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Study on the interaction between 4-thio-5-methyluridine and human serum albumin by spectroscopy and molecular modeling

Xiaohui Zhang [a,*], Hongxiu Zhai [a], Ruiqi Gao [a], Juling Zhang [b], Yue Zhang [a], Xuefang Zheng [a]

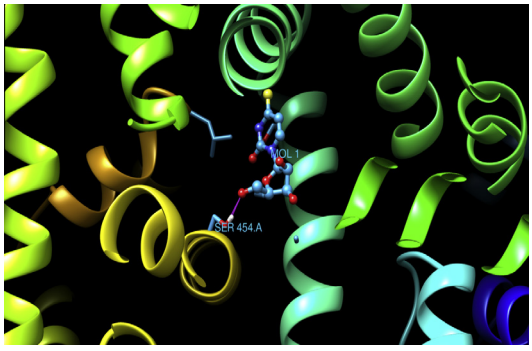
[a] College of Environment and Chemical Engineering, Dalian University, Dalian, Liaoning 116622, China
[b] MOE Key laboratory of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou, Guangdong 510631, China

HIGHLIGHTS

- The interaction between 4-thio-5-methyluridine and human serum albumin (HSA) was investigated by spectroscopy.
- The main forces of both interactions have typical static interaction from thermodynamic data to the determination.
- 4-Thio-5-methyluridine has a static fluorescence quenching on human serum albumin (HSA).
- Molecular modeling of interaction between 4-thio-5-methyluridine and HSA was investigated.
- The experimental result was in correspondence with molecular modeling theory.

GRAPHICAL ABSTRACT

Molecular modeling of interaction between 4-thio-5-methyluridine and HSA.



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ABSTRACT

The interaction between 4-thio-5-methyluridine and human serum albumin (HSA) under simulative physiological conditions has been studied by the methods of fluorescence, UV–VIS absorbance and circular dichroism (CD) spectroscopy. The results show that 4-thio-5-methyluridine has a static fluorescence quenching on human serum albumin (HSA). The main forces of both interactions have typical static interaction from thermodynamic data to the determination. The experimental result was in correspondence with molecular modeling theory.

Introduction

Nucleic acids (DNA and RNA) are fundamental biomolecules, playing crucial roles in all forms of life. Both DNA and RNA are composed of the three common moieties, i.e., bases, sugar and phosphate, but the subtle difference is that in RNA ribose is used as the sugar block while in DNA deoxyribose is used instead. The moiety containing the base and sugar is termed as ribonucleoside and deoxyribonucleoside respectively. Nucleoside and their derivatives exhibit significant anticancer, antiviral, and antibacterial activities [1–3]. Nucleosides can be modified on either the sugar or the base. Both types of modified nucleosides are of biological interest. Therefore the large number of modified nucleosides offers an attractive playfield for synthetic chemists to prepare and for others to explore their chemical, physical and biological properties.

* Corresponding author. Tel.: +86 15842639633; fax: +86 411 87402449.
E-mail address: xiaohui99@hotmail.co.uk (X. Zhang).

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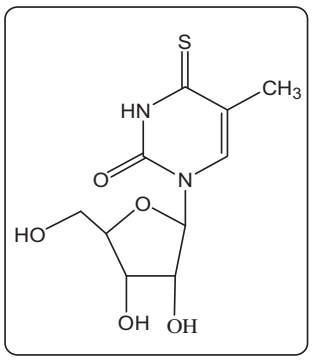

Fig. 1. The structure of 4-thio-5-methyluridine.

According to the literature [4,5], a pyrimidine ring series of compounds with thio (mercapto) group have anti-cancer activity and immune enhancement. Lipsett first separated 4-thiouridylic acid from escherichia coli, sulfur and thio analogs of DNA (such as thio-bases and thio-nucelosides) began to attract people's attention [6]. Thio DNA and thio analogs have unique properties, especially sulfur bases are very sensitivity to ultraviolet [7–11]. Nucleotides (A, G, C, T) are the main chromophoric groups of DNA, they absorb short wavelengths UVC (100–280 nm) and UVB (280–320 nm), but sulfur analogs have strong absorption in UVA (320–400 nm). Xu et al. have found that 4-thiothymidine with UVA light can selectively damage DNA and kill cancer cells, while just a little collateral damage to normal cells [12–15]. It provides a new photochemotherapy for cancer and other diseases [16–19]. We synthesized a new compound 4-thio-5-methyluridine and found that its maximum UV absorption was at 335 nm, which showed more sensitive to UVA light compared with 5-methyluridine.

Human serum albumin (HSA) is the most abundant carrier protein constituted of blood plasma and has been widely studied at the moment [20]. HSA is often used as the study for drug and protein interaction [21]. Therefore, studying the interaction between the drug molecule and HSA helps to understand the transportation, distribution and metabolism of the drug in the human body, and also has a very important significance for clarifying pharmacological effects and pharmacokinetics [22].

In this paper, a combined method of calculation and spectroscopy experiments was used to further study the mechanism of the interaction between 4-thio-5-methyluridine (4-SMeU, Fig. 1) and human serum albumin (HSA), which provided experimental data and theoretical basis to explore the influence on HSA by thionucleoside.

Experimental section

Materials and apparatus

UV–VIS spectrophotometer (JASCO, Japan); FP-6500 spectrofluorimeter (JASCO, Japan); pH-acidometer (Shanghai Lei Ci Device Works, Shanghai, China); JULABO-F12 thermostat (Germany, ±0.01 °C); J-810 circular dichroism spectrophotometer (JASCO, Japan); FA1004 Electronic balance (Shanghai Jing Ke Device Works, Shanghai, China).

HSA (Sigma) was directly dissolved in 0.05 M Tris–HCl to prepare the solution (1.0×10^{-6} M HSA), and the stock solution was kept in the dark at 0–4 °C; Tris (hydroxymethyl) aminomethane (Biochemical reagents in Shanghai); Uridine (Sigma , 98% purity); others reagents are AR; 0.05 M Tris–HCl buffer solution of pH 7.40; 4-thio-5-methyluridine was directly dissolved in 0.05 M Tris–HCl to prepare the solution (1.0×10^{-3} M); Double distilled water was used throughout the experiment.

Interaction between 4-thio-5-methyluridine and human serum albumin (HSA)

UV absorption spectroscopy experiments

2 mL 1.0×10^{-6} M HSA was pipetted into a 1 cm quartz cell, 0.05 M Tris–HCl buffer solution was used as control, then an appropriate amount of 4-thio-5-methyluridine solution was added by micro-injector. Samples were settled for 3 min, then the ultraviolet absorption spectrum was measured in 220–450 nm range (4-thio-5-methyluridine solution was added a total volume less than 50 μL).

Fluorescence spectroscopy experiments

At 290 K, 2.5 mL 1.0×10^{-6} M HSA was pipetted into a 1 cm quartz cell, an appropriate amount of 1.0×10^{-3} M 4-thio-5-methyluridine solution was added by micro-injector (a total volume less than 50 μL). Shaking and then standing for 3 min. Under conditions that 280 nm excitation wavelength, fluorescence excitation and emission slit width were 5 nm/5 nm, the scanning speed was 500 nm/min, 0.05 M Tris–HCl buffer solution was used as a blank correction, the fluorescence spectrum of HSA and HSA added 4-thio-5-methyluridine was measured in the 290–450 nm range. According to the same method, the fluorescence spectrum at the temperature of 300 K and 310 K was measured.

Circular dichroism spectra

At 290 K, 500 μL HSA was pipetted into a 1 mm round cell, an appropriate amount of 4-thio-5-methyluridine solution was added by micro-injector. Samples were settled for 3 min. Under conditions that scanning speed was 50 nm/min, response time was 2 s, the cumulative number was one time, circular dichroism spectra was measured in the 190–250 nm range.

Molecular modeling study

The initial structures of all the molecules were generated by molecular modeling software Sybyl 6.9.1. The potential of the 3D structures of HSA was assigned according to the AutoDock 4.0 force field with Kollman-all-atom charges. The geometries of the antitumor drug (4-thio-5-methyluridine) subsequently optimized using the Tripos force field with Gasteiger–Masili charges. The AutoDock 4.0 program was used to calculate the interaction modes between the drug and HSA. The Lamarckian Genetic Algorithm (LGA) applicated in AutoDock was applied to calculate the possible conformation of the drug that binds to the HSA. During the docking process, a maximum of 10 conformers was considered for the drug. The conformer with the lowest binding-free energy was used for further analysis. All calculations were performed on a SGI FUEL workstations.

Results and discussion

Ultraviolet absorption spectrum of the interaction between 4-thio-5-methyluridine and HSA

The UV–VIS absorption spectra of the unchanged absorption spectrum [HSA–4-SMeU] were also investigated. It was found that HSA absorbance increases with the increase in concentration of 4-thio-5-methyluridine, the peak position is unchanged, but HSA absorbance increases with the increased concentration of 4-thio-5-methyluridine as shown in Fig. 2. The three types