strongly quenched by tinzaparin (TP). The binding constants and binding stoichiometry can be calculated from the data obtained from fluorescence quenching experiments. The negative value of ΔG° reveals that the binding process is a spontaneous process. Thermodynamic analysis shows that the HSA-TP complex formation occurs via hydrogen bonds, hydrophobic interactions and undergoes slight structural changes as evident by far-UV CD. It indicated that the hydrophobic interactions play a main role in the binding of TP to human serum albumin. In addition, the distance between TP (acceptor) and tryptophan residues of human serum albumin (donor) was estimated to be 2.21 nm according to the Förster's resonance energy transfer theory. For the deeper understanding of the interaction, thermodynamic, and molecular docking studies were performed as well. Our docking results suggest that TP forms stable complex with HSA ($K_b \sim 10^4$) and its primary binding site is located in subdomain IIA (Sudlow Site I). The results obtained herein will be of biological significance in pharmacology and clinical medicine.

1. Introduction

Venous thromboembolism (VTE) is a disease that includes both deep vein thrombosis (DVT) and pulmonary embolism (PE). It is a common, lethal disorder that affects hospitalized and nonhospitalized patients, recurs frequently, is often overlooked, and results in long-term complications including chronic thromboembolic pulmonary hypertension (CTPH) and the post-thrombotic syndrome (PTS). VTE is a major cause of morbidity and mortality in patients with cancer [1]. Treatment with low-molecular-weight heparin (LMWH) is effective and is recommended over vitamin K antagonist therapy by clinical practice guidelines [2]. These recommendations are largely based on results from a single, large randomized trial with supportive evidence from additional smaller studies that were conducted over a decade ago in academic centers primarily in North America and Western Europe [3,4]. These limitations may partly explain why vitamin K antagonists remain frequently used worldwide in patients with cancer-associated thrombosis [5]. To provide more contemporary and global evidence for long-term LMWH therapy, we conducted the Comparison of Acute Treatments in Cancer Hemostasis (CATCH) trial to compare the efficacy and safety of tinzaparin with conventional warfarin therapy for the treatment of VTE in patients with active cancer.

Tinzaparin has the highest average molecular weight (MW; approximately 6500 Da) of all marketed Low-molecular-weight heparins (LMWHs). No dose reduction of tinzaparin is needed in patients with estimated creatinine clearance (CrCl) $\geq$20 mL/min. LMWHs are not single, (Enoxaparin, Dalteparin, Tinzaparin) well-described compounds; they are mixtures of glycosaminoglycan chains with different chain lengths, different biological activities

* Corresponding author.
** Corresponding author.
E-mail addresses: redsea001@hotmail.com (S.M.S. Abdullah), jmashraf@gmail.com (J.M. Ashraf).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.molstruc.2016.07.108
0022-2860/

and varying sulfation patterns [6]. LMWHs are partially metabolized by depolymerization and/or desulfation and excreted preferentially via the kidneys [7]. The elimination of these agents may be reduced in subjects with impaired renal function. Accumulation of LMWHs is of particular concern in populations with a high prevalence of renal impairment, such as the elderly [8] and patients with cancer [9]. Whether a reduction in renal elimination will result in a clinically relevant accumulation of anticoagulant activity (and an increased risk of bleeding after repeated dosing) depends on several factors, including the severity of renal impairment, the dose administered, the duration of treatment and the dosing frequency. These factors are not necessarily equally important for each LMWH. It is possible that the risk of clinically significant accumulation in patients with renal impairment will differ between different LMWHs.

Serum albumins are the major soluble protein constituents of the circulatory system, which have many physiological functions [10]. Human serum albumin (HSA) is a main carrier for a variety of endogenous and exogenous substances in the body therefore assists in their distribution and deposition [11]. Under physiological and pathological conditions, HSA has a pivotal role in heme scavenging [12]. In fact, although heme regulates gene expression and is the prosthetic group of heme proteins under physiological conditions, high levels of free heme: (i) catalyze the synthesis of toxic free hydroxyl radicals, (ii) affect the integrity of erythrocyte membranes, (iii) induce the enrollment to the vascular endothelium of red blood cells, platelets, and leukocytes, and (iv) cause the oxidation of low-density lipoproteins [13–15].

Protein-ligand interactions are important in the distribution and transport of small drug molecules in living systems. Therefore, understanding the molecular basis of these interactions is indispensable toward designing of new and more efficient specific therapeutic agents for improved drug action [16,17]. Fluorescence spectroscopy is one of the most widely used techniques to achieve this goal because of its high sensitivity coupled with easy operational procedure. The modular structural domain of HSA to bind a series of exogenous drugs led to an intense research toward understanding the forces responsible as well as comparative thermodynamic parameters for drug binding etc. This study is first to utilize UV–visible, far UV CD, fluorescence, FRET, and molecular docking to obtain the binding parameters, energetics and structural information for albumin binding of TP.

2. Experimental section

2.1. Materials & sample preparation

Human serum albumin (A1887; essentially globulin & fatty acid free) and tinzaparin (T1490000) were procured from Sigma Aldrich. All other reagents were of analytical grade. HSA and drug solutions were prepared in 20 mM sodium phosphate buffer (pH 7.4). The protein was dialyzed and its concentration was estimated spectrophotometerically using $E^{1\%}_{280nm} = 5.3$. All drug solutions were prepared by weight/volume (w/v).

2.2. Absorption and emission spectroscopy

The absorption spectra were acquired on a Perkin-Elmer Lambda 45 double beam UV–vis spectrophotometer attached with Peltier temperature programmer-1 (PTP-1). All the steady state fluorescence measurements were recorded on Varian, Cary Eclipse, fluorescence spectrophotometer equipped with water circulator (Julabo Eyela). During emission spectral measurements both the excitation and emission slit widths were set to 3.0 nm and an excitation wavelength of 295 nm. The concentration of the protein was maintained at 5 and 2 μM during absorption and fluorometric measurements respectively. The phenomenon of fluorescence quenching is conventionally described in terms of the following well-known Stern-Volmer equation:

$$\frac{I_o}{I} = \mathrm{Ksv[Q]} + 1 = kq\tau_o[\mathrm{Q}] + 1 \tag{1}$$

in which I_o is the original fluorescence intensity and I is the quenched intensity of the fluorophore (here intrinsic Trp and Tyr moiety of the protein), [Q] is the molar concentration of the quencher (TP), K_{SV} is the Stern-Volmer quenching constant and k_q is the bimolecular quenching rate constant, τ_o is the lifetime of the fluorophore in the absence of quencher and the fluorescence lifetime of the biopolymer is 5.78×10^{-9} s. Also the quantitative evaluation of the binding constant (K_b) and binding stoichiometry (n) for the HSA-TP interaction process rests on an analysis of the fluorescence quenching data on the following equation:

$$\log\left[\frac{Io - I}{I}\right] = \log K_b + n \log[\mathrm{Q}] \tag{2}$$

in which K_b is the binding constant and n is the binding stoichiometry. The thermodynamic parameters for the HSA-TP interaction process have also been estimated from the binding constant data as determined in various experimental temperatures. Under the assumption of no significant variation of the enthalpy change (ΔH°) within the range of temperature studied both the enthalpy change (ΔH°) and the entropy change (ΔS°) can be evaluated from the van't Hoff equation:

$$\ln K_b = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{3}$$

here R is the universal gas constant (1.987 cal K^{-1} mol^{-1}), T is the absolute temperature in Kelvin.

The free energy change (ΔG°) of the process is then estimated from the following relationship:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{4}$$

2.3. Circular dichroism (CD) spectropolarimetry

CD spectra were recorded on a Jasco J-815 spectropolarimeter at 37 °C, using a cylindrical cuvette of 0.1 cm path-length. The reported CD profiles are an average of four successive scans obtained at 50 nm min^{-1} scan rate with appropriately corrected baseline. The solutions for baseline correction have been prepared in the same way except that the protein is omitted. In order to further realize the effect of interaction with TP on the secondary structure of the protein the following analysis of the far-UV CD spectral results has been undertaken. The observed CD results are first transformed into mean residue ellipticity (MRE) according to the following equation:

$$\mathrm{MRE} = \frac{\Theta obs(m^\circ)}{10 \times n \times C \times l} \tag{5}$$

where Θ_{obs} is the CD in m°, n is the number of amino acid residues ($n = 585$-1), l is the path length of the cell in cm and C is the molar concentration of HSA (5 μM).

2.4. Molecular docking

The crystal structure of human serum albumin was extracted