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Investigation on the interaction between luteolin and calf thymus DNA by spectroscopic techniques

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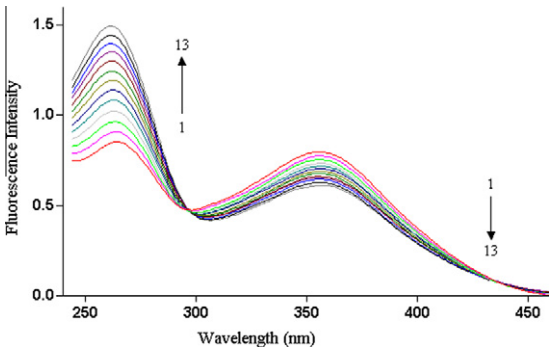
HIGHLIGHTS

- Luteolin could binds to ctDNA with a high affinity at physiological pH.
- The acting forces between luteolin and DNA mainly included hydrophobic interactions and hydrogen bonds.
- The fluorescence quenching mechanism of AO–ctDNA by luteolin was a static quenching type.
- The interaction between luteolin and ctDNA might occur via intercalative mode.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of luteolin with calf thymus deoxyribonucleic acid (ctDNA) under physiological conditions (Tris–HCl buffer solutions, pH 7.4) was studied by UV–Vis spectroscopy, fluorescence spectroscopy and viscosity measurement method, respectively. The results indicated that a complex of luteolin with ctDNA can be formed. Spectroscopic techniques together with viscosity determination provided evidences of intercalation mode of binding for the interaction between luteolin and ctDNA. The binding constant of luteolin to DNA calculated based on UV–Vis spectroscopy data was found to be 4.52×10^4 L mol^{-1} at 310 K. The thermodynamic parameters of the complex were calculated by a double reciprocal method: $\Delta_r H_m^s = -8.9 \times 10^3$ J mol^{-1}, $\Delta_r S_m^s = 60.5$ JK^{-1}mol^{-1} and $\Delta_r G_m^s = -2.76 \times 10^4$ J mol^{-1} (310 K). The interacting forces between luteolin and DNA mainly included hydrophobic interactions and hydrogen bonds. The acridine orange displacement studies revealed that luteolin had significant effect for acridine orange bounded on DNA, which was indicative of intercalation binding.

1. Introduction

As one of the most important biomacromolecules, DNA is a particularly good target for antiviral, anticancer and antibiotic drugs. Investigations of the interaction between small molecules and DNA are significant in designing new types of pharmaceutical molecules. There is growing interest in exploring the binding of small molecules with DNA to further understand the interaction mechanism at the molecular level [1–3]. Furthermore, the activity of these drugs is mainly dependent on the method and intensity of interaction between DNA and drugs. At present, studying the nature and dynamics for binding drug molecules to biomacromolecules (like DNA) is an active research area, which can lead to rational design and construction of new and more efficient drugs targeted to DNA [4].

It was well known that interaction of small molecules with DNA by three modes: (i) intercalating between stacked base pairs, thereby distorting the DNA backbone; (ii) as major or minor groove binders, causing little distortion of the DNA backbone; (iii) interaction with the external DNA double helix, which does not possess selectivity [5]. Among these interactions, intercalation is one of

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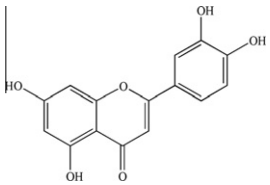

Fig. 1. The structure of luteolin.

the most important DNA-binding modes, which is related to the antitumor activity of the compound. And small molecules bounded with nucleic acids generally exhibit marked changes in absorbance and fluorescence properties, compared to that when they are free in solution.

Luteolin (3′,4′,5,7-tetrahydroxyflavone, Fig. 1), a crucial member of the flavones, widely distributes in vegetables, fruits, and natural herbal drugs, such as flos chrysanthemi, caulis lonicerae japonicae, flos lonicerae japonicae and aloe [6]. Apart from effects of vasodilation [7], anti-inflammatory [8], antioxidant [9] and anticancer activities [10–12], etc., recent studies have showed that it can enter the cellular nuclei and suppress the oxidative damage of DNA [13]. The above reports reveal that luteolin possesses a variety of pharmacological activities.

In this work, the interaction between luteolin and DNA was investigated systematically using UV–Vis and fluorescence spectroscopic techniques together with the determination of series of thermodynamic parameters, binding constants and viscosity of binding complex. Based on the results it was suggested that the binding mode between luteolin and DNA might be intercalation.

2. Materials and methods

2.1. Reagents

Calf thymus DNA were purchased from Sigma biological Co., and used without further purification. It was dissolved in doubly distilled water at concentration of 2.5×10^{-3} mol L^{-1} (as stock solutions). Working solutions (5.0×10^{-4} mol L^{-1}) were prepared by appropriate dilution with water. The purity of DNA was checked by monitoring the ratio of A_{260}/A_{280} = 1.80, and the concentration of DNA was determined by the absorption of DNA at 260 nm (ε_{260} = 6600 L mol^{-1} cm^{-1}). Luteolin (>98%) was obtained from Shanghai Jingchun Reagent Limited Company (Shanghai, China). A stock solution of luteolin (1.0×10^{-3} mol L^{-1}) was prepared by dissolving the corresponding luteolin in a mixture of ethanol and water (1:1 vol/vol). Working solutions (5.0×10^{-4} mol L^{-1}) were prepared by appropriate dilution with 1:1 ethanol water mixture. Acridine orange (AO, from Sigma) solution was prepared by dissolving AO in doubly distilled water at a concentration of 1.0×10^{-4} mol L^{-1}. Tris–HCl solution (pH = 7.40, containing 0.1 mol L^{-1} NaCl) was used as buffer solution. Other chemicals used were of analytical or higher grade. All of the stock solutions and their diluted solutions were stored in a refrigerator at 0–4 °C.

2.2. Apparatus

The absorption spectra were recorded on a CARY300 spectrophotometer (Varian, America). The fluorescence spectra and intensities were measured by an F-4600 spectrofluorophotometer (Hitachi, Japan). The pH was measured with a pHSJ-4A acidometer (Shanghai Lei Ci Device Works, Shanghai, China). In fluorescence mode, both excitation and emission bandwidths were set at 5 nm. All of the determinations were carried out at pH 7.40 remained by Tris–HCl buffer solution.

2.3. UV-spectroscopic measurements

Two milliliters of Tris–HCl and 250 μL of luteolin (5.0×10^{-4} mol L^{-1}) were added into a 1.0 cm quartz cell. The mixture was mixed thoroughly and titrated by DNA (2.5×10^{-3} mol L^{-1}) solution (10 μL each time). UV spectra were recorded from 240 to 460 nm using Tris–HCl buffer solution only as reference solution.

2.4. Fluorescence spectroscopic measurements

One milliliter Tris–HCl and 1.0 mL luteolin (1.0×10^{-3} mol L^{-1}) were added into a 1.0 cm quartz cell. The mixture was shaken up and then titrated by DNA (2.5×10^{-3} mol L^{-1}) solution (10 μL each time). Fluorescence spectra were recorded from 500 to 600 nm after these solutions had been allowed to stand for 5 min to equilibrate.

2.5. Competitive binding between AO and luteolin for DNA

Two milliliters Tris–HCl, 100 μL DNA (5.0×10^{-4} mol L^{-1}) and 25 μL AO (1.0×10^{-4} mol L^{-1}) were added into a 1.0 cm quartz cell. The mixture was shaken up and allowed to stand for 5 min then titrated by luteolin (5.0×10^{-4} mol L^{-1}) solution (10 μL each time). Fluorescence spectra were recorded from 500 to 600 nm and the intensities of 528 nm peak fluorescence were determined. In all the spectroscopic experiments the volume effects were neglectable.

2.6. Viscosity measurements method

Viscosity measurements were performed using an Ubbelohde viscometer, which was immersed in a thermostat water bath at 300 K. Different amounts of luteolin were added into the viscometer by keeping the DNA concentration constant. The flow times of the samples were repeatedly measured by using a digital stopwatch. Flow times were above 200 s, and each point measured was the average of three measurements. The data were presented as η/η_0 versus $c_{luteolin}$, where η and η_0 were the viscosity of DNA in the presence and absence of luteolin respectively.

3. Results and discussion

3.1. Absorption studies

The electronic absorption spectrum has been most commonly used to study the interaction between small molecules and DNA. Because of compound bounded with DNA, the absorbance spectrum of small molecules shows characteristic change, such as hypochromism, hyperchromism, red shift and blue shift, etc.

Due to the transition of n–π^* and π–π^* conjugated system, luteolin displayed some spectrum characters. The wavelengths of absorption bands were at 354 and 264 nm, respectively. The band I at 354 nm was related to the n–π^* transitions whereas the band II at 264 nm was related to the π–π^* chromophoric transitions [14]. Fig. 2 showed the absorption spectra of luteolin with the addition of DNA. With increasing the concentration of DNA, the absorbance at 354 nm decreased with a red shift from 354 to 355 nm, and the absorbance at 264 nm increased with a blue shift from 264 to 261 nm. Furthermore, two isoabsorptive points could be observed in absorption curves at 297 and 437 nm, respectively. Hyperchromic effect with blue shift of band II (π–π^* region) might because the reason that DNA has a maximum absorption at 260 nm. Hypochromic effect with red shift of band I (n–π^* region) attributed to the reason that luteolin could insert into DNA base pairs where it was protected against the polar solvent, forming π–π overlapping