Chemico-Biological Interactions 257 (2016) 4–13

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Investigation into the interaction of losartan with human serum albumin and glycated human serum albumin by spectroscopic and molecular dynamics simulation techniques: A comparison study

Farid Moeinpour [a, *], Fatemeh S. Mohseni-Shahri [a], Bizhan Malaekeh-Nikouei [b], Hooriyeh Nassirli [c]

[a] Department of Chemistry, Faculty of Sciences, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, 7915893144, Iran
[b] Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form
25 June 2016
Accepted 24 July 2016
Available online 25 July 2016

Keywords:
Human serum albumin
Glycated human serum albumin
Losartan
Spectroscopy
Molecular dynamics simulation

ABSTRACT

The interaction between losartan and human serum albumin (HSA), as well as its glycated form (gHSA) was studied by multiple spectroscopic techniques and molecular dynamics simulation under physiological conditions. The binding information, including the binding constants, effective quenching constant and number of binding sites showed that the binding partiality of losartan to HSA was higher than to gHSA. The findings of three-dimensional fluorescence spectra demonstrated that the binding of losartan to HSA and gHSA would alter the protein conformation. The distances between Trp residue and the binding sites of the drug were evaluated on the basis of the Förster theory, and it was indicated that non-radiative energy transfer from HSA and gHSA to the losartan happened with a high possibility. According to molecular dynamics simulation, the protein secondary and tertiary structure changes were compared in HSA and gHSA for clarifying the obtained results.

1. Introduction

Human serum albumin (HSA) is the most important plasma proteins and supplies about 80% of the colloid osmotic pressure of blood [1,2]. HSA also acts as a main depot and transport protein, serves in the transportation and distribution of a variety of substances such as fatty acids, amino acids, hormones, and pharmaceuticals. A large portion of total serum antioxidant properties can be ascribed to albumin. X-ray crystallographic analysis of HSA revealed that it is a heart-shaped globular protein comprising 585 amino acid residues in a single polypeptide chain stabilized by 17disulfide bridges [3]. It consists of three homologous domains (I–III), each of them can be divided into two subdomains (A and B). There is an exclusive tryptophan residue placed in subdomain IIA (Trp-214), which offers an advantage to investigate the ligand binding process. Two high-affinity binding sites, namely Sudlow's sites I and II, are placed within specialized hydrophobic cavities in subdomains IIA and IIIA, respectively [4]. To understand the pharmacokinetics and pharmacodynamics of drugs, the comprehension of interaction mechanisms between drugs and serum albumin are very significant. The drugs–protein interaction plays an important role in the bioavailability of drugs because the bound fraction of drugs is a storeroom, whereas the free concentration of drugs shows pharmacological effects [5]. Furthermore, if drugs have high protein binding, it may increase the half-life of drugs in vivo and lead to undesirable side effects. On the other hand, if drugs are metabolized and excreted from the body too fast because of low protein binding, drugs won't be able to provide their therapeutic effects [6]. Furthermore, very high affinity binding of drugs to serum albumin may interrupt drugs from reaching the target and resulting in insufficient tissue efficacy. In other words, the pharmacodynamics of drugs can be significantly affected as a result of their binding to HSA.

Diabetes mellitus, specified by damaged blood sugar regulation, exists in two forms: type I and type II. Type I happens when the production of insulin is compromised because of pancreatic beta cell desolation. Type II happens for 90% of all cases of diabetes and happens when insulin receptors become resistant to insulin,

* Corresponding author. Tel.: +987633665500; fax: +987633670248.
E-mail addresses: f.moeinpour@gmail.com, fmoeionpour52@iauba.ac.ir (F. Moeinpour).

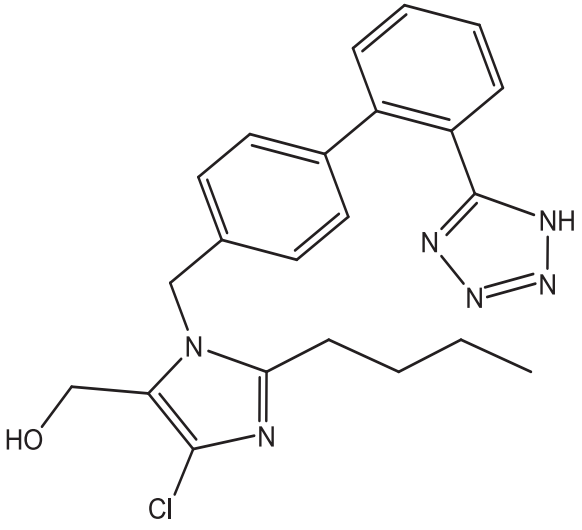
http://dx.doi.org/10.1016/j.cbi.2016.07.025
0009-2797/

requesting a high insulin discharge level, which overloads the pancreas until it be weakened [7]. It is believed one process that to affect the drugs binding to HSA is glycation, which refers to the modification of a protein by a process that starts with their action between a reducing sugar and a free NH_2 group on a protein. This action can happen for human serum albumin and becomes more enounced in diabetes when an enhanced amount of glucose is available in the blood stream. Despite, an average individual has 6–13% of HSA in glycated form; a person with diabetes may have 20–30% or more glycated HSA in the blood stream [7–10]. This action includes the addition of reducing sugars or their degradation products to free primary and secondary amine groups on proteins [11]. So, for diabetic patients in addition of HSA, glycated human serum albumin (gHSA) also is able to bind and deliver some drugs such as losartan. Mendez et al. reported that the glycation altered the local structure around Trp-214 while not significantly impacting the secondary structure, and this alteration translated into an overall change in the stability of gHSA compared to HSA [12].

Losartan(2-Butyl-4-chloro-1-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]-methyl]-1H-imidazole-5-methanol; Scheme 1) is used for the hypertension-therapy, and in earlier studies in patients with symptomatic heart damage, oral losartan produced useful haemodynamic effects both acutely and with chronic dosing [13]. Generally, the total dispersion, metabolism, and influence of many drugs can be changed on the basis of their dependency to plasma proteins, especially HSA [14].

Main methods to study protein–ligand interactions are principal hydrodynamic and spectroscopic and structural methods. In spite of having many applications, experimental techniques have certain limitations. For example, spectroscopic techniques are also limited to those conditions where the ligand binds to chromophores or to the sites that have an effect on the chromophores. Also, there would be no easily available way to estimate the contribution made by structural changes, which in most cases is associated with ligand-binding processes [15]. Using computational approaches to investigate and analyze the binding interactions of proteins with ligands can provide beneficial understandings for drug design [16,17]. Molecular dynamics (MD) simulations, first developed in the late 1970s [18], seek to reduce the computational complexity. Molecular dynamics simulations often play significant roles in drug discovery. The static models produced by NMR, X-ray crystallography provide precious insights into macromolecular structure, but molecular identification and ligand binding are very dynamic processes. Therefore, we have used experimental and theoretical methods to investigate protein-drug interaction.

In this work, the interaction between losartan with HSA and gHSA was investigated by multi-spectroscopic methods and molecular dynamics simulation. The aim of the present study was to investigate the interaction between losartan with HSA and gHSA in order to compare the two forms with regard to binding affinity and the effect of losartan on the conformation of HSA and gHSA. Because there are different binding properties in the interaction between drugs with HSA as compare to gHSA, thus each of them interact with the special concentration of drugs and determining the drug dosage should be different. Therefore, our work will provide some important information for drug usage doses [19].



Scheme 1. The chemical structure of losartan.

2. Materials and methods

2.1. Materials

Fatty acid free HSA (97–99% lyophilized powder) were purchased from Sigma - Aldrich® Chemical Co. (St. Louis, USA). Losartan (≥98.5%, in pure substance form) was provided by Darou Pakhsh Pharma. Chem. Co. All chemical compounds were analytical grade. HSA and losartan were dissolved in a potassium phosphate buffer solution (50 mM, pH = 7.4) at concentrations of 7.5×10^{-3} mM and 8.6×10^{-2} mM, respectively, for HSA and losartan.

Concentration of HSA solution was determined by UV absorbance study using known molar absorption coefficient value of 36,500 M^{-1} cm^{-1} at 280 nm [20] and concentration of losartan was calculated by using a molar absorption coefficient value of 2681 M^{-1} cm^{-1} at 232 nm [21]. gHSA sample preparation was performed in 50 mM phosphate buffer, pH 7.4, by addition of 1 ml D-glucose (100 mM) to 1 ml of HSA (80 mg ml^{-1}). Then the solution was incubated under sterilization for six days at 37 °C, and centrifuged for 1 h, at 4 °C, rotation 8000 rps. Then, the solution was dialyzed three times, each time during 8 h [9]. All solutions were stored in a refrigerator at 4 °C in the dark. The extent of glycation was estimated by the thiobarbituric acid reaction to be 0.35 mM 5-hydroxymethylfurfural equivalents (approx. 1 mol 5-hydroxymethylfurfural/mol HSA) [22].

2.2. Methods

2.2.1. Fluorescence spectroscopy

Fluorescence spectra and intensities were recorded on a FP-6200 spectrofluorometer (Jasco, Japan) equipped with a 1-cm quartz cell and a xenon lamp. The widths of both the excitation and emission slits were set to 5 nm. A 2.5 ml solution containing an appropriate concentration of HSA and gHSA (7.5×10^{-3} mM) was titrated manually by successive addition of stock solution of losartan to attain a series of final concentrations (0–9.62×10^{-3} mM) with trace syringes. The excitation wavelength was 280 nm, and the emission wavelength was 300–500 nm.

2.2.2. Synchronous spectroscopy

Synchronous fluorescence spectra were obtained by simultaneously scanning the emission and excitation monochromators. The synchronous fluorescence spectra showed the Tyr and Trp residues of HSA only when the wavelength interval was 15 and 60 nm, respectively. A 2.5 ml solution containing 7.5×10^{-3} mM of protein was injected by successive additions of losartan to a maximum concentration of 9.62×10^{-3} mM.