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Spectroscopic studies and molecular docking on the interaction of organotin antitumor compound bis[2,4-difluoro-N-(hydroxy-⟨κ⟩O)benzamidato-⟨κ⟩O]diphenyltin(IV) with human cytochrome P450 3A4 protease

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ABSTRACT

A novel organotin DFDPT was synthesized and characterized by elemental analysis, IR, ^{1}H, ^{13}C, ^{119}Sn, NMR techniques,etc. In order to investigate profoundly the relationship between DFDPT with human CYP3A4 proteaset and anticancer molecular mechanism of DFDPT, the intercalative mode of binding of DFDPT with CYP3A4 under physiological conditions were comprehensively evaluated using steady state, synchronous, three-dimensional fluorescence spectroscopy,circular dichroism and molecular docking. Fluorescence emission data showed that CYP3A4 fluorescence affected by DFDPT was a static quenching procedure, which implied that DFDPT-CYP3A4 complex had been formed. Apparent binding constants K_b of CYP3A4 with compound at 298 and 310 K were 2.51×10^7 and 3.09×10^5, respectively. The binding sites number n was 1.64 and 1.22, respectively. The thermodynamic parameters ΔH and ΔS of the DFDPT-CYP3A4 complex were negative, which indicated that their interaction was driven mainly by hydrogen bonding and van der Waals force. The binding of DFDPT-CYP3A4 was spontaneous process in which ΔG was negative. The synchronous results showed DFDPT induced conformational changes of CYP3A4 protein. Three-dimensional fluorescence and circular dichroism spectra results also revealed conformation of CYP3A4 protein had been possible changed in the presence of DFDPT. Molecular docking was used to study the interaction orientation between DFDPT and CYP3A4 protease. The results indicated that DFDPT interacted with a panel of amino acids in the active sites of CYP3A4 protein mainly through formation of hydrogen bond. Furthermore, the predicted binding mode of DFDPT into CYP3A4 appeared to adopt an orientation with interactions among Arg105, Ser119 and Thr309.

1. Introduction

In the past several decades, organotin (IV) compounds were studied as the probable antitumor drug [1]. Our research group synthesized and structurally analyzed a potential organotin candidate for the clinical application, bis[2,4-difluoro-N-(hydroxy-⟨κ⟩O)benzamidato-⟨κ⟩O]diphenyltin(IV) (DFDPT) which exhibited the strong antitumor activity against seven human cancer cell lines including HepG-2, SHSY5Y, HEC-1-B, EC, T24, HeLa and A549 along with human liver HL-7702, a human normal hepatocytes cell [2]. The mechanism of great *anti*-cancer activity of DFDPT may be relevant to the inhibition effect on CYP3A. The studies of our previous reports suggested both mRNA and protein expression of CYP450 were inhibited by organotin compounds. The inhibition of the key isoenzyme CYP3A could cause the change of metabolism. Cytochrome P450 (CYP) proteins play significant roles in metabolism, both the endogenous molecules and exogenous substances are detoxified by these enzymes. A crucial fraction of the CYP family is CYP3A4, which composes up to 30% of the total liver CYP enzyme pool in humans [3]. The interaction between proteins and organotin compound was imperative for investigating the pharmacodynamics, pharmacokinetics and activity of organotin compound. Moreover, we can further clarify the relationship between the effective antitumor activity and the interaction of DFDPT with CYP3A4. The mechanism of interactions of organotin compounds and CYP3A4 was studied in present work. The structure of DFDPT and is shown in Fig. 1 A, and the structure of CYP3A4 protease is shown in Fig.1 B, which is divided into two regions, namely α helix and β fold region. Heme is the binding site of oxygen and substrate in the oxidation reaction. Central iron atom of heme is the non-covalent binding form in the CYP3A4 molecule.

Various techniques had been employed to investigate the complex-protein interaction, including nuclear magnetic resonance (NMR), UV–Vis spectrophotometry, fourier infrared spectrophotometry (FT-IR),

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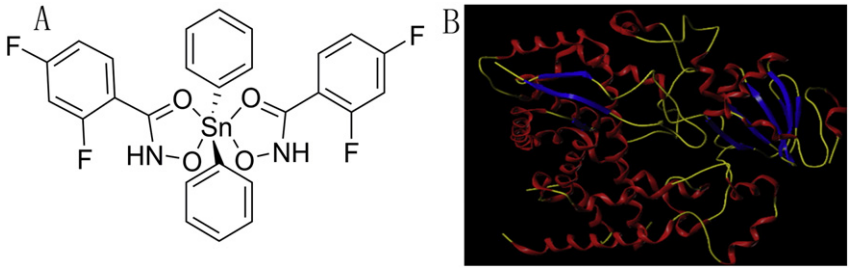

Fig. 1. Structure of DFDPT and CYP3A4 protease A DFDPT B CYP3A4 protease.

fluorescence [4–8], circular dichroism (CD) and molecular docking and so on. Among these methods, fluorescence spectroscopy has a variety of superior advantages over other techniques, including its potency for sensing any minimal changes in the local environment of a fluorophore. Fluorescence spectroscopy is an effective method to reveal the interaction between small molecules and proteins [9]. In present work, we use fluorescence spectroscopy to investigate the quenched mechanism, the binding constant, the binding site and the thermodynamic parameters [10–13]. The synchronous fluorescence and three-dimensional fluorescence were performed to confirm the changes of conformation [14,15]. The CD spectrum can effectively reflect the secondary structure changes of protein. The present paper would deal with the binding mechanism of organotin compound DFDPT with CYP3A4 protein by fluorescence, CD and the molecular docking measurement which was reported for the first time. Molecular docking can be used to study the metabolic behavior of the compounds through docking the compounds into the activity sites of drug-metabolizing enzymes. In addition, understanding the interaction of DFDPT with CYP3A4 could contribute to the clinical employment of the organotin compounds and the relief of the organotin pollutant. Therefore, the aim of the present study is to determine the binding of DFDPT towards the activity cavity of CYP3A4 protein. Through these data, the metabolic behavior of DFDPT by CYP3A4 protease could be deeply understood.

2. Materials and methods

2.1. Materials

DFDPT was synthesized by Shanxi Medical University with purity over 99% by HPLC analysis. CYP3A4 protein was purchased from Becton, Dickinson and Company (BD Company, New Jersey, USA) and stored at −80 °C. The solvent of organotin (IV) compound was 90% propanediol (Tianjin Fengchuan Chemical Reagent Science And Technology Co., Ltd. Tianjin, China), 1% ethanediamine and 9% normal saline (Shijiazhuang pharmaceutical, Shijiazhuang, China). The stock solution of CYP3A4 was prepared in the PBS buffer solution (pH 7.4) with a final concentration of 5.0×10^{-9} mol·L^{-1}. The disodium hydrogen phosphate dodecahydrate was purchased from Tianjin Fengchuan Chemical Reagent Science and Technology Co., Ltd (Tianjin, China). The potassium phosphate monobbasic was purchased from Tianjin Beichen Fangzheng Company. The pH measurement was made with a Leici pHS-2 digital pH-meter (Shanghai, China) with a combinational glass calomel electrode. All other materials were of analytical reagent grade and double distilled water was used throughout all the experiments.

2.2. Synthesis of DFDPT

Di-phenyltin dichloride (0.344 g, 1.0 mmol) was added to an anhydrous methanol solution (20 mL) of 2, 4–di-fluorbenzohydroxamic acid (0.346 g, 2.0 mmol) and potassium hydroxide (0.112 g, 2.0 mmol). The solution was stirred at room temperature per overnight. Water (20 mL) was added and a white precipitate was formed. Then filtrate, vacuum dry. Yield: 62%; m.p.116–118 °C. Calcd (%) for $C_{26}H_{18}N_2O_4F_4Sn$: C, 50.57; H, 2.92; N, 4.54. Found (%): C, 50.30; H, 3.09; N, 4.33. IR: ν = (N—H) 3422 (s), ν = (CO/NC) 1611(s), (N—O) 950 (s), (Sn—C) 515 (m), (Sn—O) 476 (m) cm^{-1}. ^{1}H NMR($CDCl_3$): δ = 13.23 (s, 2H, NH), 7.90–7.08 (m, 16H, Harom) ppm. ^{13}C NMR ($CDCl_3$): δ = 161.8 (CO); 159.2, 158.5, 149.5, 147.6, 136.1 ~ 127.8, 112.5, 104.4 (Carom) ppm. ^{119}Sn NMR ($CDCl_3$): δ = −432.3 ppm. ESI-MS, m/z = 616.9 [M]$^+$.

2.3. Fluorescence quenching spectra

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with a quencher molecule. In the present study, the emission wavelength was performed from 300 nm to 400 nm. The excitation wavelength was at 280 nm. The concentration of CYP3A4 was constant (5×10^{-9} mol·L^{-1}) while varying the compound DFDPT concentration (0, 0.5×10^{-5}, 1×10^{-5}, 1.5×10^{-5}, 2×10^{-5}, 2.5×10^{-5}, 3×10^{-5}, 3.5×10^{-5} and 4×10^{-5} mol·L^{-1}). The excitation and emission band widths were 5 nm. Fluorescence spectra were recorded (n = 3 replicates) on LS-55 fluorophotometer (Perkin Elmer, USA) equipped with a 150 W Xenon lamp, a HH-2 waterbath (Changzhou Guohua Electric Applance Co. Ltd, Changzhou, China) and1.0 cm quartz cells.

2.4. Synchronous fluorescence

The synchronous fluorescence spectroscopy (SFS) is a kind of multi-dimensional fluorescence technique that gives information about the conformational change of protein molecular environment in a vicinity of the fluorophore functional groups. When the D-value ($\Delta\lambda$) between excitation and emission wavelength are stabilized at 60 nm, SFS gives the characteristic information of tryptophan residues. The variation in the tryptophan emission is the consequence of the protein conformational changes. Therefore, synchronous fluorescence spectra of CYP3A4 (1×10^{-8} mol·L^{-1}) with complex DFDPT (0, 0.5×10^{-5}, 1×10^{-5}, 1.5×10^{-5}, 2×10^{-5}, 2.5×10^{-5}, 3×10^{-5}, 3.5×10^{-5} and 4×10^{-5} mol·L^{-1}, respectively) at $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm were recorded on LS-55 fluorescence spectrophotometer.

2.5. Three-dimensional fluorescence

The three-dimensional fluorescence spectra have become a popular fluorescence analysis technique in recent years. It can comprehensively exhibit the fluorescence information of the sample, which makes the investigation of characteristic conformational change of protein to be more scientific and credible. If there is a shift at the excitation or emission wavelength around the fluorescence peak, or appearance of a new peak or disappearance of existing peak, it could be an important hint a conformational change of the researched protein. In this research, the three-dimensional fluorescence spectroscopy of CYP3A4 protein (5×10^{-9} mol·L^{-1}) treated with DFDPT (0 and 3×10^{-5} mol·L^{-1}) was measured on F-320 fluorophotometer (Tianjin Gangdong Sci. &Tech. Development Co. Ltd., Tianjin, China). The emission wavelength was recorded between 320 nm and 450 nm with an increment of