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Interaction of coumarin with calf thymus DNA: Deciphering the mode of binding by *in vitro* studies

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ABSTRACT

DNA is the major target for a wide range of therapeutic substances. Thus, there has been considerable interest in the binding studies of small molecules with DNA. Interaction between small molecules and DNA provides a structural guideline in rational drug designing and in the synthesis of new and improved drugs with enhanced selective activity and greater clinical efficacy. Plant derived polyphenolic compounds have a large number of biological and pharmacological properties. Coumarin is a polyphenolic compound which has been extensively studied for its diverse pharmacological properties. However, its mode of interaction with DNA has not been elucidated. In the present study, we have attempted to ascertain the mode of binding of coumarin with calf thymus DNA (Ct-DNA) through various biophysical techniques. Analysis of UV–visible absorbance spectra and fluorescence spectra indicates the formation of complex between coumarin and Ct-DNA. Several other experiments such as effect of ionic strength, iodide induced quenching, competitive binding assay with ethidium bromide, acridine orange and Hoechst 33258 reflected that coumarin possibly binds to the minor groove of the Ct-DNA. These observations were further supported by CD spectral analysis, viscosity measurements, DNA melting studies and *in silico* molecular docking.

1. Introduction

There has been considerable interest in the binding studies of small molecules with DNA owing to their diverse applications [1]. DNA is the pharmacological target of many drugs that are currently in clinical use or are in advanced clinical trials [2,3]. DNA has been the recognition and characterization site for the interaction of small molecules as they yield effective information for the development of therapeutic agents for controlling gene expressions [4,5]. Studying the interaction of pharmaceutical agents with DNA is also essential for understanding their mode of action and structural specificity of their binding reactions [6]. Interaction between small molecules and DNA provides a structural guideline in rational drug designing. It helps in the synthesis of new and improved drug entities with more selective activity, greater clinical efficacy and lower toxicity. Small molecules may bind to DNA double helical structures through three different modes (i) Electrostatic binding: occurs due to interaction between negatively charged DNA phosphate backbone and positively charged end of small molecules (ii) Intercalative binding: occurs when small molecules intercalate within stacked base pairs thereby distorting the DNA backbone conformation [7] (ii) Groove binding: occurs due to hydrogen bonding or van der Waals interaction with nucleic acid bases and small molecules in the deep major groove or the shallow minor groove. Groove binders cause no or little distortion of the DNA backbone [8]. However, many small molecules can directly interact with DNA, and the factors for these interactions are quite complex. Studying DNA as a drug target is attractive due to the availability of the genome sequence, well-studied three-dimensional DNA structure and the predictability of their accessible chemical functional groups. However, the number of known DNA-based drug targets is still very limited as compared to the protein-based drug targets [9].

Coumarin (1,2-benzopyrone), the parent molecule of coumarin derivatives, is the simplest compound (Fig. 1A) of a large class of naturally occurring polyphenolic substances made of fused benzene and apyrone rings [10]. Coumarin is present in a wide variety of plants including cassia, lavender, yellow sweet clover, tonka beans, green tea, woodruff and in fruits such as bilberry and cloudberry. Coumarins have recently attracted much attention because of their broad pharmacological properties. Coumarin has been

Abbreviations: Ct-DNA, calf thymus DNA; EB, ethidium bromide; AO, acridine orange.

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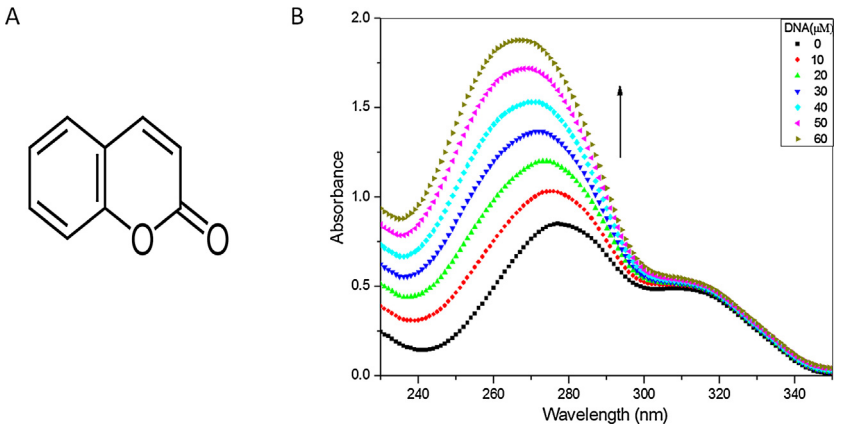

Fig. 1. (A) Structure of coumarin. (B) Interaction of coumarin with Ct-DNA using UV–visible spectroscopy. UV–visible absorption spectra of coumarin (50 μM) in presence of increasing concentrations of Ct-DNA (0–60 μM) in 10 mM Tris–HCl buffer (pH 7.2). Spectra were recorded in the range of 230–350 nm.

reported to exhibit antioxidant, anti-inflammatory, anti-mutagenic and anti-cancer properties [11–13]. Inspite of vast pharmacological properties of coumarin, its mode of binding with DNA has not been elucidated. It is thus pertinent to study the interaction of coumarin with DNA to reveal how this compound may be further modified to enhance its biological activities. The source being natural dietary constituents, an understanding of the interactions of coumarin and other related derivatives has the potential to provide guidelines for the development of more potent compounds.

In the present study, the mode of binding of coumarin with DNA was studied by UV–visible absorbance and fluorescence spectroscopy. Nucleic acids that bind small molecules generally exhibit marked changes in absorbance and fluorescence properties compared to their spectral characteristics when free in solution. In order to get into the deeper insight of coumarin–DNA interaction, circular dichroism (CD) spectroscopy, viscosity measurement and DNA melting study was performed. *In silico* molecular docking further revealed the relative binding energy of the complex formed between coumarin and DNA.

2. Experimental procedures

2.1. Materials

Coumarin, calf thymus DNA (Ct-DNA), acridine orange (AO) and Hoechst 33258 were purchased from Sigma Aldrich, USA. Ethidium bromide (EB) was purchased from Himedia, India. All the other chemicals and solvents were of reagent grade and were used without further purification.

2.2. Sample preparation

Stock solution of coumarin (10 mM) was prepared in 5% ethanol. Ct-DNA was dissolved in 10 mM Tris–HCl buffer (pH 7.2) at room temperature with occasional stirring to ensure the formation of a homogeneous solution. Ratio of UV absorbance at 260 and 280 nm for Ct-DNA solution was more than 1.8. Thus, indicating that Ct-DNA was sufficiently free from protein and no further purification was needed [14]. The concentrations of DNA solutions were determined by using the average extinction coefficient value of 6600 M^{-1} cm^{-1} of a single nucleotide at 260 nm [15].

2.2.1. UV spectroscopic method

The UV spectra were recorded with a Shimadzu dual beam UV-visible spectrophotometer UV-1800 (Japan) using a 1 cm × 1 cm quartz cuvettes. The UV–visible spectra of coumarin and coumarin–Ct-DNA complex were recorded in the wavelength range 200–350 nm. Experiment was carried out in presence of fixed concentration of coumarin (50 μM) in a total volume of 3 ml and titrated with varying concentration of DNA (0–60 μM). DNA solutions of same concentrations without coumarin were used as the blank to observe the UV-spectra specific to coumarin–DNA complex.

2.2.2. Fluorescence studies

Fluorescence emission spectra of coumarin were recorded on a Shimadzu RF-5301PC spectroflurophotometer (Japan) equipped with xenon flash lamp using 1.0 cm quartz cells. Samples were excited at 278 nm and emission spectra were recorded from 300 nm to 500 nm after setting the widths of both the excitation and the emission slits at 5 nm. Appropriate blanks corresponding to the buffer were subtracted to correct the background fluorescence. The fluorescence titration was carried out by keeping the concentration of coumarin constant (50 μM) and varying Ct-DNA concentration (0–90 μM). In case of EB displacement assay, a solution containing 5 μM of EB and 50 μM of Ct-DNA was titrated with increasing concentration of coumarin. EB–Ct-DNA complex was excited at 476 nm and emission spectra were recorded from 530 to 700 nm. In another set of displacement assays, AO–Ct-DNA complex was excited at 480 nm while Hoechst–Ct-DNA complex was excited at 343 nm and emission spectra were recorded from 490 to 600 nm and 360 to 600 nm respectively. They were subsequently titrated with increasing concentration of coumarin. Iodide quenching experiments were carried out by adding increasing concentrations of potassium iodide (0–150 mM) to coumarin (50 μM) alone and coumarin–Ct-DNA complex (50 μM each). Excitation was done at 278 nm and emission spectra were recorded from 300 to 500 nm. Effect of ionic strength was studied by varying the concentration of NaCl between 0 and 133 mM on the fluorescence spectra of coumarin–Ct-DNA complex (50 μM each). Excitation was done at 278 nm and emission spectra were recorded between 315 and 520 nm. Final reaction mixture in all the above experiments was 3 ml containing 10 mM Tris–HCl (pH 7.2).

2.2.3. Circular dichroism studies

CD spectra of DNA alone and coumarin–Ct-DNA complex were recorded using Applied Photophysics CD spectrophotometer (model CIRASCAN, U.K.) equipped with a Peltier temperature controller to keep the temperature of the sample constant at 25 °C. All the CD spectra were recorded in far-UV range (200–300 nm) with a scan speed of 200 nm per min with a spectral band width of 10 nm. Each spectrum was the average of four scans. Background spectrum