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The binding behavior of itraconazole with hemoglobin: Studies from multi-spectroscopic techniques

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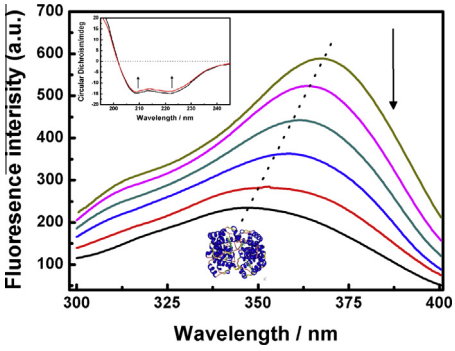
HIGHLIGHTS

- Itraconazole induces the heme group of Hb exposed to the aqueous medium.
- Percentages of α-helix and β-sheet are calculated according to CD spectra.
- Steady state/synchronous/three-dimensional/time-resolved fluorescence are employed.
- Thermogravimetric suggest hydrogen bonds and van der Waals are the main force.

GRAPHICAL ABSTRACT



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ABSTRACT

The interactions between hemoglobin (Hb) and itraconazole (ITZ) are investigated in details using UV–vis spectra, circular dichroism spectroscopy, steady state fluorescence, three-dimensional fluorescence spectra, synchronous fluorescence and time-resolved fluorescence spectra at molecular level. The UV–vis studies represent that ITZ can access into heme group and lead to it explored in aqueous medium. CD spectra suggest ITZ could combine with amino acid residues in polypeptide chain and cause a partial unfolding of Hb (reducing of the α-helix content). Steady state fluorescence/synchronous fluorescence (taking into account inner filter effects) and three-dimensional fluorescence/time-resolved fluorescence spectroscopy results reveal that ITZ alters polarity and conformation around the fluorophore molecule. The interaction processes are static quenching mechanisms. The negative of ΔH^0 and ΔS^0 indicate that hydrogen bonds and van der Waals are the main force.

Introduction

Itraconazole (ITZ) is one of synthetic trizole antifungal drugs which are used for broad-spectrum antibacterial and it has a notable effect on superficial and deep fungal infection [1]. When clinical application by injection becomes possible, facilitating treatment with injection and oral preparation is generated. There are many cases on ITZ clinical experiments and the mechanisms of preclinical and mechanistic effect have been explored on the pharmacological profile. However, recent preclinical cardiovascular evaluations demonstrate that ITZ administration (intravenous or oral) can cause a significant reduction in cardiac contractility in conscious dogs [2,3]. The scientists concerned with the mechanism by ITZ in vitro antimicrobial activity and exploring the activity profile of ITZ in pharmacological and biochemical models to better understand its activity [4].

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Hemoglobin (Hb) plays an important role in many biologically relevant processes in life science, clinical medicine and environment. It is well known for its function in the vascular system of animals, being a carrier of oxygen. It also aids, both directly and indirectly, the transportation of carbon dioxide and regulation of the pH in blood [5]. Drug associations at protein binding level will in most cases evidently influence the apparent distribution volume of the drugs and affect the elimination rate of drugs. It can be announced that the effectiveness of drugs as pharmaceutical agents is relied on their binding ability and they can also alter the drug stability and toxicity during the treatment [6,7].

Thus, the objective of the current work is to use different analytical techniques to study the binding mechanisms of ITZ with protein at molecular level. The protein conformation changes, interaction modes, binding sites and thermodynamic parameters are discussed. According to the study, we expect it can offer a further insight on interpretations for the pharmacological and toxicological activities of ITZ in theory.

Materials and methods

Materials

Itraconazole (ITZ) and hemoglobin (Hb, from bovine blood, Mr = 64,500, Sigma, USA) were purchased from Sigma (USA). Hb solution (c_{Hb} = 5 μmol/L) was prepared in phosphate buffer solution (PBS) at pH 7.4 as reserve. ITZ was dissolved by DMSO and diluted in deionized water (c_{Hb} = 10 mmol/L) as reserve and the final concentration of interacting was 50 μmol/L. All other reagents were of analytical grade and they were dissolved with deionized Milli-Q water. All solutions were kept in the dark at 4 °C.

Characterization

The UV–vis absorption spectra were recorded on Varian Cary 5000. The steady state fluorescence emission spectra, 3D-fluorescence and synchronous fluorescence spectra were performed on PerkinElmer LS-50B fluorescence spectrophotometer. The excitation of steady state fluorescence was 280 nm. The *D*-value ($\Delta\lambda$) between excitation wavelength and emission wavelength was 20 nm and 60 nm, respectively in synchronous fluorescence spectroscopy. The emission wavelengths range of 3D-fluorescence was 220–520 nm, and the excitation wavelength scan range was 200–400 nm at 10 nm increment. Time-resolved fluorescence spectra were recorded on a spectrofluorimeter (FM-4P-TCSPC, HORIBA JOBIN YVON FLUORCUBE). The fluorescence lifetimes were determined from time-resolved intensity decay by time-correlated single photon counting (TCSPC) method. The excitation of the sample was carried out by a nanosecond diode laser at 265 nm as a light source. The decay was analyzed by IBH DAS-6 decay 1 mm path length in a wavelength. The fluorescence decay was acquired with a peak preset of 10,000 counts.

The secondary structures of samples were obtained with an Applied Photophysics Chriascan circular dichroism spectrometer, and spectra are scanned with 1 nm spectral bandwidth and 0.5 nm step resolution in the UV-region 190–260 nm. The CD data were calculated using the CDNN program. CDNN is a program which can analyze circular dichroism spectra used by Applied Photophysics. This program, written by Dr. Gerald Böhm, used neural networks along with known reference spectra [8]. In the far-UV region, the different secondary structures give rise to characteristic CD spectra. By analyzing the CD spectrum in this region and comparing with reference spectra of known protein structures, an approximation of the composition of a protein can be found with respect to these secondary structures. This process was more commonly known as CD spectra deconvolution. In order to avoid the influences of buffer signal of CD spectra, buffers of Hb were replaced by Milli-Q water in the experiment of CD.

All experiments were carried out at room temperature 25 °C except fluorescence dynamic experiments.

Results and discussion

The UV–vis absorption spectroscopy

The UV–vis absorption spectra reflect the conformational changes of proteins. Moreover, the peak shape and peak position of protein could indicate the important changes of structure such as unfolding and denaturation. Fig. 1 showed the UV–vis absorption spectra of Hb with increasing of ITZ. There are several characteristic peaks of Hb: the phenyl group of Trp and Tyr residues (273 nm), ε-band (360 nm) and the porphyrin-Soret band (404 nm, strongly allowed π–π* electronic transition) [9,10]. The UV–vis spectra of ITZ were also examined. With increasing of ITZ, the intensities of wavelength from 250 to 350 nm were increasing, while the Soret band at 404 nm decreased. The results indicated that ITZ can access the heme group and ITZ could directly involve producing disturbance of the structures and induced the exposure of the heme group to the aqueous medium [11].

Circular dichroism spectroscopy study

In order to further study the influence of ITZ on the conformation of Hb, CD measurement, which provides information of the secondary and tertiary structures of proteins are performed [12]. As it is known, CD spectroscopy allows the detection and quantitation of the chirality of molecular structures. Fig. 2 showed the comparisons of Hb and ITZ interactions with Hb at the far-UV–vis region (190–250 nm). There are two negative bands at 209 nm (corresponds to π–π* transfer for the peptide bound of α-helix) and 222 nm (contributed to π–π* transfer for both the α-helix and random coil), and a positive band at 195 nm [13,14]. The intensity at 209 nm and 222 nm bands decreased with addition of ITZ, showing the loss of α-helix content and the changes of some secondary structure. Under the experimental conditions, ITZ showed no CD signal in the range 190–250 nm which did not interfere with Hb signal. Moreover, the solvent (DMSO) also had no interference on Hb. The decrease α-helix content suggested that ITZ was combined with the amino acid residues of the main polypeptide chain of the protein and further caused partial unfolding of Hb [15]. There was no significant change of Hb before and after addition

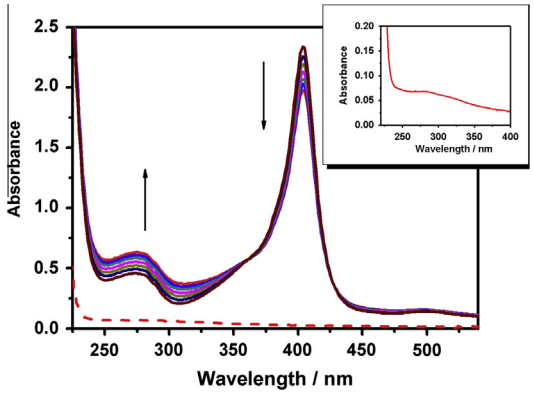

Fig. 1. UV–vis absorption spectra of Hb in addition of ITZ (the red dash line and inset are the spectra of ITZ). [Hb] = 5 × 10^{-6} mol/L; [ITZ] = 0.0, 1.7, 3.3, 5.0, 6.7, 8.3, 10.0 × 10^{-6} mol/L; *T* = 298 K. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)