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Molecular spectroscopic studies on the interaction of ferulic acid with calf thymus DNA

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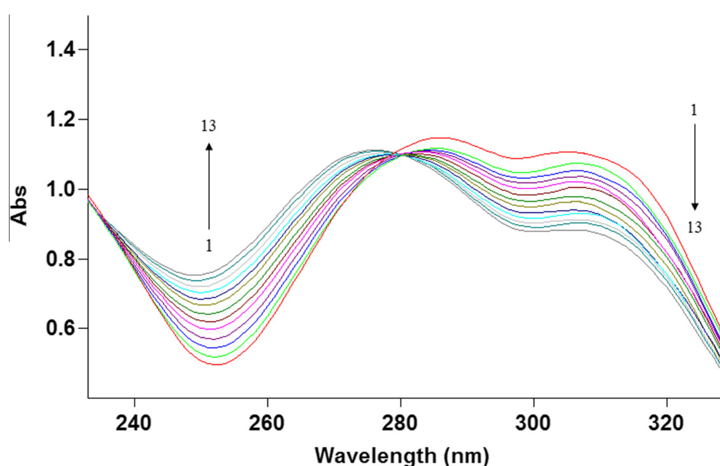
HIGHLIGHTS

- Ferulic acid could insert into DNA base pairs to form a binary complex at physiological pH.
- The acting forces between ferulic acid and DNA mainly included hydrophobic interactions and hydrogen bonds.
- Ferulic acid could substitute for AO probe in the AO-DNA complex by intercalation mode.
- The stabilization of the ctDNA helix was increased in the presence of ferulic acid.
- The interaction between ferulic acid and ctDNA might occur via intercalative mode.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction between ferulic acid and calf thymus deoxyribonucleic acid (ctDNA) under physiological conditions (Tris–HCl buffer solutions, pH 7.4) was investigated by UV–Vis spectroscopy, fluorescence spectroscopy, DNA melting techniques, and viscosity measurements. Results indicated that a complex of ferulic acid with ctDNA was formed with a binding constant of $K_{290K} = 7.60 \times 10^4$ L mol^{-1} and $K_{310K} = 4.90 \times 10^4$ L mol^{-1}. The thermodynamic parameters enthalpy change ($\Delta H°$), entropy change ($\Delta S°$) and Gibbs free energy ($\Delta G°$) were calculated to be -1.69×10^4 J mol^{-1}, 35.36 J K^{-1} mol^{-1} and -2.79×10^4 J mol^{-1} at 310 K, respectively. The acting forces between ferulic acid and DNA mainly included hydrophobic interaction and hydrogen bonds. Acridine orange displacement studies revealed that ferulic acid can substitute for AO probe in the AO-DNA complex which was indicative of intercalation binding. Thermal denaturation study suggested that the interaction of ferulic acid with DNA could result in the increase of the denaturation temperature, which indicated that the stabilization of the DNA helix was increased in the presence of ferulic acid. Spectroscopic techniques together with melting techniques and viscosity determination provided evidences of intercalation mode of binding for the interaction between ferulic acid and ctDNA.

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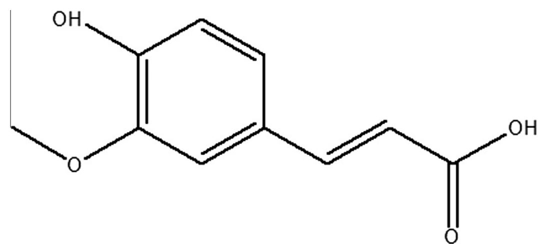

Fig. 1. The structure of ferulic acid.

Introduction

Some phenolic compounds in edible plants have received much attention as powerful antioxidants to protect against oxidative deterioration of food [1]. Ferulic acid (FA, 4-hydroxy-3-methoxy-cinnamic acid, Fig. 1) is one of the antioxidatively active phenolic acids, which is widely distributed in various plants such as cereals [2,3], citrus fruits [4], spinach [5], sugar beet [6] and pineapple [7,8]. It has many important applications in the field of medicine because of its variety of pharmacological activities, such as antioxidant [9], cholesterol-lowering activity [10], preventing against atherosclerosis [11], anticancer activities [12] and anti-inflammatory [13]. However, as far as our knowledge there is no report about detailed interaction mechanism of ferulic acid with DNA yet.

In recent years, many researches have been focusing on interaction of small molecules with DNA, which is generally the primary intracellular target of anticancer drugs. The interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells, and resulting in cell death [14]. Small molecule can interact with DNA through the following three non-covalent modes: intercalation, groove binding and electrostatic interaction. Among these interactions, intercalation is the most important mode in which small molecules can intercalate between the pair-bases of double helix DNA, forming π–π overlapping interaction, simultaneously lengthening and unwinding the helix [15]. And small molecules binded with DNA generally exhibit marked changes in absorbance and fluorescence properties compared to when they are free in solution. Furthermore, intercalation is related to the antitumor activity of the compound. The activity of the compound is mainly dependent on the method and intensity of interaction between DNA and compound. 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 [16].

In the present study, the interaction between ferulic acid and DNA was investigated systematically using UV–Vis and fluorescence spectroscopic techniques, as well as DNA melting techniques and viscosity measurements. Based on the results it was suggested that the binding mode between ferulic acid and DNA might be intercalation.

Materials and methods

Chemicals and reagents

ctDNA were purchased from Sigma biological Co. and used as received, which was used without further purification and dissolved in doubly distilled water at concentration of 0.80×10^{-3} mol L^{-1} (as stock solutions). The purity of ctDNA was checked by monitoring the ratio of A_{260}/A_{280} = 1.80, and the concentration of ctDNA was determined by the absorption of ctDNA at 260 nm (ε_{260} = 6600 L mol^{-1} cm^{-1}) [14]. Ferulic acid (>98%) was obtained from HongLi chemical plant corporation (Qufu, Shandong). A stock solution of ferulic acid (1.0×10^{-3} mol L^{-1}) was prepared by dissolving the corresponding ferulic acid in doubly distilled water. 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 4 °C.

Instrumentations

The absorption spectra were recorded on a CARY300 spectrophotometer (Varian, America) with a thermostat bath (Hengping Instrument Factory, Shanghai, China), using 1.0 cm quartz cell. The fluorescence spectra and intensities were measured on a F-4600 spectrofluorophotometer (HITACHI, Japan) with a thermostat bath and a 1.0 cm quartz cells. The widths of both the excitation slit and emission slit were set at 5.0 nm. The pH was measured on a pHSJ-4A acidometer (Shanghai Lei Ci Device Works, Shanghai, China). All of the determinations were carried out at pH 7.40 remained by Tris–HCl buffer solution.

UV-spectroscopic measurements

To a 1.0 cm quartz cell, 2.0 mL of Tris–HCl and 100 μL of ferulic acid (1.0×10^{-3} mol L^{-1}) were added. The mixture was mixed thoroughly and titrated by ctDNA (0.80×10^{-3} mol L^{-1}) solution (10 μL each time). UV spectra were measured and recorded from 220 nm to 350 nm using Tris–HCl buffer solution only as reference solution.

Fluorescence spectroscopic measurements

To a 1.0 cm quartz cell, 1.0 mL of Tris–HCl and 100 μL of ferulic acid (1.0×10^{-3} mol L^{-1}) were added. The mixture was shaken up and then titrated by ctDNA (2.7×10^{-4} mol L^{-1}) solution (10 μL each time). Fluorescence spectra were recorded from 340 nm to 500 nm at an excitation wavelength of 315 nm after these solutions had been allowed to stand for 5 min to equilibrate.

Competitive binding between AO and ferulic acid for DNA

To a 1.0 cm quartz cell, solutions were added in the following order: 1.0 mL of Tris–HCl, 25 μL of ctDNA (0.80×10^{-3} mol L^{-1}) and 25 μL of AO (1.0×10^{-4} mol L^{-1}). The mixture was shaken up and allowed to stand for 5 min then titrated by ferulic acid (1.0×10^{-3} mol L^{-1}) solution (10 μL each time). Fluorescence spectra were recorded from 500 nm to 600 nm at an excitation wavelength of 480 nm and the intensities of 528 nm peak fluorescence were determined.

DNA denaturation studies

DNA denaturation experiments were carried out by monitoring the absorption of ctDNA at 260 nm by a CARY300 spectrophotometer in the absence and presence of ferulic acid at various temperatures. The absorbance intensities were then plotted as a function of temperature ranging from 30 °C to 95 °C. The denaturation temperature (T_m) was determined as the transition midpoint. All measurements of T_m were repeated three times and the data presented were the average values.

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