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Probing the binding mode of psoralen to calf thymus DNA

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ABSTRACT

The binding properties between psoralen (PSO) and calf thymus DNA (ctDNA) were predicted by molecular docking, and then determined with the use of UV–vis absorption, fluorescence, circular dichroism (CD) and Fourier transform infrared (FT-IR) spectroscopy, coupled with DNA melting and viscosity measurements. The data matrix obtained from UV–vis spectra was resolved by multivariate curve resolution–alternating least squares (MCR–ALS) approach. The pure spectra and the equilibrium concentration profiles for PSO, ctDNA and PSO–ctDNA complex extracted from the highly overlapping composite response were obtained simultaneously to evaluate the PSO–ctDNA interaction. The intercalation mode of PSO binding to ctDNA was supported by the results from the melting studies, viscosity measurements, iodide quenching and fluorescence polarization experiments, competitive binding investigations and CD analysis. The molecular docking prediction showed that the specific binding most likely occurred between PSO and adenine bases of ctDNA. FT-IR spectra studies further confirmed that PSO preferentially bound to adenine bases, and this binding decreased right-handed helicity of ctDNA and enhanced the degree of base stacking with the preservation of native B-conformation. The calculated thermodynamic parameters indicated that hydrogen bonds and van der Waals forces played a major role in the binding process.

1. Introduction

Deoxyribonucleic acid (DNA) is an important genetic substance in the organism and a significant component of cell. It not only carries and expresses the hereditary information but also decides the type and function of cells. It plays a decisive role in growth, breeding, heredity, variation and transformation and a series of life phenomena [1]. DNA is a common intracellular target for antiviral, antitumor, anticancer and antibiotic drugs, therefore to explore the binding mechanisms of drugs with DNA for guiding the rational design and construction of new and more efficient drugs targeted to DNA is of great importance [2]. Drugs can interact with DNA through the following three non-covalent modes: (i) intercalation between the base pairs; (ii) interactions with DNA groove; and (iii) electrostatic attractions with the anionic sugar–phosphate backbone of DNA [3]. Intercalative binding and groove binding are related to the grooves in DNA double helix, while the electrostatic binding takes place out of the groove.

Psoralen (PSO, structure shown in Fig. 1) is known as a furocoumarin, which is naturally occurring or synthetic tricyclic aromatic compound deriving from the condensation of a coumarine nucleus with a furan ring [4]. It is the main active ingredient extracted from the fruits of *Psoralea corylifolia* L. [5]. PSO exhibits many therapeutic effects, for example, promoting MCF-7 cell proliferation significantly as ERα agonists [6], showing multidrug resistance effect on leukemia cell [7], acting through the activation of BMP signaling to promote osteoblast differentiation [5], etc. Also, it was reported that derivates of PSO could cause a linkage to DNA which lead to some changes in DNA properties [8,9]. In recent years, some studies have shown that PSO exerts activity in preventing proliferation of bladder carcinoma, mucoepidermoid carcinoma and mammary cancer cells in vitro [10]. Owing to a variety of biological and pharmacological activity of PSO, it is necessary to clarify the interaction mechanism between PSO and DNA. Various techniques have been adopted to investigate the interaction of small molecules with DNA, including fluorescence spectroscopy, electrochemistry, UV–vis absorption [11], circular dichroism (CD), Fourier transform infrared (FT-IR), nuclear magnetic resonance (NMR) [12], and atomic force microscopy (AFM) [13]. Though spectroscopic techniques possess the advantages of reproducibility, selectivity and convenience among these methods, it is difficult to obtain more information about the composition in complex system of more than two components due to the overlap of their response signals. Therefore, a chemometrics method, multivariate curve resolution–alternating least squares (MCR–ALS), has been used to analyze the overlapping response signals in recent years because it

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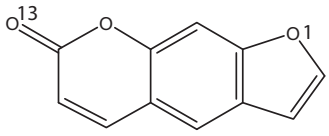

Fig. 1. Molecular structure of psoralen (PSO).

can extract simultaneously the concentration information and the pure spectra of all species involved in interaction [14–16].

In this work, the molecular docking was employed to predict the probable binding site and binding mode of PSO with ctDNA, and then multispectroscopic methods including UV–vis absorption, fluorescence, CD and FT-IR spectroscopy along with DNA melting and viscosity measurements were used to determine the interaction between PSO and ctDNA at physiological buffer (pH 7.4). Furthermore, the MCR–ALS method was applied to decompose the expanded UV–vis absorption spectral data collected from the PSO–ctDNA mixtures, and then the corresponding pure spectra of each component and their concentration profiles were simultaneously extracted from composite responses to evaluate the interaction process of PSO with ctDNA.

2. Experimental

2.1. Chemicals

PSO (analytical grade) was obtained from the National Institute for the Control of Pharmaceutical Biological Products (Beijing, China). The stock solution (4.94×10^{-3} mol L^{-1}) of PSO was prepared in absolute ethanol. The ctDNA was purchased from Sigma Chemical Co., and it was dissolved in 0.10 mol L^{-1} NaCl solution to get ctDNA solution. As the ratio of absorbance at 260 and 280 nm (A_{260}/A_{280}) was 1.86, the ctDNA solution was deemed to be free from protein sufficiently [17]. Also, the concentration of ctDNA solution was determined to be 3.61×10^{-3} mol L^{-1} by UV absorption at 260 nm using the molar absorption coefficient $\varepsilon_{260} = 6600$ L mol^{-1} cm^{-1} [18]. All other reagents and solvents were of analytical reagent grade, and ultrapure water was used throughout the experiment. All the solutions used in the experiments were kept in cold storage at 0–4 °C.

2.2. Procedures

2.2.1. Molecular docking studies

The molecular modeling investigation was aimed at predicting the binding mode and binding site of PSO with ctDNA. Docking studies were carried out with the use of Autodock (version 4.2) software. The structure of 24 bp long DNA used for docking was acquired from the Protein Data Bank with identifier 453D [19]. Then the structure of macromolecule was optimized for docking by adding polar hydrogen atoms and Gasteiger charges. The 3D structure of the ligand PSO was generated in Sybyl × 1.1 (Tripos Inc., St. Louis, USA) and its conformation was energy-minimized using MMFF94 force field. Rotatable bonds in the ligands were assigned with AutoDock Tools and docking carried out by the AutoDock 4.2 Lamarckian Genetic Algorithm (LGA) [20]. DNA was enclosed in a grid possessing 0.375 Å spacing and PSO molecule was allowed to move within the whole region. The output from AutoDock was rendered with PyMol.

2.2.2. UV–vis absorption measurements

UV–vis absorption spectra measurements were conducted to investigate the interaction of PSO with ctDNA over a wavelength range of 215–390 nm, and two different experiments were performed in pH 7.4 Tris–HCl buffer at room temperature. For experiment 1: the concentration of PSO constant was kept at 1.98×10^{-5} mol L^{-1}, and different amounts of ctDNA (0–1.62×10^{-4} mol L^{-1} in increment of 6.01×10^{-6} mol L^{-1}, total 27 solutions) were added successively to the solution. For experiment 2: the concentration of ctDNA was kept at 7.22×10^{-5} mol L^{-1}, and PSO was added into the solution in increment of 8.23×10^{-7} mol L^{-1} (total 27 solutions) with a final concentration of 2.22×10^{-5} mol L^{-1}. All the solution samples were allowed to stand for 3 min to equilibrate after addition, and then the UV–vis absorption spectra were collected every 1 nm on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan). Thus, two data matrices $\boldsymbol{D}^{\mathrm{PSO}}$ (27×176) and $\boldsymbol{D}^{\mathrm{ctDNA}}$ (27×176), were obtained from these measurements, and column-wise expanded data matrix for the two experiments were constructed.

2.2.3. Fluorescence measurements

A quantitative analysis of the interaction between PSO and ctDNA was carried out by fluorimetric titration with a Hitachi spectrofluorometer model F-7000 (Hitachi, Japan) equipped with a 150 W xenon lamp. The diluent PSO solution (1.21×10^{-5} mol L^{-1}) was pipetted to a 1.0 cm quartz cuvette, and then successive titrated by the 3.61×10^{-3} mol L^{-1} ctDNA solution to give a final concentration of 1.20×10^{-4} mol L^{-1}. After equilibrating for 3 min, the fluorescence spectra of the solution were recorded at four temperatures (292, 298, 304 and 310 K) in the wavelength range of 370–550 nm with an exciting wavelength at 280 nm. Both the excitation and emission bandwidths were set at 5.0 nm. The background of fluorescence was corrected by subtracting appropriate blanks of the Tris–HCl buffer.

The fluorescence data were corrected for absorption of excitation light and emitted light to eliminate the re-absorption and inner filter effect caused by UV–vis absorption according to the relationship [21].

$$F_c = F_m e^{(A_1+A_2)/2} \quad (1)$$

where F_c and F_m represent the corrected and measured fluorescence, respectively. A_1 and A_2 are the absorbance of the ctDNA solutions at excitation and emission wavelengths, respectively.

Iodide quenching effects were compared according to the quenching constants calculated from fluorescence data of titrating KI to PSO and PSO–ctDNA complex solutions.

Fluorescence polarization was measured by keeping the concentration of PSO at 1.21×10^{-5} mol L^{-1} while varying ctDNA concentrations from 0 to 1.20×10^{-4} mol L^{-1}. After setting a pair of polarizers, the samples were performed at the corresponding excitation and emission wavelengths: 280 and 463 nm, respectively.

2.2.4. DNA melting studies

DNA melting experiments were conducted by determining the absorption of ctDNA at 260 nm in the absence and presence of PSO at temperatures varying from 20 to 100 °C with an interval of 4 °C. The transition midpoints of the curves of $f_{ss} = (A - A_0)/(A_f - A_0)$ versus temperature (T) were regarded as the melting temperatures (T_m) of ctDNA and PSO–ctDNA complex, where A_0 and A_f represent the absorbance intensities at 20 and 100 °C, respectively, and A is the absorbance intensity of the corresponding temperature [22].

2.2.5. Viscosity measurements

Viscometric titrations were performed on an Ubbelohde viscometer (Φ 0.7–0.8 mm, Shanghai Qianfeng Rubber and Glass Co., Shanghai, China), which was kept at 25 ± 0.1 °C by a constant temperature bath. Each flow time of the solutions was measured with a digital stopwatch for five times after being added appropriate amounts of PSO to give a certain r (r = [PSO]/[ctDNA]) value while the ctDNA concentration was kept in constant. The average times