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Investigation on the protein-binding properties of icotinib by spectroscopic and molecular modeling method

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ABSTRACT

Icotinib is a highly-selective epidermal growth factor receptor tyrosine kinase inhibitor with preclinical and clinical activity in non-small cell lung cancer, which has been developed as a new targeted anti-tumor drug in China. In this work, the interaction of icotinib and human serum albumin (HSA) were studied by three-dimensional fluorescence spectra, ultraviolet spectra, circular dichroism (CD) spectra, molecular probe and molecular modeling methods. The results showed that icotinib binds to Sudlow's site I in subdomain IIA of HSA molecule, resulting in icotinib-HSA complexes formed at ground state. The number of binding sites, equilibrium constants, and thermodynamic parameters of the reaction were calculated at different temperatures. The negative enthalpy change (ΔH^{θ}) and entropy change (ΔS^{θ}) indicated that the structure of new complexes was stabilized by hydrogen bonds and van der Waals power. The distance between donor and acceptor was calculated according to Förster's non-radiation resonance energy transfer theory. The structural changes of HSA caused by icotinib binding were detected by synchronous spectra and circular dichroism (CD) spectra. Molecular modeling method was employed to unfold full details of the interaction at molecular level, most of which could be supported by experimental results. The study analyzed the probability that serum albumins act as carriers for this new anticarcinogen and provided fundamental information on the process of delivering icotinib to its target tissues, which might be helpful in understanding the mechanism of icotinib in cancer therapy.

1. Introduction

Icotinib (ICTN; formula: $C_{22}H_{21}N_3O_4$; CAS registry number: 1204313-51-8), belongs to first generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, whose chemical name is 4-((3-ethynylphenyl)amino)-6,7-benzo-12-crown-4-quinazoline. As a quinazoline derivative, icotinib can bind reversibly to ATP's binding site on EGFR protein and thus prevent completion of the signal transduction cascade [1]. The molecular structure of icotinib is constructed by modification on that of erlotinib. With similar structure, mechanism of action and curative effect to erlotinib, icotinib is of superior safety and tolerance. Icotinib was approved in China by the China Food and Drug Administration in June, 2011. It was firstly indicated for the treatment for EGFR mutation-positive, advanced or metastatic non-small cell lung cancer [2,3]. More than 40,000 patients in China have been treated successfully by icotinib since its approval in 2011. In American, Beta Pharma, Inc. has also been granted by the US FDA in 2014, to conduct a phase I study which aimed at evaluation of icotinib as a treatment for EGFR mutation-positive Non-Small Cell Lung Cancer.

One of the significant factors to be considered on the pharmaceutical action of drugs is their binding tendency to plasma proteins [4]. As the most abundant proteins in the circulatory system (0.63 mmol·L^{-1}, 60% of the total serum protein level) [5], human serum albumin (HSA) can bind with a wide variety of molecules, acting as a transport vehicle [6]. It is an important tool in the development of novel therapeutic agents as drug-protein binding can affect pharmacokinetics as well as pharmacodynamics of drugs [7,8].

The purpose of this paper was to investigate the binding reaction of icotinib with HSA under simulated physiological conditions (ionic strength = 0.1 mol·L^{-1}; pH 7.40) by multiple spectroscopy methods, to determine the binding constant, number of binding sites, binding location, nature of binding forces and HSA's structural changes, and to model the molecule structure according to experimental data.

2. Experimental

2.1. Materials

HSA was purchased from Sigma (USA, 99%). warfarin and ibuprofen were obtained from Hubei Biocause Pharmaceutical Co., Ltd. (Hubei, China) with the purity no less than 99.7%. Tris, HCl, and NaCl were purchased from Shanghai Chemical Reagent Company (China). Icotinib was obtained from Betta pharmaceuticals Co., ltd (Zhejiang, China) and had a purity of no less than 99.5%. All other chemicals were of analytical grade. Stock solutions of HSA (10^{-5} mol·L^{-1}), ICTN (10^{-4} mol·L^{-1}),

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NaCl (0.5 mol·L^{-1}) and Tris–HCl buffer (0.05 mol·L^{-1} Tris, 0.15 mol·L^{-1} HCl) of pH 7.40 were prepared by directly dissolving the original reagents. Water used to prepare solutions was double-distilled.

2.2. Instrumental methods

All fluorescence spectra were recorded on F-4600 Fluorescence spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. To obtain smooth emission spectra with moderate intensity, the widths of excitation slit and emission slit were both set at 5 nm. The scanning speed was set at 2400 nm per minute. An excitation wavelength of 275 nm was chosen and appropriate blanks corresponding to the buffer were subtracted to correct the background. Circular dichroism (CD) measurements were performed at 311 K on a J-810 Spectropolarimeter (Jasco, Japan) equipped with 1.0 cm quartz cells over a wavelength range of 260–200 nm and under constant nitrogen flush at a scanning speed of 200 nm/min. The pH measurements were made with a pHS-3 digital pH-meter (Shanghai, China). All weight measurements were performed with an AY-120 electronic analytic weighing scale (Shimadzu, Japan).

2.3. Molecular modeling

Molecular modeling calculations were carried out using Sybyl8.1. The crystal structure of HSA was from Brookhaven Proteins Data Bank (PDB) database (entry codes: 1h9z for site I, 2bxg for site II). All ligands and water molecules were removed before the analysis. H atoms were added and the biopolymer was charged using AMBER7 FF99 method. The site of the protein was defined with ligand (A/WRR2001 for site I, A/IBP2001 for site II); the principal regions of ligand binding to HSA were analyzed with Sybyl 8.1 software. The structure of ICTN was generated with sybyl8.1 package and the molecule was optimized (energy minimization) using Tripos Force Field after being charged with Gasteiger and Marsili method. The docking mode of ICTN with HSA was conducted by a Surflex-Dock program in Sybyl 8.1 package.

3. Results and discussions

3.1. Quenching mechanism analysis

Fluorescence quenching can be caused by a lot of physical and chemical processes [9], but can be classified as either dynamic or static mechanism [10]. Dynamic and static quenching can be distinguished by their different dependence on temperature and viscosity. In dynamic quenching, interaction between quencher and phosphor takes place during the lifetime of the excited state. Dynamic quenching is caused by collision and higher temperature will result in larger diffusion coefficients, the dynamic quenching constants are expected to increase with increasing temperature [11,12]. In order to ascertain the quenching mechanism, the emission spectra of HSA in presence of different amounts of ICTN were recorded when the excitation wavelength was stabilized at 275 nm, as shown in Fig. 1.

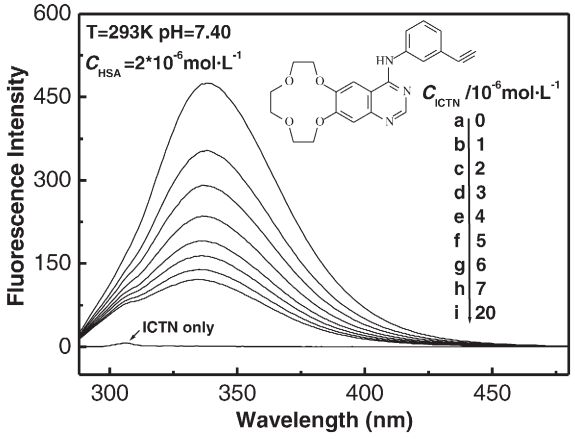

Fig. 1. Fluorescence emission spectra of HSA system (inset is structure of ICTN).

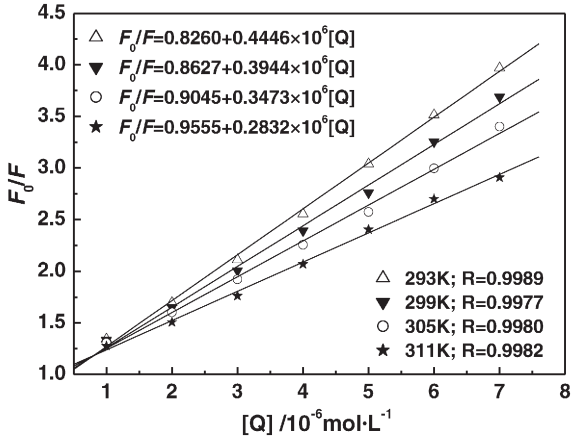

Fig. 2. Stern–Volmer plots for the quenching of HSA by ICTN.

Supposing the quenching was dynamic quenching, the fluorescence data at different temperatures should be analyzed with the well-known Stern–Volmer equation [13,14]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \tag{1}$$

where F_0 and F are the fluorescence intensity in the absence and in the presence of quencher, respectively, K_{SV} is the Stern–Volmer quenching constant, [Q] is the concentration of the quencher. Eq. (1) could be applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q]. Fig. 2 displayed the Stern–Volmer plots of the fluorescence quenching of HSA by ICTN at four different temperatures, the corresponding values of K_{SV} at each temperature were calculated and listed in Table 1. It was obvious that the value of K_{SV} was inversely correlated with temperature, which indicated that the quenching mechanism of the HSA-ICTN interaction could not be dynamic collision. Alternatively, the present interaction should belong to static quenching with ICTN-HSA complex formation at ground-state. This quenching mechanism could also be supported by 3D spectra (Fig. 9), in which Stokes shift of fluorescence peaks significantly changed when ICTN was added (Table 4).

3.2. Binding equilibrium and thermodynamics

For a static quenching process, the binding constant (K_b) and the number of binding sites (n) can be determined by the following equation [15,16]:

$$\log\left(\frac{F_0 - F}{F}\right) = \log K_b + n\log[Q] \tag{2}$$

Table 1
Stern–Volmer constants.

pH	T(K)	K_{SV}(10^5 L·mol^{-1})	SD[a]
7.4	293	4.446	0.049
	299	3.944	0.063
	305	3.473	0.053
	311	2.832	0.040

[a] SD is the standard deviation for K_{SV}.