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Interaction of rofecoxib with human serum albumin: Determination of binding constants and the binding site by spectroscopic methods

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Abstract

The interaction of rofecoxib with human serum albumin (HSA) under physiological condition was investigated by fluorescence, UV–vis absorbance and Fourier transfer infrared (FT-IR) spectroscopy. Fluorescence data revealed that the fluorescence quenching of HSA by rofecoxib was the result of the formed complex of HSA–rofecoxib, and the site binding constants (K_a) were 4.840×10^4, 3.450×10^4, and 2.325×10^4 M^{-1} at 298, 304, and 310 K, respectively. The thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) for the reaction were calculated to be -46.90 kJ mol^{-1} and -67.59 J mol^{-1} K^{-1} according to van't Hoff equation. The spectroscopic measurements and the thermodynamic parameters suggested that van der Waals interaction and hydrogen bonds were the predominant intermolecular forces to stabilize the complex. The distance $r = 5.1$ nm between donor (Trp^{214}) and accepter (rofecoxib) was obtained according to the Förster theory of non-radiative energy transfer. FT-IR spectra and UV–vis absorbance showed that the change of protein secondary structures resulted from the rofecoxib binding to several amino acids on the hydrophobic pocket of HSA. Furthermore, it is observed from the probe of competitive experiments that the binding location of rofecoxib with HSA could be the same as the warfarin site I of HSA, which was also revealed by fluorescence anisotropy.

Keywords: Rofecoxib; Human serum albumin; Fluorescence quenching; Site competitive binding

1. Introduction

Serum albumin, as the most abundant protein constituent of blood plasma [1], facilitates the disposition and transportation of varieties of exogenous and endogenous ligands. The protein is capable of binding an extraordinarily broad range of pharmaceuticals, including fatty acids, amino acids, steroids, metal ions, etc. It is also responsible for the maintenance of blood pH [2], the drug disposition and efficacy [3], and the contribution of colloid osmotic blood pressure. Much of the clinical and pharmaceutical interest in the serum albumin derives from its effects on the drug pharmacokinetics [4]. HSA is considered to have at least three specific binding sites for high-affinity binding of drugs, sites I, II and III [5,6] and a single tryptophan (Trp^{214}) in the subdomain IIA. X-ray measurements have revealed that ligands binding to HSA were located in hydrophobic cavities in subdomains IIA and IIIA. It is important to study the interaction of the drugs with HSA owing to the interaction of drugs with HSA influence the drugs' pharmacology and pharmacodynamics.

Rofecoxib(4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5*H*)-furanone), a prescription COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) was proved by FDA in May 1999 for the relief of signs and symptoms of osteoarthritis, for the management of acute pain in adults, and for the treatment of menstrual symptoms, and was later approved for the relief of signs and symptoms of rheumatoid arthritis in adults and children. As its structure shown in Fig. 1, it has been developed for treating acute pain and chronic inflammatory disorders without gastric side effects associated with the use of COX-1 inhibitors [7–9].

It is currently approved for the treatment of acute and chronic symptoms of osteoarthritis, rheumatoid arthritis, acute pain and menstrual pain [10]. Furthermore, rofecoxib can also slow the

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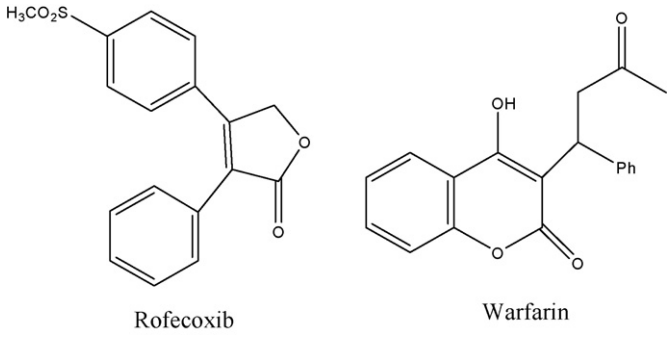

Fig. 1. Molecular structure of rofecoxib and warfarin.

growth of human pancreatic cancer by changing the gene expression that favors cell cyclic arrest [11]. On 30 September 2004, however, Merck & Co., Inc. announced a voluntary withdrawal of rofecoxib (Vioxx) from the U.S. and worldwide market due to the safety concerns of an increasing risk of cardiovascular events (including heart attack and stroke) in patients on rofecoxib [12].

It is widely accepted that the distribution, metabolism, and efficacy of many drugs can be altered based on their affinity to serum albumin. Significantly, the determination and understanding of rofecoxib interacting with serum albumin are important for the therapy and design of the drug [2]. The knowledge of the interaction and binding to HSA is poorly understood but may open new avenues for the design of the most suitable rofecoxib derivatives without side-effects. Investigating the influence of the drug on protein not only provide the pharmacological action of rofecoxib, but also can illuminate its binding mechanisms.

In this paper, we have studied the interaction of rofecoxib with human serum albumin (HSA) at three temperatures (298, 304, and 310 K) under physiological condition. Spectroscopic data were used to quantify the binding constants of rofecoxib to HSA and the action distance which was based on the Förster energy transference (FET). UV–vis and FT-IR spectroscopy revealed that the change of protein secondary structure resulted from the rofecoxib binding to several amino acids on the hydrophobic pocket of HSA. What is more, the interaction of the mainly acting forces and the binding site of the location were characterized by optical spectroscopy.

2. Materials and methods

2.1. Materials and solutions

Human serum albumin (HSA, fatty acid free), purchased from Sigma Chemical Company, was used without further purification. Tris–Base had a purity of no less than 99.5%, and NaCl, HCl, etc., were all of analytical purity. HSA was dissolved in Tris–HCl buffer solution (50mM Tris–Base, 100mM NaCl, pH 7.4 ± 0.1). Rofecoxib was obtained from USA of Merck & Co., Inc., prepared by absolute dimethyl sulfoxide (DMSO) to form 5 mM solution, and the DMSO in the solution of HSA was at a concentration of 0.1–1.5% (v/v). Warfarin was obtained from Medicine Co. Ltd., Jiangshu (China), prepared by the Tris–HCl buffer (pH 7.4 ± 0.1) to form 5 mM solution. All other solutions were also prepared in a Tris–HCl buffer (pH 7.4 ± 0.1).

To evaluate the effect of DMSO on the conformation of HSA and fluorescence quenching, a 2.0 mL of 10 μM HSA was titrated by DMSO which was in the range of concentration 0.1–1.5% (v/v), and the spectra of UV–vis absorbance and fluorescence quenching was studied. There was no change observed in the spectral profiles (data not shown), which suggests no change in HSA conformation and can be considered negligible in the amount used [13].

2.2. Fluorescence measurements

Fluorescence spectra were measured with a F-2500 Spectrofluorimeter (Hitachi, Japan) equipped with a 1.0 cm quartz cell and a thermostat bath. The excitation wavelength was 295 nm, and the emission spectra was read at 300–450 nm, using 2.5 nm/2.5 nm slit widths. To quantify the binding constants of rofecoxib to HSA, a 2.0 mL solution containing 10 μM HSA was titrated by successive additions of rofecoxib solution using trace syringes (to give a concentration ranging from 0 to 50 μM), and the fluorescence intensity was measured ($\lambda_{ex} = 295$ nm). All experiments were measured at each temperature (298, 304, and 310 K) with recycle water keeping the temperature constant. The appropriate blanks corresponding to the buffer were subtracted to correct background of fluorescence. The results obtained were analyzed by using the Stern–Volmer equation or modified Stern–Volmer equation to calculate binding constants.

2.3. UV absorbance measurement

UV–vis absorption spectra of 10 μM of free rofecoxib in buffer solution (pH 7.4 ± 0.1, 0.2% of DMSO), and as well as the UV–vis absorption spectra of rofecoxib/HSA varieties of molar ratio complexes were recorded on a TU-1901 UV–vis spectrometer (Puxi Analytic Instrument Ltd. of Beijing, China) from 220 to 400 nm. To accomplish this, a 2.0 mL solution of 10 μM HSA was titrated by successive additions of rofecoxib solution.

2.4. FT-IR measurements

FT-IR was measured on a Perkin-Elmer spectrometer equipped with a MCT-B detector. Solution spectra were taken, using CaF_2 windows with resolution of 2 cm^{-1} and 400 scans. HSA was prepared using a deuterated water to prevent the interference of hydrogen bonds in the amide region of the protein spectra. The amount of DMSO (0.5–1.0%) used as solvent in the HSA solution in the spectra of IR has not absorbance in the range 1600–1700 cm^{-1}. The subtraction of D_2O from the protein solution was carried out as Ref. [14].

2.5. Fluorescence anisotropy measurement

The steady state anisotropy, however, gives information about the rotational rate of the solute molecule. Upon excitation with vertically polarized light the emitted fluorescent light is