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Multi-spectroscopic method study the interaction of anti-inflammatory drug ketoprofen and calf thymus DNA and its analytical application

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ABSTRACT

Interactions of the anti-inflammatory drug ketoprofen with calf thymus DNA (ctDNA) in aqueous solution have been studied by multi-spectroscopic method including resonance light scattering (RLS) technique, ultraviolet spectra (UV), ^{1}H NMR, etc. The characteristics of RLS spectra, the effective factors and optimum conditions of the reaction have been unequivocally investigated. Mechanism investigations have shown that ketoprofen can bind to ctDNA by groove binding and form large particles, which resulted in the enhancement of RLS intensity. In Critic acid-Na_2HPO_4 buffer (pH = 6.5), ketoprofen has a maximum peak 451.5 nm and the RLS intensity is remarkably enhanced by trace amount of ctDNA due to the interaction between ketoprofen and ctDNA. The enhancement of RLS signal is directly proportional to the concentration of ctDNA in the range of 1.20×10^{-6}–1.0×10^{-5} mol/L, and its detection limit (3σ) is 1.33×10^{-9} mol/L. The method is simple, rapid, practical and relatively free from interference generated by coexisting substance, and was applied to the determination of trace amounts of nucleic acid in synthetic samples with satisfactory results.

1. Introduction

The study of the interaction of DNA with small molecules such as drugs, organic dyes and metals has been an intensive topic for decades because it provides insight into the screening design of new and more efficient drugs targeting to DNA, which can speed up the drug discovery and development processes [1]. DNA is one of the most important biological molecules targeted by many small molecules. Also, during the past decades, along with more attention has been given on the research of the interaction between DNA and small molecular, the molecular mechanisms of the action of some drugs and origins of some diseases have generally been understood [2–5]. The investigation interaction based on small molecules bind to DNA is important to understand molecular mechanisms of drug action and contribute to the design of several DNA-targeted drugs.

Ketoprofen, 2-(3-benzoylphenyl)-propionic acid (The structure of ketoprofen is given in Fig. 1.) is one of the propionic acid class of non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic effects. This medicine works by reducing hormones that cause inflammation, stiffness and pain in the body. So far, the molecular mechanisms underlying its anti-inflammatory effect are still unknown. Moreover, it is unclear whether classical resistance mechanisms might limit the use of ketoprofen. In addition, new specific antibiotics are in scope of interest because of their potential anticancer activity [6]. Therefore, it is important to study the binding characteristics and molecular mechanism of ketoprofen. In recent years, resonance light scattering (RLS) technique has gained growing interests in the investigation of DNA–drug interactions [7–9], due to its simplicity, rapidness and economy. It also applied to study biomacromolecules such as proteins [10,11] and heparin [12], and has been used for the determination of some trace inorganic ions [13,14], organic compounds [15] and pharmaceuticals [16,17]. There has not yet any report about the detection of the ketoprofen–DNA interaction based on RLS technique.

As a new spectral analysis technique, the light scattering measurements by using a common spectrofluorometer on supramolecular assemblies of chromophores is sensitive and selective. This technique is generally coupled to other spectral analysis techniques such as absorption, fluorescence and NMR to study the interaction of drug molecules with DNA, whose results are very valuable in designing drugs for clinical use and developing sensitive chemical probes of nucleic acid structure [18].

Studying the interaction between ketoprofen and DNA may help to simplify the mechanism of action of this important class of antibiotic agents, and may perhaps at last lead to the design of better and more effective drugs with fewer side effects. Lately, structure based design strategies exploiting drug–DNA interaction have yielded new DNA binding agents with clinical assurance.

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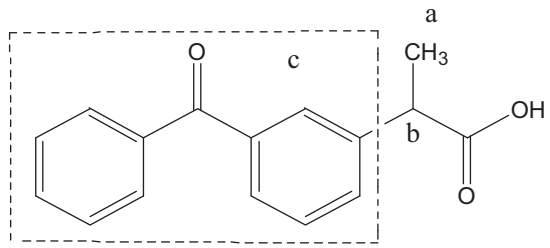

Fig. 1. Molecular structures of ketoprofen.

2. Experiment

2.1. Chemicals

Ketoprofen was purchased from Sigma and used without further purification. Calf thymus double-stranded DNA, purchased from Sigma, was directly dissolved in water without further purification and stored at 4 °C. The concentrations of nucleic acids were determined according to the absorbance at 260 nm after establishing that the absorbance ratio A260/A280 was in the range of 1.80–1.90 for ctDNA. Critic acid–Na_2HPO_4 solution was used as buffer solution to control the acidity of the reaction system. All the reagents are analytical reagent grade and used without further purification. Milli-Q purified water (18.2 MΩ) was used for all sample preparations.

2.2. Apparatus

The RLS spectra and the intensity were measured with a RF-5301PC (Shimadzu, Japan) fluorescence spectrometer by using 1.00 cm quartz fluorescence cell. All absorption spectra were measured on a La 25 UV/vis spectrometer (PE, USA). ^{1}H NMR was recorded on a BRUKER 400 spectrometer for solution in $D_2O + CD_3OD$ with tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported downfield in part per million (ppm) and *J*-values were in Hz. A pHs-3C digital pH meter (Leici, Shanghai) was utilized to detect the pH values of the aqueous solutions. Viscosity experiments were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 25.0 ± 0.1 °C in a thermostatic water-bath.

2.3. Experimental procedure

Into a 10 mL volumetric flask were added appropriate ketoprofen solution, 1.5 mL of Critic acid–Na_2HPO_4 buffer solution, and an appropriate ctDNA solution, then the mixture was shaken by hand after each addition of the interacting additives, and then it was diluted to 10 mL with water and mixed thoroughly for RLS, absorption or fluorescence measurements. The RLS spectra were recorded with synchronous scanning the excitation and emission monochromators ($\Delta\lambda = 0$ nm) from 250.0 to 700.0 nm. The intensity measurement and the spectrum scanning of the RLS were both made by keeping the slit width for excitation and emission at 3.0 nm. Based on the spectra, the RLS intensity was measured at 451.5 nm. The RLS intensity of the ketoprofen increased by ctDNA was represented by $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$, where I^0_{RLS} and I_{RLS} were the intensities of the ketoprofen without and with ctDNA, respectively. All measurements were made at an ambient temperature of 25 °C.

3. Results and discussion

3.1. RLS spectrum characteristic

Fig. 2 shows that the RLS spectra of ctDNA, ketoprofen and the mixture of ctDNA with ketoprofen at Critic acid–Na_2HPO_4 buffer (pH = 6.5) solution, respectively. It can be seen that both ctDNA and ketoprofen have weak RLS signals when they exist separately in aqueous solution over the scanning wavelength range of 250.0–700.0 nm. However, when the DNA is mixed with ketoprofen, the I_{RLS} is remarkably enhanced and the maximum scattering peak is located at 451 nm. This indicates that interactions between ctDNA and ketoprofen have occurred [19]. Moreover, it was found that in the RLS spectrum, there was a linear relationship between the enhanced intensity and the concentration of nucleic acid. It was concluded that the RLS technique was based on the aggregation of probe molecules on the surface of nucleic acid, which resulted in enhanced RLS intensity. According to the RLS theory [19], the intensity of a RLS signal should depend sensitively on the size of the aggregate and the extent of the electronic coupling among chromotophores. The increscent size of the particles may lead to great enhancement of I_{RLS} of ketoprofen. Considering the sensitivity of detection, the maximum scattering wavelength at 451.5 nm is selected for the subsequent work.

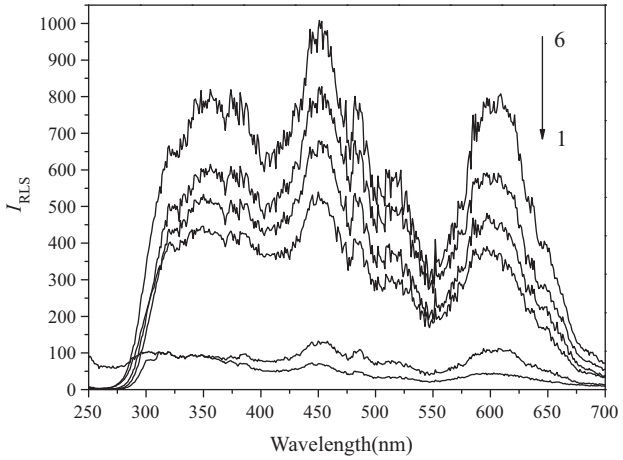

Fig. 2. Resonance Light scattering spectra of ketoprofen–ctDNA system. Condition: (1) ctDNA, 8.64×10^{-6} mol/L; (2) ketoprofen, 1.6×10^{-5} mol/L; and (3–6) ketoprofen–ctDNA complex. Concentration of ctDNA: 3, 4.92; 4, 6.16; 5, 7.40; 6, 8.64×10^{-6} mol/L; and ketoprofen, 1.6×10^{-5} mol/L; pH 6.5.

3.2. Absorption characteristic

The application of electronic absorption spectroscopy in DNA-binding studies is a most useful technique. In Fig. 3, the absorption spectra of ketoprofen (at a constant concentration) is shown in the absence and presence of ctDNA in Critic acid–Na_2HPO_4 buffer solution. From the Fig. 3 it is seen that with each addition of ctDNA to ketoprofen solution, the entire absorption spectrum undergoes a hyperchromic effect without any noticeable spectral shift. According to the literatures [20] intercalation into DNA base pairs is characterized by a red shift and the hypochromic effects in the absorption spectra, the outside groove binding is characterized by no (or minor) changes of UV–vis spectra, occasionally with some hyperchromicity. Again, outside binding with self-stacking shows quite similar characteristics as the intercalative binding mode but to a lesser extent [21,22]. Thus, from the UV–vis absorption study indicated that ketoprofen forms a ground-state complex with ctDNA could not be via intercalative interaction. Perhaps, the binding between ketoprofen and DNA is in the mode of outside groove binding or electrostatic binding.