Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 153 (2016) 612–618

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Binding properties of drospirenone with human serum albumin and lysozyme in vitro

Qing Wang, Xiangling Ma, Jiawei He, Qiaomei Sun, Yuanzhi Li, Hui Li *

College of Chemical Engineering, Sichuan University, Chengdu 610065, Sichuan, China

ARTICLE INFO

Article history:
Received 25 October 2014
Received in revised form 28 July 2015
Accepted 26 September 2015
Available online 30 September 2015

Keywords:
Drospirenone
Human serum albumin (HSA)
Lysozyme (LYZ)
Main binding site
Molecular modeling

ABSTRACT

The interaction of drospirenone (DP) with human serum albumin (HSA)/lysozyme (LYZ) was investigated using different optical techniques and molecular models. Results from the emission and time resolved fluorescence studies revealed that HSA/LYZ emission quenching with DP was initiated by static quenching mechanism. The LYZ–DP system was more easily influenced by temperature than the HSA–DP system. Displacement experiments demonstrated that the DP binding site was mainly located in site 1 of HSA. Based on the docking methods, DP was mainly bound in the active site hinge region where Trp-62 and Trp-63 are located. Conformation study showed that DP had different effects on the local conformation of HSA and LYZ molecules.

1. Introduction

Drospirenone (DP, Fig. 1) is an analogue of spironolactone, which is an aldosterone antagonist. DP is a unique novel progestogen with antimineralocorticoid and anti-androgenic characteristics [1,2]. Drospirenone can counteract the estrogen-induced stimulation of the renin–angiotensin–aldosterone system and block testosterone from binding to androgen receptors [3]. DP may reduce body weight, blood pressure, and low-density lipoprotein levels, as well as enhance the high-density lipoprotein levels [4].

Human serum albumin (HSA), the most abundant protein in blood plasma, binds and transports several exogenous and endogenous substances and serves a key function in several biologic systems and processes [5,6]. HSA has been a research focus in the pharmaceutical industry because of its ability to bind various drug molecules and alter their pharmacokinetic properties [7]. HSA has been widely used as a model protein for the study of ligand interaction principles [8]. Drug distribution is mainly controlled by HSA because most drugs travel in plasma and reach target tissues by binding to protein [9]. Lysozyme (LYZ) is pervasive in various body fluids, such as tears, saliva, mucus, urine, lymphatic tissues, human milk, and cells of the innate immune system [10]. This protein with high natural abundance is an enzyme known for its unique ability to damage bacterial cell wall, thereby providing protection against bacterial infections [11]. When diverse endogenous and exogenous ligands enter the human body, ligand–LYZ conjugation can be observed within the recursion to restrain precocious systemic effects regardless of the effects of the free ligand [12]. LYZ is also a commonly used protein model because of its natural abundance, high stability, and small size [13]. Therefore, HSA and LYZ are selected to investigate DP binding characteristics, which are critical in understanding possible delivery, consequent availability, and relevant health risks of this drug.

In the current study, fluorescence spectrometry was used to investigate DP interaction with HSA and chicken egg white LYZ at different temperatures. Displacement experiments were performed to establish the main binding sites of HSA for DP. Based on the displacement experiments results, the CDOCKER program, a molecular dynamics (MD) simulated-annealing-based algorithm, was used to specify DP placement in the active site using a binding site sphere. This program can determine whether DP binds to the site. However, the CDOCKER program does not require prior knowledge of the binding site. Currently, no specific probe has been reported for LYZ. The CDOCKER program was selected to identify the main binding sites of LYZ for DP. Drug binding to a protein may affect the conformation and the stability of the protein. Thus, 3D fluorescence and circular dichroism (CD) were used to investigate whether the local conformation of HSA/LYZ molecule was affected by DP.

2. Materials and methods

2.1. Materials

Fatty acid-free HSA was purchased from Sigma Aldrich (USA) and used directly. LYZ from chicken egg white was purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). The stock solutions (20.0 μM) prepared in Tris–HCl buffer at pH 7.40 and containing 0.10 M NaCl. DP, warfarin, and ibuprofen were purchased from J&K

* Corresponding author.
E-mail address: lihuilab@sina.com (H. Li).

http://dx.doi.org/10.1016/j.saa.2015.09.017
1386-1425/

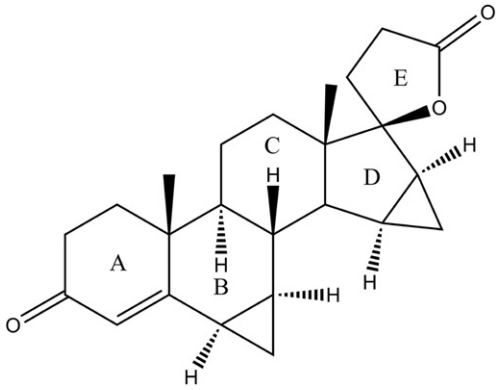

Fig. 1. Drospirenone (DP) molecular structure.

Scientific Ltd. (Beijing, China). These drugs were dissolved in anhydrous ethanol to obtain a 2.0×10^{-4} M stock solution. All stock solutions were stored at 0 °C to 4 °C.

To evaluate the effect of ethanol on HSA and LYZ conformation and fluorescence quenching, 2.0 mL 1.0 μM HSA and LYZ were titrated with 0.1% to 1.0% (V/V) ethanol while its UV–vis and fluorescence spectra were being monitored. No changes were observed in the spectral profiles (data not shown), indicating that HSA and LYZ conformation remain unchanged in the presence of 0.1% to 1.0% (V/V) ethanol. In addition, this observation suggested that the effects of ethanol on the structural changes in HSA and LYZ were negligible.

2.2. Fluorescence experiments

Fluorescence measurements were conducted on a Cary Eclipse fluorescence spectrophotometer (Varian, U.S.) equipped with 1.0 cm quartz cells. Both HSA and LYZ concentrations were kept at 2.0 μM, and DP concentration varied from 0 μM to 6.0 μM with a 1.0 μM gradient growth based on preliminary experiments. Fluorescence spectra were measured using 10/5 nm (excitation/emission) slit widths. The excitation wavelength was fixed at 280 nm, and the fluorescence spectra were recorded in the 300 nm to 500 nm range at 298, 304, and 310 K.

Displacement experiments were performed using the site probes, warfarin and ibuprofen. HSA/LYZ and DP concentrations were stabilized at 2.0 μM. Warfarin and ibuprofen were then gradually added to the DP–HSA/LYZ binary mixture. An excitation wavelength of 280 nm was selected, and the fluorescence spectra were recorded in the range of 300 nm to 500 nm. Every spectrum was recorded as the mean of three scans; data were corrected for the buffer solution signal.

Fluorescence lifetime measurements were executed using a Horiba Jobin Yvon FluoroMax-4 spectrofluorometer (HORIBA, FRA). The time-resolved HSA/LYZ fluorescence quenching by the drug was recorded by fixing 280 nm as the excitation wavelength and 338 nm as the emission wavelength. The HSA/LYZ concentration was fixed at 2.0×10^{-6} mol·L^{-1}, and DP concentration was varied from 2.0×10^{-6} mol·L^{-1} to 6.0×10^{-6} mol·L^{-1} at room temperature.

The 3D fluorescence spectra of HSA/LYZ (2.0×10^{-6} mol·L^{-1}) and DP–HSA/LYZ complex (molar ratio, 4:1) were recorded at a 200 nm to 400 nm excitation wavelength range at 5 nm increments. Emission spectra were also monitored between 200 and 500 nm.

In the present study, the fluorescence intensities were corrected for the absorption of excited light and the re-absorption of emitted light. The following relationship was used to correct the inner-filter effect:

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

where F_{corr} and F_{obs} are the corrected and observed fluorescence intensities, respectively, and A_{ex} and A_{em} are the absorption of the system at the excitation and emission wavelengths, respectively.

2.3. CD spectrum measurements

CD spectra were recorded on a CD spectrometer (Model 400, AVIV, USA). The spectra were obtained at 298 K in a 0.2 cm path-length quartz cell from 250 to 200 with a 1 nm step size, 1 nm band width, and 0.5 s averaging time. An average of three scans was obtained for all spectra.

2.4. Molecular modeling preparation

The CDOCKER docking program implemented in Discovery Studio 3.1 (DS 3.1) was used in the present study. DS 3.1 (Accelrys Co., Ltd., U.S.) was provided by the State Key Laboratory of Biotherapy (Sichuan University, China). The HSA (PDB ID: 1H9Z) and LYZ (PDB ID: 2LYZ) crystal structures were obtained from a protein data bank for docking simulations. Water and other small molecules were removed during protein preparation. In the protein structure, missing bond orders, hybridization states, charges, and angles were assigned; moreover, explicit hydrogen was added with a pH of 7.40. The protein structure energy was minimized using 200 steps of the smart minimize method [14,15]. For ligand preparation, the DP 3D structure was generated using ChemBioOffice 2010, optimized with DS 3.1, and subjected to CHARMm force field simulation before docking [16].

3. Results and discussion

3.1. Fluorescence-quenching measurements

In HSA, intrinsic fluorescence was caused by tryptophan (Trp) and tyrosine (Tyr) because phenylalanine has a very low quantum yield [17,18]. However, the Tyr emission wavelength was not usually found at approximately 304 nm to 310 nm because its fluorescence was almost completely quenched by Trp, which was caused by the efficient energy transfer from Tyr to Trp (i.e., the internal quenching effect) [19]. In LYZ, Trp-62 and Trp-63 are responsible for most of its intrinsic fluorescence [20,21]. The effect of DP on HSA/LYZ and the resulting conformational change in HSA/LYZ was evaluated by measuring the intrinsic fluorescence intensity of the protein before and after DP addition. The fluorescence intensity significantly decreased when HSA/LYZ concentration was fixed at 2.0×10^{-6} M at room temperature after the addition of DP (Fig. 2). This finding suggested that the interaction occurred between DP and the protein; in addition, this observation revealed that the non-fluorescent complex was formed. In addition, DP quenched LYZ higher than HSA. A slight blue shift of the maximum emission peak was also found in the HSA/LYZ emission spectra when the DP concentration was increased continuously, which suggested that Trp was located in a more hydrophobic environment.

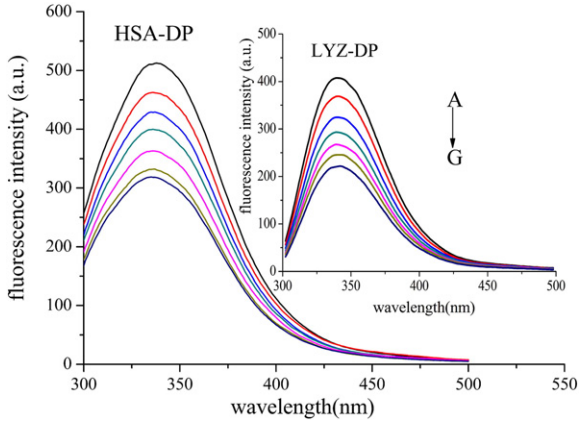

Fig. 2. HSA and LYZ fluorescence spectra (inset): (A) c(HSA) = c(LYZ) = 2.0 μM, (B–G) c(DP) = 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 μM.