International Journal of Biological Macromolecules 92 (2016) 593–599

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Short communication

Affinity of miriplatin to human serum albumin and its effect on protein structure and stability

Hongmei Zhang [1], Peirong Wu [1], Yanqing Wang*, Jian Cao

Institute of Applied Chemistry and Environmental Engineering, Yancheng Teachers University, Yancheng City, Jiangsu Province, 224002, People's Republic of China

ARTICLE INFO

Article history:
Received 14 May 2016
Received in revised form 16 July 2016
Accepted 21 July 2016
Available online 22 July 2016

Keywords:
Miriplatin
Human serum albumin
Protein stability

ABSTRACT

In this report, circular dichroism (CD) along with steady-state fluorescence spectroscopy and molecular modeling investigations were carried out to better understand the interaction of miriplatin with human serum albumin (HSA). The presence of miriplatin in solution is found to destabilize the native structure of HSA: The tertiary structure of HSA was changed and the microenvironment of Trp residue became more hydrophobic; the binding affinity of HSA with miriplatin indicating by 8-Anilino-1-naphthalenesulfonic acid (ANS) fluorescence study was 1.74×10^6 L/mol; miriplatin induced the denaturation and unfolding of HSA and disrupted the polar contacts and decreasing the reversibility of the unfolding process of protein. In addition, molecular modeling studies indicated miriplatin bound to domain II of HSA by hydrophobic force, hydrogen bonds, and electrostatic force interactions. HSA retained most of its esterase activity even after its binding with miriplatin. These results provide valuable insight into the binding mechanism between miriplatin and a plasma protein that is known to play an important role in the drug delivery of medicinal drugs to target organs.

1. Introduction

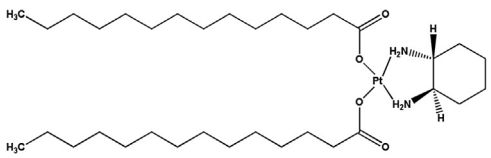
Platinum-based drugs, such as cisplatin, carboplatin and oxaliplatin play a prominent role in the treatment of human and animal cancers [1]. However, since each of these platinum drugs do have severe toxic side effects [2,3], novel platinum-based drugs are being developed which may offer reduced toxicity and improved therapeutic efficacy [4–6]. For example, miriplatin (Scheme 1), an analog of oxaliplatin, contains myristates as leaving groups and diaminocyclohexane as a carrier ligand [7]. It was approved in Japan to treat hepatocellular carcinoma by transcatheter arterial chemoembolization [8]. As a new platinum-based drug, the binding interaction of miriplatin with the major carrier protein of the circulatory system has not been reported to date.

Human serum albumin (HSA), the most important and the major protein in human blood, has been widely used as drug delivery model protein for many drugs [9–15]. The interaction between platinum-based drugs and serum albumin is important on the basis of both drug delivery and possible development of acquires resistance/side effects [16]. For example, cisplatin is administered intravenously, 65–98% of the drug is bound to blood plasma proteins, particularly albumin [17]. Protein interaction with cisplatin studies indicate that cisplatin is believed to be therapeutically active only when it is not bound to proteins [17]. In addition, the HSA conformation and stability was also affected after the binding of cisplatin derived hydrolysis products [18]. This included the loss of helical content, the cleaved S—S bonds, the formation of a molten globule-like state, and the aggregation of albumin [19,20]. Therefore, binding of novel platinum-based drugs or their hydrolysis products to HSA as well as the effect of this binding on the tertiary structure of HSA is of considerable pharmacological relevance. However, as far as we know, the interaction of miriplatin with HSA has not been reported until now.

Herein, we report on the results of studies to delineate the interaction between miriplatin and HSA under near physiological conditions. Experimental studies were conducted to assess the binding affinity as well as the thermal stability of miriplatin-HSA complex. In addition, studies using 8-Anilino-1-naphthalenesulfonic acid (ANS) that the binding sites in albumin molecule have been well defined may be helpful in more accurate identification of miriplatin binding with HSA. This research may provide valuable information to the growing concerns regarding the drug delivery and drug design procedures of new platinum-based drugs.

* Corresponding author.
E-mail address: wyqing76@126.com (Y. Wang).
[1] Hongmei Zhang and Peirong Wu contributed equally.

http://dx.doi.org/10.1016/j.ijbiomac.2016.07.073
0141-8130/



Scheme 1. The molecular structure of miriplatin.

2. Material and methods

2.1. Reagents

Miriplatin (≥98%) was obtained from Sunlidabio Corporation (Nanjing city, China). HSA (≥96%, agarose gel electrophoresis) was obtained from Sigma–Aldrich Company (St. Louis, MO). Analytical standard of ANS (≥98%) was obtained from Aladdin Industrial Corporation (Kaplan Ave, City of Industry). All other chemicals used were of analytical purity or higher. HSA solution was prepared in pH 7.40 phosphate buffer (0.05 mol/L). Water was purified with a Milli-Q purification system to a specific resistance >18.2 MΩ/cm.

2.2. Methods

2.2.1. Fluorescence experimental studies

LS–50B Spectrofluorimeter (Waltham, Massachusetts, USA) equipped with 1.0 cm quartz cells and a thermostat bath was used to detect the fluorescence quenching of HSA induced by miriplatin. HSA concentration was kept constant at 1.0×10^{-6} mol/L and miriplatin concentrations have been varied from 0 to 40.0×10^{-6} mol/L. All steady fluorescence spectra were measured with the excitation at 295 nm and the emission wavelengths at 300–500 nm with 5.0 nm/5.0 nm slit widths. In addition, ANS was measured by exciting at 370 nm and emission wavelength at 400–600 nm with 10.0 nm/10.0 nm slit widths.

2.2.2. CD experiments

The CD spectra were measured by a Chirascan spectrometer (Applied Photophyysics Ltd., Leatherhead, Surrey, UK). For the experiments, the 1.0×10^{-6} mol/L HSA solution in the presence and absence of miriplatin were recorded from 200 to 320 nm with three scans averaged and scanning speed was set at 30 nm/min for each CD spectrum. The program CD spectra deconvolution program (CDNN) was used to analyze CD spectra [http://bioinformatik.biochemtech.uni-halle.de/cdnn]. The thermal denaturation of HSA in the absence and presence of miriplatin was varied from 20 to 90 °C in 5 °C steps, with 240 s increments.

2.2.3. Esterase activity measurement

The UV–vis absorption measurements were carried out on a SPECORD S600 spectrophotometer (Jena, Germany) to analyze the influence of miriplatin on the esterase activity of HSA that was examined with the *p*-nitrophenyl acetate by following the formation of *p*-nitrophenyl at 400 nm (λ_{abs} = 405 nm, ε = 17,700 L/mol/cm) [17]. The reaction mixtures contained 1.0×10^{-5} mol/L HSA and 4.0×10^{-5} mol/L *p*-nitrophenyl acetate in the absence and presence of miriplatin at pH = 7.4 and T = 310 K.

2.2.4. Molecular modeling study

In this work, Density functional theory (DFT) calculations with B3LYP/LanL2DZ base were used to obtain the optimized geometrical structure of miriplatin in Gaussian 09 [21]. The structure of HSA (PDB ID 1E78) was taken from RCSB Protein Data Bank [22]. Autodock 4.2.3 program was used to perform a computational investigation on the interaction of miriplatin with HSA [23]. According to the molecular structure of HSA, the grid boxes with 126 Å × 126 Å × 126 Å size were selected in case of blind docking search spaces covering the whole protein to cover miriplatin molecule. In addition, the GA population size, the maximum number of energy evaluation, the number of GA runs were set at 100, 2,500,000, and 100, respectively. Others used were default parameters. When the docking calculation finished, Molegro Molecular Viewer software (Molegro-a CLC bio company, Aarhus, Denmark) was used to analysis the predicated binding mode [24].

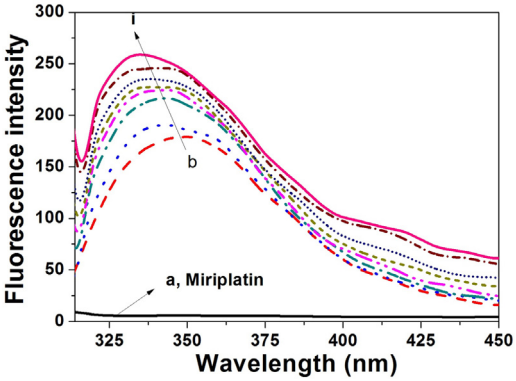


Fig. 1. The fluorescence spectra of miriplatin and HSA-miriplatin system. Line a, c(miriplatin) = 20.0×10^{-6} mol/L, from line b to I, c (HSA) = 1.0×10^{-6} mol/L, c (miriplatin) = 0.0×10^{-6} mol/L, 4.0×10^{-6} mol/L, 8.0×10^{-6} mol/L, 12.0×10^{-6} mol/L, 16.0×10^{-6} mol/L, 20.0×10^{-6} mol/L, 24.0×10^{-6} mol/L, and 28.0×10^{-6} mol/L, respectively. pH = 7.40, T = 298 K, λ_{ex} = 295 nm.

3. Results and discussion

3.1. Trptophan fluorescence enhancing of HSA by miriplatin

In this paper, fluorescence spectral experiment was performed to reveal the effect of miriplatin on the tertiary structures of HSA. HSA has one Trp residue (Trp-214). When excited at 295 nm, the emission spectra of HSA in the absence of miriplatin exhibits an emission maximum at 349 nm mainly coming from Trp residue. As Fig. 1 indicated, miriplatin has not fluorescence emission peak near the wavelength of 349 nm when excited at 295 nm (Fig. 1 line a), it can not cause an obvious enhancement of fluorescence signal of the HSA with increasing of miriplatin concentration. the fluorescence intensity of HSA is increased gradually with increasing of miriplatin concentration. The increase of fluorescence of Trp residues in HSA indicated at the binding site of miriplatin maybe near to Trp-214 which locates in Subdomain IIA of HSA. miriplatin has two hydrophobic carbon chains, which not only are more like to interact with the hydrophobic amino residues in HSA, but also penetrate into the hydrophobic cavity. It is evidence for the presence of Trp in a hydrophobic environment. In addition, the λ_{max} of Trp residue shift to blue wavelength (shifted 15 nm). The blue shift of Trp residue implied that the microenvironment of Trp residue become more hydrophobic. Due to binding miriplatin with HSA, the Trp residue become less accessible for the environment provided by the water molecule. In a word, the tertiary structure of HSA is changed by miriplatin.

3.2. ANS fluorescence studies of HSA in the presence of miriplatin

ANS fluorescence study was performed to obtain a much deeper understanding of these conformational changes induced by miriplatin. ANS is a dye molecule, that is one of the most used organic probes in the analysis of protein by fluorescence method [25–27]. Bound into hydrophobic sites of HSA and surrounded by nonpolar residues, ANS gives high fluorescence intensity. In HSA, the binding sites of ANS locate at subdomain IIA and IIIA, one of