Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 204 (2018) 209–216

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Exploration of intermolecular interaction of calf thymus DNA with sulfosulfuron using multi-spectroscopic and molecular docking techniques

Jie-Hua Shi *, Yan-Yue Lou, Kai-Li Zhou, Dong-Qi Pan

College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310032, China

ARTICLE INFO

Article history:
Received 1 February 2018
Received in revised form 21 May 2018
Accepted 14 June 2018
Available online 18 June 2018

Keywords:
Sulfosulfuron
Calf thymus DNA
Groove binding
Spectroscopy
Molecular modeling

ABSTRACT

As a sulfonylurea herbicide, sulfosulfuron is extensively applied in controlling broad-leaves and weeds in agriculture. It may cause a potential risk for human and herbivores health due to its widely application and residue in crops and fruits. The study of the binding characteristics of calf thymus DNA (ct-DNA) with sulfosulfuron was performed through a series of spectroscopic techniques and computer simulation. The experimental results showed sulfosulfuron interacted with ct-DNA through the groove binding. The negative values of thermodynamic parameter (ΔH^0, ΔS^0 and ΔG^0) revealed that the reaction of sulfosulfuron with DNA could proceed spontaneously, and the hydrogen bonding and van der Waals forces were essential to sulfosulfuron–ct-DNA binding, which was further verified by molecular docking study. Meanwhile, the electrostatic and hydrophobic interactions also played a supporting function for the interaction of sulfosulfuron with ct-DNA. The circular dichroism (CD) results exhibited a minor change in the secondary structure of ct-DNA during interaction process. Moreover, the conformation of sulfosulfuron had the obvious change after binding to DNA, which suggested that the flexibility of sulfosulfuron contributed to stabilizing the sulfosulfuron–ct-DNA complex.

1. Introduction

Deoxyribonucleic acid (DNA) is an important carrier for genetic information and attends dispensable life activities such as gene transcription, translation and expression, protein synthesis, and cell death. However, small molecules binding interaction with DNA could artificially change and/or inhibit the structure and function of DNA [1]. In general, small molecules interact with ct-DNA via the non-covalent patterns [2]: (i) intercalation into the stacked base pairs of DNA, (ii) interaction on the DNA groove via hydrogen bonding or van der Waals interaction, and (iii) electrostatic binding with the anionic sugar phosphate of DNA. Among these interaction ways, the intercalative binding can cause the most damage to DNA and influence the duplication of DNA. This is often the initiation stage of carcinogenicity when damaging the repair system [3]. As is well known, the long-term effects of pesticide (such as herbicide, insecticide and fungicide) exposure are associated with many diseases. And, many studies results reveal that there is an association between pesticide exposure and incidence of certain types of cancers such as lung and prostate cancers [4]. In other words, there are concerns about the genotoxic risk associated with long-term exposure to pesticides as these compounds may damage DNA, resulting in mutations that eventually may lead to cancer, neurological, and reproductive adverse health effects. Therefore, the exploration of the intermolecular interaction of pesticides (such as herbicide, insecticide and fungicide) with DNA has an important sense for further exploring their possible genotoxicity and has aroused great attention in the field of life sciences and chemistry [5–9].

Sulfosulfuron is a sulfonylurea herbicide, which is widely applied in controlling broad-leaves and weeds from crops for ascertaining quality and quantity production [10]. In particular, sulfosulfuron has an adaptation to all major wheat production areas including Europe, Australia, India, North America, parts of Africa, and so on [11]. The significance of evaluating chronic exposures and cumulative risk assessments has grown a focus issue over the years [12]. There is evidence that sulfosulfuron has side effects in rats (pathology of the urinary bladder, kidney and ureter) [13]. In addition, the use of sulfosulfuron at more than recommended does has raised a major concern regarding the health hazards for animals and humans due to its higher residues in soil and crop [14]. Recently, sulfosulfuron has being detected in natural rain and air [15]. Besides, sulfosulfuron could be able to leach to deeper in the soil profile and leading accumulation due to its high solubility property [16]. However, in alkaline, dry and low-organic matter soils, the degradation of sulfosulfuron is quietly slow [17]. Previous study presented that sulfosulfuron has phytotoxicity to some sensitive crops like lentil and pea [18]. Hence, the residue of sulfosulfuron in the soil could

* Corresponding author.
E-mail address: shijh@zjut.edu.cn (J.-H. Shi).

https://doi.org/10.1016/j.saa.2018.06.054
1386-1425/

cause the succeeding sensitive crops damage as well as have a side effect on human and herbivores wellness owing to accumulation of its residues in grains. To the best of our knowledge, there is not the report regarding whether it can overcome the barrier of the cell membrane to get to the cellular DNA. However, we found some sulfonylurea herbicides have the character of overcoming the barrier of the cell membrane to damage the cellular DNA. For example, research results revealed that tribenuron methyl has a marginal DNA damaging effect on the leukocytes [19] and there was chromatin and probable gene decompaction whening acting nicosulfuron on 3rd-instar D. Daccharalic [20]. Sulfosulfuron as one of sulfonylurea herbicides maybe overcome the barrier of the cell membrane to get to the cellar DNA and to damage DNA. The exploration of intermolecular interaction of sulfosulfuron with DNA is of great significance to further elucidate whether it has genotoxicity.

The present study was mainly to investigate the interaction mechanism of sulfosulfuron and calf thymus DNA (ct-DNA), which could help us to elucidate its toxicological mechanism. Various spectroscopies and computer simulating technology were implemented to clarify the mode of binding sulfosulfuron with ct-DNA. Circular dichroism was performed for exploring conformational change of ct-DNA. Thermodynamic parameters were determined to provide effective support of binding forces of sulfosulfuron–ct-DNA complex. Molecular docking was also carried out to get valid information regarding the binding mode and binding forces.

2. Materials and Methods

2.1. Reagents and Solutions

Sulfosulfuron (≥97%) was purchased from Shanghai Tengzhun Biotechnology Co., Ltd. Ct-DNA was obtained from Sigma Chemical Co. Ltd. Ethidium bromide (EB) was purchased from Shanghai Titan Scientific Co., Ltd. and Hoechst 33258 that is called as bisBenzimide H 33258 was purchased from Aladdin Industrial Corporation. All other chemicals and solvents belong to analytical reagent grade.

The ct-DNA was dissolving in 0.050 M Tris–HCl buffer solution (pH 7.40) with a proper concentration and used within five days. Its absorbance ratio (A_{260}/A_{280}) was 1.80, implying that the ct-DNA was sufficiently free from protein [21]. The final concentration of ct-DNA was determined by UV spectrophotometry with the $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$. Sulfosulfuron was dissolving in 0.050 M Tris–HCl buffer solution (pH 7.40) with the concentration of 1.5×10^{-2} M. EB (3×10^{-3} M) was prepared in anhydrous ethanol, and Hoechst 33258 (3×10^{-3} M) was prepared in redistilled water. All stock solutions were maintained in the dark at 4 °C. And, all the fresh working solutions were prepared daily from the stock solutions by proper dilution.

2.2. UV Spectra Measurements

All absorption spectra were performed on Shimadzu UV-1601 spectrophotometer (Kyoto, Japan) with 1.0 cm quartz cuvette. The UV spectra of ct-DNA with addition of sulfosulfuron were determined at 298 K. The corresponding free sulfosulfuron solutions were as reference solution. The change in the net volume could be ignored because the relative deviation caused by the change in the cumulative volume of the stock solution of sulfosulfuron was ≤1.8%. While the UV spectra of sulfosulfuron with increment of ct-DNA were determine at three temperatures (298, 303, and 310 K) to measure the binding constant and to illustrate the impact of temperature on the interaction between sulfosulfuron and ct-DNA. The corresponding free ct-DNA solutions were as reference solution and the relative deviation caused by the change in the cumulative volume of the stock solution of DNA was ≤1.8%.

2.3. Fluorescence Spectra Determination

When varying the concentration of herbicide sulfosulfuron, fluorescence spectra of all mixture solutions of ct-DNA (5×10^{-5} M) and fluorescence probes such as EB (3×10^{-6} M), Hoechst 33258 (3×10^{-6} M) and Rhodamin B (0.2×10^{-6} M) were measured by a LengGuang F97pro Spectrofluorimeter in the range of 400–800 nm using a 1.0 cm quartz cuvette (Shanghai, China) at 298 K. Here the emission and excitation slits were fixed at 10 and 5 nm, respectively. And the excitation wavelengths for EB, Hoechst 33258, and Rhohamin B were fixed at 535, 345, and 520 nm, respectively.

The fluorescence intensity was corrected due to inner filter effects (IFE) on the basis of the following equation [22]:

$$F_{cor} = F_{obs} \cdot e^{(A_1+A_2)/2} \quad (1)$$

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensities at the emission wavelength (λ_{em}), respectively. A_1 and A_2 are the sum of the absorbance of all components at the λ_{ex} and λ_{em}, respectively.

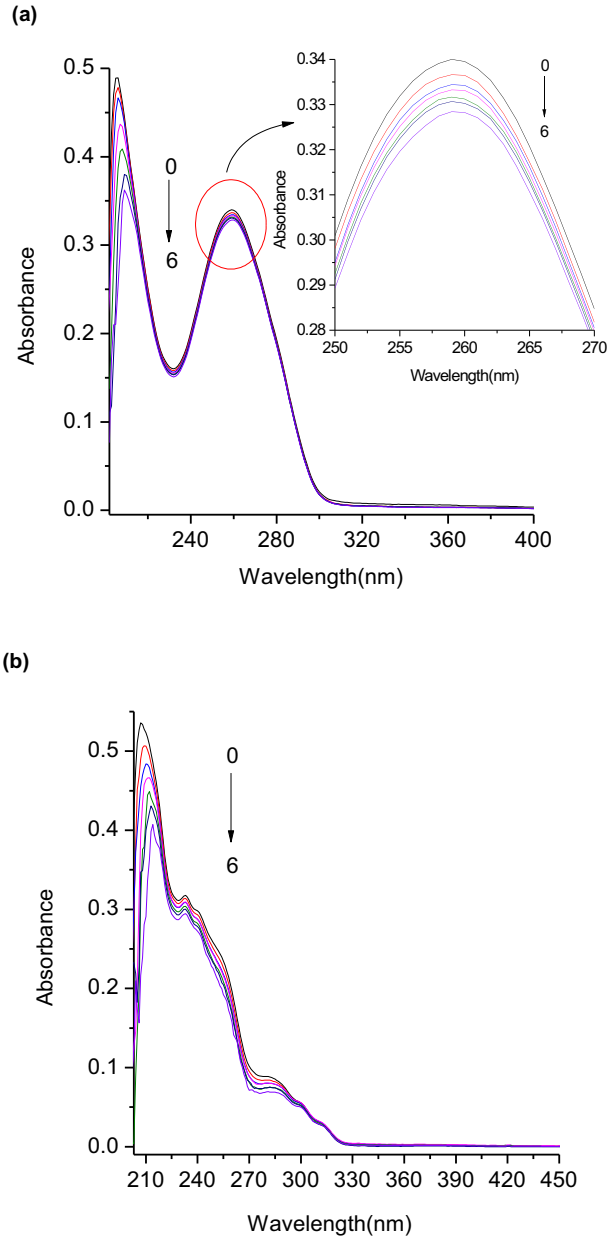

Fig. 1. (a) UV absorption spectra of ct-DNA (5.0×10^{-5} M) with increasing the concentrations of sulfosulfuron from 0 to 3.0×10^{-5} M at intervals of 5×10^{-6} M in Tris-HCl buffer (pH 7.40) at 298 K. (b) UV absorption spectra of sulfosulfuron (1.0×10^{-5} M) with the addition of ct-DNA from 0 to 24.1×10^{-5} M at intervals 4.02×10^{-5} M at 298 K.