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Intercalation of herbicide propyzamide into DNA using acridine orange as a fluorescence probe

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ABSTRACT

The binding action of PRO associated with calf thymus DNA (ctDNA) was determined using multi-spectroscopic methods combined with parallel factor analysis (PARAFAC) and molecular docking. The fluorescence titration suggested a static fluorescence quenching of PRO was induced by ctDNA. The positive values of enthalpy changes and entropy changes suggested that the binding reaction was predominantly driven by hydrophobic interactions. Increases in melting temperature and viscosity of ctDNA and decrease in iodide-quenching effect indicated that an intercalative binding of PRO to ctDNA occurred. Moreover, the three-way synchronous fluorescence spectra data from the competitive interaction between PRO and fluorescence probe acridine orange (AO) with ctDNA were resolved by PARAFAC modeling to provide the concentration information and spectra of the three components (PRO, AO and ctDNA–AO complex) for the reaction system, which further supported the intercalation of PRO molecule into ctDNA double-helix by replacing bound AO probe. The molecular docking predicted that the A–T base pairs of ctDNA were the main binding sites for PRO, which was confirmed by Fourier transform infrared analysis, and the conformational change of ctDNA from B-form to A-form induced by PRO was inferred from the circular dichroism spectra studies.

1. Introduction

DNA plays a vital role in the process of human life as it is the carrier of genetic information and the material basis of gene expression [1,2]. DNA is the main target of exogenous hazardous substances within an organism [3]. Hence, evaluating the interactions of pollutants with DNA is of great importance because the formation of pollutant–DNA adducts is closely related to abnormalities, such as cancer and genetic diseases [4,5]. Nowadays, pesticides have been extensively used in agriculture throughout the world and become major environmental pollutants. Many pesticides such as paraquat, fenitrothion and chloridazon have been reported to interact with DNA and induce serious genetic damage [6–8]. Exploring the binding action between pesticides and DNA may provide useful information in understanding the structural properties of DNA and toxic mechanism of pesticides as well as designing new and more efficient pesticides with low toxicity [9].

Propyzamide (PRO, chemical structure shown in Fig. 1) is an effective and widely used herbicide, which is applied in weed control during the cultivation process of lettuce, endive, witloof and so on [10]. However, previous studies have found that PRO exerted chronic health effects on human involving in cancer, endocrine disruption and reproductive toxicity [11]. PRO has been assessed as the most likely human carcinogen (B-type) by US Environmental Protection Agency [12]. To the best of our knowledge, no investigation was conducted on the interaction between PRO and DNA, which may provide new insights into the toxicity mechanism of this herbicide.

Spectroscopic approaches were considered as sensitive techniques to investigate the interaction of small molecules and DNA. However, when the spectra of small molecules highly overlapped with that of DNA, it is difficult to get the accurate information for the formation process of small molecules–DNA complex, and the concentration changes of reaction components during the reaction process are unavailable by conventional spectroscopic methods. The application of spectroscopic techniques combined with chemometrics methods has attracted much attention in recent years, because it can provide crucial information of the interaction between small molecules and biomolecules. Parallel factor analysis (PARAFAC), a soft modeling algorithm, has been introduced to investigate the interaction of small molecules and DNA with the aid of fluorescence probes [13,14]. The pure spectra and equilibrium concentrations of coexisting species can be directly processed by

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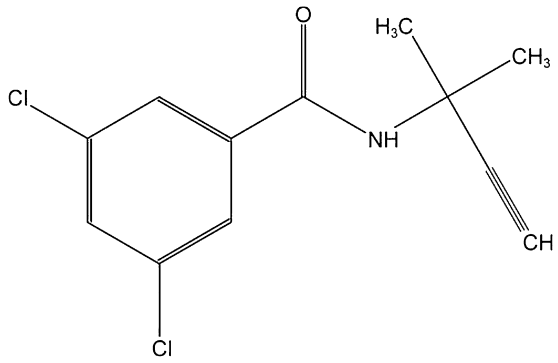

Fig. 1. Molecular structure of propyzamide (PRO).

PARAFAC, which is helpful to identify the reacting species involved and quantitatively evaluate the interaction process [15].

In this study, the binding action of PRO to calf thymus DNA (ctDNA) in physiological buffer (pH 7.4) was investigated with the aid of acridine orange (AO) dye as a fluorescence probe by the application of multispectroscopic methods including UV–vis absorption, fluorescence, circular dichroism (CD) and Fourier transform infrared (FT-IR) spectroscopy along with DNA viscosity and melting measurements. Moreover, the PARAFAC method was used to resolve the three-way synchronous fluorescence spectral data of the competitive interaction between PRO and AO probe with ctDNA so as to extract the concentration and spectra profiles of the reacting species, and further understand the complex kinetic process. In addition, molecular docking technique was employed to predict the probable binding sites and binding mode of PRO with ctDNA, which can in turn validate the results of spectroscopic studies.

2. Materials and methods

2.1. Chemicals

PRO and ctDNA were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Sigma–Aldrich Co. (St. Louis, MO, USA), respectively. The stock solution of PRO (3.90×10^{-3} mol L^{-1}) was prepared by dissolving its crystals in 95% (v/v) ethanol. An appropriate amount of ctDNA was dissolved in a 0.1 mol L^{-1} NaCl solution to give a final concentration of 3.61×10^{-3} mol L^{-1}. The monitored absorbance at 260 and 280 nm gave a ratio of $A_{260}/A_{280} > 1.8$, indicating that ctDNA was sufficiently free from protein [16]. The stock solution of AO (1.0×10^{-3} mol L^{-1}, Sigma Chem. Co., USA) was prepared in ultrapure water (18.2 MΩ). Tris–HCl buffer of pH 7.4 was used to control the acidity of the reaction system. All other chemicals were of analytical reagent grade, and ultrapure water was used throughout the work.

2.2. Spectroscopic measurements

Fluorescence intensities were measured using a Hitachi spectrofluorimeter (Model F-7000, Hitachi, Japan) equipped with a 150 W Xenon lamp and a thermostat bath. The excitation wavelength was set at 254 nm and the fluorescence spectra were measured with a constant concentration of PRO while gradually increasing the concentration of ctDNA. The mixed solutions were equilibrated for 5 min before the spectral measurement at different temperatures (292, 298, 304 and 310 K). To correct background fluorescence, appropriate blanks concerning with the Tris–HCl buffer were subtracted. All the fluorescence data in this study were corrected for absorption of excitation light and reabsorption of emitted light [17].

The competitive synchronous fluorescence spectral measurements were recorded as follows: a fixed amount of ctDNA–AO mixture was titrated with increasing amounts of PRO solution (total of nine samples). All these solutions were allowed to stand for 5 min to equilibrate, and then the three-way synchronous fluorescence spectra were collected over the excitation wavelength range of 200–700 nm at 1 nm increments (total of 501 wavelengths) and $\Delta\lambda$ (a constant wavelength difference between emission and excitation wavelengths) changed in the range of 10–200 nm at 10 nm intervals (total of 20 $\Delta\lambda$ increments). Thus, the three-way data matrix, $\boldsymbol{X}_{I\times J\times K}$, the dimensions of 20 ($\Delta\lambda$ wavelengths) × 501 (excitation wavelengths) × 9 (samples) were defined by $I \times J \times K$, was obtained and then decomposed by PARAFAC. The synchronous fluorescence spectra were employed in the Tris–HCl buffer of pH 7.4 at 298 K.

The CD spectra of ctDNA with increasing concentration of PRO were recorded at room temperature on a Bio-Logic MOS 450 CD spectrometer (Bio-Logic, Claix, France) using a 1.0 mm quartz curette. The samples of the ctDNA–PRO mixture at the molar ratios ([PRO]/[ctDNA]) of 0, 1/20 and 1/10 were measured at wavelengths between 230 and 320 nm, and the Tris–HCl buffer background was subtracted from the samples.

Infrared spectroscopic measurements of ctDNA and the ctDNA–PRO complexes were conducted using a FT-IR spectrometer (Thermo Nicolet-5700, USA) equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter in pH 7.4 Tris–HCl buffer at room temperature. The spectra of ctDNA and the ctDNA–PRO complexes after 2 h of incubation were collected by the ATR method over the range of 1800–800 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans. The spectrum of Tris–HCl buffer (pH 7.4) background was subtracted from the spectra of free ctDNA and ctDNA–PRO complexes. The difference spectra [(ctDNA solution + PRO solution) – (PRO solution)] were obtained referring to the sharp ctDNA band at 968 cm^{-1}. The association band of water around 2200 cm^{-1} was used as a reference for subtraction of water and achieved to a flat baseline. The relative intensity (R) of several peaks of ctDNA-in-plane vibrations were normalized using $R_i = I_i/I_{968}$, where I_i denotes the intensity of absorption peak at i cm^{-1} for pure ctDNA and the ctDNA–PRO complex at different concentrations of PRO, and I_{968} is the intensity of the 968 cm^{-1} peak as the internal reference.

2.3. Molecular simulation

The docking studies were carried out using AutoDock docking software (version 4.2) to predict the preferred binding sites and mode of PRO on ctDNA. The structure of PRO was constructed using software SYBYL × 1.1 (Tripos Inc., St. Louis, USA) and its geometry optimization was refined with the aid of the MMFF94 force field using MMFF94 charges. The crystal structure of DNA (PDB code: 453D) was taken from the Protein Data Bank [18]. Then the models of the ligand and macromolecule were processed with the aid of AutoDock tools (ADT) and submitted to conduct docking. To determine the preferred binding sites on DNA, PRO molecule was allowed to move within the whole region of DNA via 100 runs to obtain the possible binding gesture.

2.4. DNA melting measurements

DNA melting studies were performed by monitoring the absorption of ctDNA at 260 nm in the absence and the presence of PRO at various temperatures (from 20 to 100 °C). All measured values of A_{260} were substituted into the following equation [19]:

$$f_{ss} = \frac{A - A_0}{A_f - A_0} \quad (1)$$