Journal of Luminescence 134 (2013) 515–523

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

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

Comparing the effects of Fe(III) and Cu(II) on the binding affinity of erlotinib to bovine serum albumin using spectroscopic methods

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ARTICLE INFO

Article history:
Received 10 May 2012
Received in revised form
20 July 2012
Accepted 30 July 2012
Available online 8 August 2012

Keywords:
Erlotinib
Bovine serum albumin
Metal ions
Spectroscopic methods
Thermodynamic parameters

ABSTRACT

The interactions between erlotinib (ET) and bovine serum albumin (BSA) in the absence and presence of Cu(II) and Fe(III) in aqueous solution were investigated by using fluorescence, circular dichroism and three-dimensional (3D) fluorescence spectroscopic methods under simulative physiological conditions. Erlotinib effectively quenched the intrinsic fluorescence of BSA with slight redshifts in the absence and presence of Cu(II) and Fe(III). Cu(II) decreased the binding affinity and reduced the binding sites of erlotinib to BSA, while Fe(III) increased the binding affinity and binding sites of erlotinib to BSA. The negative values of ΔH and ΔS illustrate that the binding is mainly driven by the hydrogen bond and van der Waals force. The conformation of BSA was changed through ET binding in the presence of Cu(II) and Fe(III), which was revealed by circular dichroism, synchronous fluorescence and 3D fluorescence spectroscopic methods. The results indicate that the binding capability of erlotinib to BSA is affected by the types of metal ions.

1. Introduction

Erlotinib [*N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine] (ET, structure in Fig. 1), a member of a class of targeted anticancer drugs that inhibit the activity of the epidermal growth factor receptor, is the first drug to demonstrate an increase in patient survival in Phase III trials with advanced non-small-cell lung cancer [1,2]. Moreover, ET has also shown effects on treating pancreatic cancer and several other types of cancer [3,4]. In addition, this anticancer drug has recently been reported to be a potent inhibitor of JAK2/V617F activity, which is a mutant of tyrosine kinase JAK2 [5,6]. However, the researches on this drug are mainly focused on its mechanism as a reversible tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR); few have been done on the interaction of ET to serum albumins.

The interaction between drug and plasma protein is an important factor to understand the pharmacokinetics and pharmacodynamic properties of drugs because distribution and metabolism of drugs in the body are correlated with their affinities to plasma protein [7,8]. Effectiveness and efficacy of new drugs in therapeutic applications may be modified on the basis of its toxicity and distribution in plasma proteins [8,9]. Investigation on the binding activities of drugs with plasma protein is fundamentally important and helpful in drug design and therapy.

Serum albumins (SAs) are the major soluble plasma proteins of the circulatory system in vivo [10,11]. It has been shown that the distribution, free concentration and metabolism of many biologically active compounds are correlated with their binding to serum albumins [12–14]. Furthermore, it has also been demonstrated that conformational changes of serum albumins can be caused by the interactions between serum albumins and small-molecule ligands, which may influence serum albumins′ biological functions as carrier proteins [15,16]. Bovine serum albumin (BSA) is studied extensively as a model protein due to 76% structural homology with human serum albumin (HSA) [17–19]. The binding activities of small molecules (i.e. drugs or fatty acids) to BSA have been studied for years with different techniques in order to understand the functions of this unique carrier, and to disclose its structural basis for designing new therapeutic agents [20–24].

Metal ions are vitally essential in biological systems and the importance of metals to life has been demonstrated by their crucial roles in variety of bioprocesses [25–29]. Copper and iron both are the common metallic elements in nature and it has been reported that Cu(II) and Fe(III) are the most essential trace minerals to human beings [29–31]. The blood plasma is considered to be suitable for the study of physiological activities of metal ions in human body [32]. Many metal ions, such as Cu(II) or Fe(III), can affect the binding activities of drugs with SA, thereby affecting some of the characteristics of the drugs in vivo

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http://dx.doi.org/10.1016/j.jlumin.2012.07.036

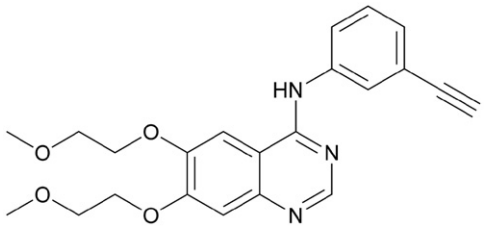

Fig. 1. Molecular structure of erlotinib (ET).

[33,34]. The study on the interaction between ET and SA in a ternary system of drug–protein–metal ion will be helpful in supplying basic information on the pharmacological properties, metabolism, and bio-distribution of ET.

In this work, the interaction between ET and BSA under simulative physiological conditions with and without Cu(II) or Fe(III) was comprehensively investigated by using combined methods of fluorescence spectrum, circular dichroism (CD) spectrum and three-dimensional (3D) fluorescence. The information related to the binding mechanism of ET to BSA in the presence of common metal ions was obtained. The perturbative information aimed at the microenvironment and conformation of BSA–ET system affected by Cu(II) or Fe(III) is rationally discussed. These results clarify the impacts of metal ions on the binding activity between ET and BSA in vitro, which is not only helpful for understanding the metabolism and distribution of this anticancer drug in vivo, but also useful for further elaborating the effects of ET on protein function during the blood transportation process.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA) was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). Erlotinib hydrochloride (ET) with 98% purity was obtained from Shanghai Institute of Pharmaceutical Industry, China. All other reagents used were of analytical purity and double distilled water was used throughout experiments. BSA was dissolved in 0.05 M phosphate buffered saline solution (PBS) to form a 2.0×10^{-6} M solution, and then preserved at 4 °C for later use.

2.2. Apparatus

Fluorescence data were obtained on a Cary Eclipse Spectrofluorimeter (Varian Corporate, America) equipped with 1.0 cm quartz cells. Absorption spectra were recorded on a Lambda-35 spectrophotometer (PerkinElmer Corporate, America) equipped with 1.0 cm quartz cells at room temperature. Circular dichroism (CD) spectra were measured on a MOS-450/AF-CD Spectropolarimeter (Bio-Logic corporate, France) with a 1 mm quartz cell at room temperature under constant nitrogen flush. An isothermal titration calorimeter (Microcal ITC-200, USA) was used to measure the enthalpy and entropy changes. All pH measurements were made with a PHS-3C acidity meter.

2.3. Procedures

For the fluorescence measurement, ET solution was added into the protein solutions in sequence. Both ET and BSA concentrations were calculated by weight for consistency. The systems were excited at 280 nm, and the emission wavelength was adjusted from 300 to 500 nm with a scanning speed of 600 nm min^{-1}. The excitation and emission slit widths were both set at 5.0 nm. The detailed binding behavior of drug to plasma protein was characterized by fluorescence experiments with fixed concentrations of BSA and ET. All the experiments were conducted in triplicate.

For UV–vis absorption spectra, ET and BSA solutions with fixed concentration were added to 10 mL volumetric flasks in sequence, and then diluted with PBS solution to the mark. The equilibrated solution was poured into quartz cells and scanned in the ultraviolet range 190–500 nm using PBS solution (pH 7.40) as reference.

Circular dichroism (CD) spectra were measured on a MOS-450/AF-CD Spectropolarimeter at room temperature under constant nitrogen flush over a wavelength range of 260–200 nm. The path length and volume of quartz cells were 0.1 cm and 400 μL, respectively. The scanning speed was set at 100 nm min^{-1}. Each spectrum was the average of three successive scans and appropriate buffer solutions running under the same conditions were taken as blank and their contributions were subtracted from the experimental spectra. The concentration of BSA was kept at 2.0×10^{-6} mol L^{-1} and the molar ratios of BSA to ET were varied from 1:0 to 1:4. The contents of different secondary structures of BSA were analyzed.

Synchronous fluorescence spectra of solutions prepared as described above were measured on the Cary Eclipse fluorescence spectrophotometer. The excitation wavelength (λ_{ex}) was 280 nm. The excitation and emission slit widths were both set at 5 nm. The *D*-value ($\Delta\lambda$) between the excitation and emission wavelengths was set at 15 or 60 nm for studying the characteristics of intrinsic amino residues tyrosine (Tyr) and tryptophan (Trp), respectively.

The three-dimensional fluorescence spectra were measured under the following conditions: the excitation wavelength scan range was recorded between 200 nm and 400 nm; the emission wavelength scan range was recorded from 200 nm to 500 nm at 5 nm increments. The number of scanning curves was 41.

3. Results and discussion

3.1. Binding properties determined by fluorescence titration assays

The fluorescence quenching can be used to obtain valuable information on the binding activities between drugs and proteins [35–37]. In order to investigate the binding activity of ET to BSA affected by metal ions, the fluorescence spectra of BSA with ET in the absence and presence of Cu(II) or Fe(III) were measured with an excitation wavelength of 280 nm (Fig. 2). As shown in Fig. 2, BSA had a fluorescence peak located at 348 nm for pH 7.40. Without or with metal ion, the fluorescence intensity of BSA decreased gradually with the addition of ET. Moreover, the position and shape of the emission peak with Cu(II) or Fe(III) at different concentrations of ET were similar to those without Cu(II) or Fe(III). Additionally, further fluorescence attenuation of BSA was detected when ET was gradually added into BSA–metal ion solution. The redshifts of the λ_{em} of BSA with the addition of ET were also observed with and without metal ions. When the concentration of ET reached 10×10^{-6} L mol^{-1}, the fluorescence intensities of BSA decreased by 28.15% and 32.26% in the presence of Cu(II) and Fe(III) respectively, compared with 30.3% decrease of the fluorescene intensity of ET–BSA without metal ions. The result shows that the quenching effect of ET on BSA can be enhanced by Fe(III), but weakened by Cu(II).

To further clarify the binding activity between ET and BSA in the presence of metal ions, the fluorescence quenching data were analyzed using the Stern–Volmer equation [36]

$$\frac{F_0}{F}=1+K_q\tau_0[Q]=1+K_{SV}[Q] \quad (1)$$