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Exploration of association of telmisartan with calf thymus DNA using a series of spectroscopic methodologies and theoretical calculation

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ABSTRACT

Telmisartan (TMST) can block selectively and irreversibly an angiotensin II receptor type 1 (AT1), but has no effect on other receptor systems and also induce human prostate cancer cell apoptosis. In this work, domain specific association of TMST with calf thymus DNA was explored through multi-spectral methodologies and theoretical calculation. The results of absorption spectroscopy revealed that TMST interacting with ct-DNA formed the TMST–ct-DNA complex through non-covalent interaction and the association constant (K_a) was of $2.71 \times 10^3\ M^{-1}$ at 298 K. Based on the thermodynamic analysis, it can be inferred that the interacting TMST with ct-DNA was an enthalpy-driven, spontaneous, and exothermic process due to the $\Delta G^0 < 0$, $\Delta H^0 < 0$, and $|\Delta H^0| > T|\Delta S^0|$ under the studied temperature ranges. The domain interacting forces for interacting TMST with ct-DNA were van der Waals forces and hydrogen bonding interaction due to the $\Delta H^0 < 0$ and $\Delta S^0 < 0$. Meanwhile, the electrostatic and hydrophobic interactions also played a supporting function. Additionally, from experiments and theoretical calculation (molecular docking), it was also confirmed that TMST interacted to a minor groove of the C-G rich region of ct-DNA and resulted in the slight alteration in the secondary structure of ct-DNA whereas the obvious alteration in the geometry of TMST after binding to DNA, indicating that the flexibility of TMST contributed to stabilizing the TMST–ct-DNA complex.

1. Introduction

Telmisartan (TMST, Fig. 1) is an angiotensin II receptor antagonist, which can block selectively and irreversibly the AT1 receptor and don't affect other receptors in cardiovascular regulation [1, 2]. It can also act as a partial agonist for peroxisome proliferator activated receptor gamma (PPAR-γ) that affects the gene expression involved in carbohydrate metabolism [3]. As a result, TMST has been widely used in treating hypertension, diabetes and cardiovascular disease in patients with hypertension, and congestive heart failure [4–13]. It was also found that TMST could reduce bone mineral density for male spontaneously hypertensive rats [14]. Moreover, it was confirmed from in vitro experimental results that TMST is active for a variety of cancers such as human prostate cancer cell [15], human renal cancer cell [16], and adult T-cell leukemia/lymphoma (ATL) [17]. However, many research results have also confirmed that there are three active pathways for anticancer drugs: (1) the control of transcription factors and polymerases in which drug interacts with proteins that bind to DNA; (2) RNA binding either to DNA to form nucleic acid triple helix structures or to exposed DNA single strand to form DNA–RNA hybrids resulting in interfering with transcriptional activity; (3) the binding of small molecules to DNA double helical structures [18, 19]. In other words, the efficacy of most of anticancer drugs is related with the binding mode and affinity between anticancer drugs and DNA. Therefore, study of the interaction of TMST with DNA has great significance for further investigate its anticancer mechanism.

DNA is an important biological macromolecule, which has two main functions: transcription and replication [20]. Transcript refers to the transformation of genetic information from DNA to RNA to synthesize proteins in the body including hormones, enzymes, carriers, receptors, regulators, etc. replication is the regeneration of DNA itself. Transcription and replication are essential for the survival and proliferation of cells and the smooth operation of all physiological processes. DNA begins to transcribe or replicate only when it receives the signal. It is usually carried out through binding the regulatory protein to a specific region of DNA. If small molecules/drugs can be associated to the specific region of DNA, DNA function can be artificially regulated through substituting small molecules/drugs for the regulatory protein, resulting in inhibiting or activating the DNA function [21]. As a result, the synthetic or natural small molecules can be used as drugs to inhibit or activate the DNA function to cure or control the disease. Thus, the exploration of the intermolecular association between small molecules/drugs and DNA using spectroscopic and theoretical methods

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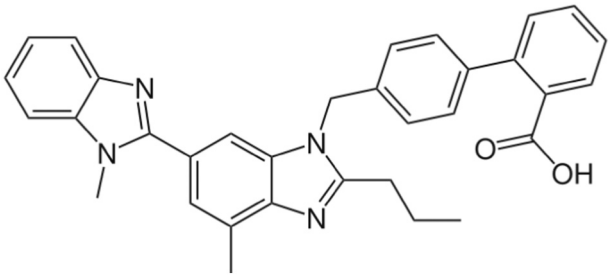

Fig. 1. Structure of TMST.

gradually becomes a topic of interest for many researchers [22–33]. However, to the best of our knowledge, the domain specific association of TMST with DNA has not been studied yet.

Calf thymus DNA (ct-DNA), extracted from calf thymus is one of the common model of DNA and is widely used to investigate the binding interaction of small molecules with DNA. The objective of this work was to research the domain specific association of TMST with ct-DNA, which could be help to further clarify its anticancer activity and anticancer mechanism. For this, various spectroscopic methodologies and computer simulating technology were applied to elucidate the specific association mechanism of TMST with ct-DNA including the information regarding the affinity of TMST on ct-DNA, the binding mode, the conformational change of ct-DNA after associating TMST, and so on.

2. Materials and methods

2.1. Reagents and solutions

Ct-DNA was purchased from Sigma Chemical Co. Ltd. without any purification before use. Ethidium bromide (EB, 95% purity) was obtained from Sigma Aldrich. Rhodamin B (>95% purity) was provided from Aladdin Industrial Corporation. Telmisartan (TMST, >98% purity) was provided from Adamas Reagent Co., Ltd. Other chemicals and solvents were analytical reagent grade.

The stock solution of ct-DNA was prepared in Tris–HCl buffer solution (0.050 M, pH 7.40) and its concentration was determined using absorption spectroscopy according to the previous literature [22]. The TMST was dissolved in dimethyl sulfoxide to prepare TMST stock solution. EB and Rhodamin B were dissolved in anhydrous ethanol, respectively, to obtain the corresponding stock solutions. All solutions were stored in the refrigeration at 4 °C. The working solutions were prepared through diluting stock solution using Tris–HCl buffer solution (0.050 M, pH 7.40).

2.2. Absorption spectral measurements

All absorption spectral measurements were implemented on Shimadzu UV-1601 spectrophotometer (Kyoto, Japan) with 1.0 cm quartz cuvette.

The absorption titration spectra of ct-DNA (5×10^{-5} M) were measured through the titration of TMST solution into the 3 mL of DNA solution in the interval of 5 μL at 298 K, and the corresponding TMST solution was used as reference. The absorption titration spectra of TMST (9×10^{-4} M) upon the increase of ct-DNA ($0 \rightarrow 2.68 \times 10^{-3}$ M) were determined through the titration of DNA stock solution into the 3 mL of TMST solution in the interval of 5 μL at 298 K, 303 K, and 310 K based on the corresponding ct-DNA solution as reference solution and the data were applied for estimating the association constant (K_a) according to Benesi-Hildebrand equation [29, 34]

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_T}{\varepsilon_{T-DNA}-\varepsilon_T} + \frac{\varepsilon_T}{\varepsilon_{T-DNA}-\varepsilon_T} \times \frac{1}{K_a \cdot C_{DNA}^n} \quad (1)$$

where, A_0 and A represent the absorption of TMST without and with ct-DNA, respectively, at 207 nm. C_{DNA} denotes the initial concentration of ct-DNA. ε_T and ε_{T-DNA} are the absorption coefficient of TMST and TMST–ct-DNA system, respectively. n is the stoichiometry of the TMST–ct-DNA complex.

Additionally, the absorbance of the TMST (9×10^{-4} M) and ct-DNA (5×10^{-5} M) system at 259 nm upon the addition of NaCl ($0 \rightarrow 0.05$ M) was monitored to estimate the effect of ionic strength on the interacting forces.

2.3. Steady-state fluorescence spectral determination

The steady-state fluorescence spectra of the fluorescence probes and ct-DNA system with the increase of TMST ($0 \rightarrow 2.5 \times 10^{-5}$ M) were monitored on a LengGuang F97pro Spectrofluorimeter with 10 mm quartz cuvette and the emission and excitation slits of 10/10 nm (Shanghai, China) to estimate the association mode of TMST on ct-DNA. For the EB (3×10^{-6} M) and ct-DNA (4.87×10^{-5} M) system, the excitation wavelength was fixed at 535 nm while the excitation wavelength was fixed at 520 nm for Rhodamin B (0.2×10^{-5} M) and ct-DNA (4.87×10^{-5} M) system.

As is well known, in the determination of steady-state fluorescence spectrum, the fluorescence intensity must be corrected using the following equation because of the inner filter effect (IFE) [22]:

$$F_{cor} = F_{obs} \cdot e^{(A_1+A_2)/2} \quad (2)$$

where, F_{cor} and F_{obs} denote the fluorescence intensities after and before correction at the emission wavelength (λ_{em}). A_1 and A_2 are the total absorbance of all components at the λ_{ex} and λ_{em}, respectively. In this work, the IFE was ignored due to the very low absorbance of the studied system at the λ_{ex} and λ_{em}.

2.4. Assay of ct-DNA viscosity

The viscosities of ct-DNA (2.6×10^{-5} M) solution upon the increase of TMST ($0 \rightarrow 2.25 \times 10^{-5}$ M) were monitored on an Ubbelohde viscometer at 298 K. And, the flow time of the studies solutions through the capillary was determined using a digital stopwatch and the data were used to calculate the viscosity values ($\eta = (t - t_0) / t_0$) (t is the flow times of the TMST and ct-DNA system and t_0 denotes the corresponding Tris–HCl buffer) [22, 29].

2.5. Estimation of CD spectra

The determination of circular dichroism (CD) was implemented on JASCO J-815 Spectrophotometer (Tokyo, Japan) with 10 mm quartz cuvette at ambient temperature. In this work, the concentration of ct-DNA was fixed at 6.7×10^{-5} M while altering the TMST concentration (r = [TMST] / [ct-DNA] = 0, 0.1, and 0.2). And, the Tris–HCl buffer was used as baseline correction.

2.6. Molecular simulation

The initial structure of TMST was downloaded from the PubChem Compound Database and was optimized using Gaussian 03 software package by the density functional theory (DFT) method with B3lyp/6-31G as reported in reference [22, 26]. The optimized structure of TMST was introduced in molecular simulation. The geometries of two B-DNA fragments (PDB ID: 1BNA and 1D29) were downloaded from Protein Data Bank. Their sequences of base pairs are of d(CGCGAATTCGCG)2 and d(CGTGAATTCACG)2, respectively. The association of TMST with B-DNA fragment was simulated through molecular docking in AutoDock 4.2 software package. The molecular docking was carried out according to the previous literature [31].