Colloids and Surfaces B: Biointerfaces 84 (2011) 272–279

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Exploring the binding mechanism of dihydropyrimidinones to human serum albumin: Spectroscopic and molecular modeling techniques

Gongke Wang, Dongchao Wang, Xiang Li, Yan Lu*

School of Chemistry and Environmental Science, Key Laboratory of Green Chemical Media and Reactions, Ministry of Education, Henan Normal University, Xinxiang, Henan 453007, PR China

ARTICLE INFO

Article history:
Received 15 November 2010
Accepted 13 January 2011
Available online 20 January 2011

Keywords:
Human serum albumin
Dihydropyrimidinones
Binding mechanism
Fluorescence quenching
UV-absorption spectroscopy
Molecular modeling

ABSTRACT

The binding mechanism of molecular interaction between 5-(ethoxycarbonyl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (a dihydropyrimidinones derivative, EMPD) and human serum albumin (HSA) was studied using spectroscopic methods and modeling technique. The quenching mechanism was investigated in terms of the binding constants and the basic thermodynamic parameters. The results of spectroscopic measurements suggested that EMPD have a strong ability to quench the intrinsic fluorescence of HSA through static quenching procedure. The drug–protein complex was stabilized by hydrophobic forces and hydrogen bonding as indicated from the thermodynamic parameters and synchronous fluorescence spectra, which was consistent with the results of molecular docking and accessible surface area calculation. Competitive experiments indicated that a displacement of warfarin by EMPD, which revealed that the binding site of EMPD to HSA was located at the subdomains IIA. The distance between the donor and the acceptor was 4.85 nm as estimated according to Förster's theory of non-radiation energy transfer. The effect of metal ions on the binding constants was also investigated. The results indicated that the binding constants between EMPD and HSA increased in the presence of common metal ions.

1. Introduction

Dihydropyrimidinones and their derivatives are well known heterocyclic units in the realm of natural and synthetic organic chemistry due to their therapeutic and pharmacological properties. Furthermore, they have attracted considerable interest in recent years because of their medicinally important as calcium channel blockers [1], antihypertensive agents [1], α1a-antagonists [2], mitotic kinesin Eg5 inhibitors [3], melanin concentrating hormone receptor antagonists [4], neuropeptide antagonists [5,6] and strong resistance HIVgp120-CD4 function [7]. Moreover, several alkaloids containing the dihydropyrimidinones as a core unit have been isolated from marine source, which also showed interesting biological properties [8]. As a member of the derivatives of dihydropyrimidinones, EMPD (structure shown in Fig. 1) is regarded as the potential new protein targeted drug, which brings its wide applicability in biochemistry and biomedicine.

Human serum albumin (HSA) is the most abundant plasma protein, which plays key roles in transportation, distribution and metabolism of many endogenous and exogenous ligands [9]. The unique feature of HSA is its ability to bind a wide variety of compounds such as nutritional components of foods, metabolites, drugs, dyes, fatty acids and metal ions [10,11], mainly because of the availability of hydrophobic cavities inside the protein network and the flexibility of the albumins to adapt its shape. Crystallographic analysis revealed that HSA is a single polypeptide chain of 585 amino acid residues with a largely helical triple-domain structure that assemble to form a heart-shaped molecule. The protein contains three homologous α-helix domains (I–III) [12]. Each domain contains 10 α-helices and is divided into six-helix and four-helix subdomains (A and B). Serum albumin has two well-known ligand binding sites, site I and site II, which are corresponded to the subdomains IIA and IIIA, respectively [12]. The remarkable binding properties of HSA account for the central role in both the efficacy and rate of delivery of drugs, so it is used to enucleate the transportation and distribution of drug in the body. The binding of a drug to HSA influences its metabolism, distribution, toxicity and elimination from the circulation. Co-binding of two drugs or displacement of one drug to another may alter therapeutic drug level and can lead to serious health conditions. Thus, the detailed knowledge of the binding interaction of a drug with HSA and of their relative strengths becomes an important research field in chemistry, life science and clinical medicine. Recently, extensive investigations into the interaction between HSA and internal compound or pharma-

* Corresponding author. Tel.: +86 373 3325249; fax: +86 373 3325249.
E-mail addresses: yanlu2001@sohu.com, wanggongke@126.com (Y. Lu).

0927-7765/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2011.01.016

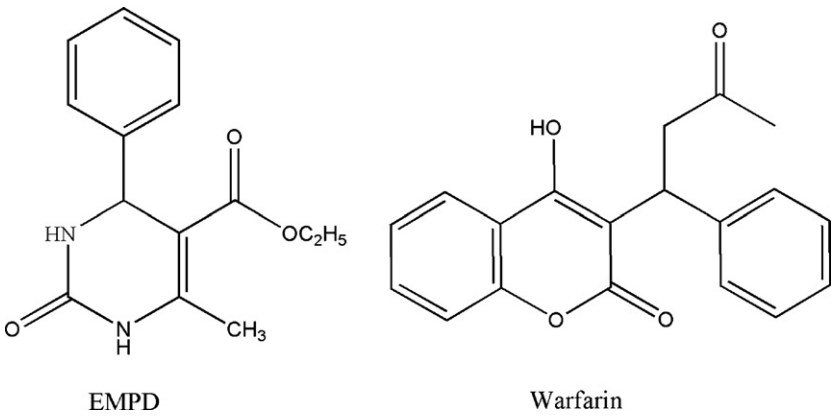

Fig. 1. The chemical structure of EMPD and warfarin.

ceutical molecules have been made [13,14], but interaction of HSA with dihydropyrimidinones and their derivatives has seldom been reported.

The purpose of the present study is to investigate the binding mechanism of EMPD to HSA by fluorescence spectrum, UV-absorption spectrum and molecular modeling techniques. Fluorescence spectroscopy has been widely used to investigate the interaction of drug with protein. After the protein is treated by quenchers, quenching of the protein intrinsic fluorescence can be used to infer the binding mechanism and to calculate the number of binding site, binding constant and binding distance from the tryptophan residues [15]. The molecular interactions are often monitored by spectroscopic techniques [16,17], because these methods are also sensitive and relatively easy to use. They have advantages over conventional approaches such as affinity and size exclusion chromatography, equilibrium dialysis, ultrafiltration and ultracentrifugation, which suffer from lack of sensitivity, long analysis time or both and use of protein concentrations far in excess of the dissociation constant for the drug–protein complex and for drug–protein interaction studies [18–22]. In this work, mechanisms and characters of interaction between EMPD and HSA were investigated systematically by spectroscopic and molecular modeling methods for the first time. We hope that this work will be helpful for realizing the distribution, transportation and metabolism of drugs in vivo, elucidating the binding mechanism, toxicity and dynamics of drugs at the molecular level.

2. Materials and methods

2.1. Materials

HSA (66,000 Da) and warfarin were purchased from Sigma Chemical Company (USA). Tris base was from Beijing Solarbio Science & Technology Co., Ltd. 0.1 M Tris–HCl buffer solution (0.1 M NaCl) was used to keep the pH of the solution at 7.40. The 2.0×10^{-4} M stock solution of HSA was always prepared freshly by dissolving the solid samples in the buffer. The 2.0×10^{-3} M stock solution of EMPD was prepared by dissolving appropriate amount of EMPD in 10 ml ethanol and diluted to the experimental concentration with the buffer. All other chemicals were of analytical grade and used without further purification. Doubly distilled water was used throughout the work and all stock solutions were stored in the dark at 0–4 °C.

2.2. Apparatus and methods

All fluorescence spectra and synchronous fluorescence spectra were performed on a Cary Eclipse fluorescence spectrophotometer (Varian, America), using 5 nm/5 nm slit widths. The UV-absorption spectroscopy was recorded on a TU-1810 UV spectrophotometer (Beijing Puxi Analytic Instruments Co., Ltd, Beijing, China) equipped with 1.0 cm quartz cells. The pH values were measured on a pHS-3C digital pH-meter (Shanghai Yilan Instruments Co., Ltd, Shanghai, China) with a combined glass electrode. All the spectral measurements were thermostatically controlled by a SHP DC-0515 circulating water thermostat (Shanghai Hengping Instruments Co., LTD, Shanghai, China). All calculations were performed on SGI FUEL workstations while carrying out the molecular docking and accessible surface area.

2.3. Synthesis and characterization of EMPD

EMPD was successfully synthesized by the literature procedure [23]. A mixture of benzaldehyde (0.530 g, 5 mM), ethyl acetoacetate (0.975 g, 7.5 mM), urea (0.450 g, 3 mM) and $FeCl_3{\cdot}6H_2O$ (0.337 g, 1.25 mM, 25 mol%) in 6 ml ethanol was refluxed for 6 min under microwave irradiation (200 W). After cooling, the reaction mixture was washed with cold water (2×20 ml) and residue recrystallized from ethyl acetate: n-hexane (1:3) to afford the pure product (1.240 g, 95%). M_p 202–204 °C; ^{1}H NMR (DMSO-d_6): δ_H 9.18 (s, 1H), 7.73 (s, 1H), 7.22–7.34 (m, 5H), 5.14 (d, J = 3.2 Hz, 1H), 3.98 (q, J = 7.0 Hz, 2H), 2.25 (s, 3H), 1.09 (t, J = 7.0 Hz, 3H).

2.4. Measurements of spectrum

The fluorescence quenching of HSA at increasing concentration of EMPD was recorded in the wavelength range 300–500 nm after exciting the protein solution at 280 nm. The HSA concentration was fixed at 2 μM and the drug concentration was varied from 1 to 10 μM. To evaluate the effect of temperature on HSA–EMPD interaction, fluorescence spectrum was recorded at three different temperatures (18, 25 and 37 °C).

For the investigation on the effects of metal ions, NaCl, KCl, $CuCl_2$, $HgCl_2$, $ZnCl_2$, $NiCl_2$, $FeCl_3$, $AlCl_3$ and $CrCl_3$ were used as foreign substances. The anions were the same chloride ions, which did not affect the protein fluorescence. The fluorescence measurements of HSA–EMPD complex were recorded in the absence and presence of various ions in the range of 300–500 nm upon excitation at 280 nm. The concentration of HSA was fixed at 2 μM and that of ions were maintained at 4 μM.

Synchronous fluorescence spectra of HSA in the absence and presence of the increasing amount of EMPD (1–10 μM) were recorded at λ_{em} = 250–400 nm and the constant differences of $\Delta\lambda$ = 60 nm and 15 nm, respectively, were maintained between excitation and emission fluorescence monochromators.