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Interaction of capsaicin with calf thymus DNA: A multi-spectroscopic and molecular modelling study

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ABSTRACT

Studying the mode of interaction between small molecules and DNA has received much attention in recent years, as many drugs have been reported to directly interact with DNA thereby regulating the expression of many genes. Capsaicin is a capsaiciniods family phytocompound having many therapeutic applications including diabetic neuropathy, rheumatoid arthritis, prevention of DNA strand breaks and chromosomal aberrations. In this study, we have investigated the interaction of capsaicin with calf thymus DNA using a number of biophysical techniques to get an insight and better understanding of the interaction mechanism. Analysis of UV–vis absorbance spectra and fluorescence spectra indicates the formation of complex between capsaicin and Ct-DNA. Thermodynamic parameters ΔG, ΔH, and ΔS measurements were taken at different temperatures indicated that hydrogen bonding and van der Waal's forces played major role in the binding process. Additional experiments such as iodide quenching, CD spectroscopy suggested that capsaicin possibly binds to the minor groove of the Ct-DNA. These observations were further confirmed by DNA melting studies, viscosity measurements. Molecular docking provided detailed computational interaction of capsaicin with Ct-DNA which proved that capsaicin binds to Ct-DNA at minor groove. Computational molecular docking also revealed the exact sites and groups to which capsaicin interacted.

1. Introduction

A living organism has genetic material containing the coded information for its functioning that makes the DNA an obvious target of study for many researchers [1,2]. The drug-DNA interaction is an important area since it provides valuable information in the development of drugs and controlling gene expression [3–5]. DNA is also target molecule for many drugs including those under advanced clinical trials, especially anticancer drugs [6,7]. Small molecules like drugs interact with DNA via mainly three different binding modes: intercalation, groove binding and ionic interactions [8]. Many such molecules may directly interact with DNA and the factors controlling these interactions are still not very well understood. Studying these interactions has become simpler due to the availability of well-known three-dimensional structure of DNA, availability of genomic sequence and many automated computer programs. Studying these interactions also enables us to understand the mechanism of action of drugs at molecular level.

Capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide) and dihydrocapsaicin constitute upto 90% of total capsaiciniods in which capsaicin accounts for approximately 71% [9]. Capsaicins have many therapeutic applications including those in diabetic neuropathy and rheumatoid arthritis [10]. This is one of the important dietary phytocompound having not only anticarcinogenic effects [11], but has also been reported to prevent DNA strand breaks and chromosomal aberrations [12]. Studies have revealed that capsaicin inhibits the activity of ethylmorphine-*N*-demethylase and many drug metabolizing enzymes of liver by interacting with cytochrome P-450 [13]. On the contrary, it is also evident that capsaicin has tumor promoting effects and people consuming large amount of chilli peppers are more prone to get gastric cancer [14,15].

Such compound needs to be investigated to get insights into their molecular mechanism of interaction with DNA. Therefore, in the present study, we have tried to explore the mode of interaction between capsaicin and Ct-DNA by using various biophysical and molecular modelling techniques. *In silico* molecular modelling complemented the *in vitro* interactions results and confirmed binding mode as minor groove binder.

Abbreviations: Ct-DNA, calf-thymus DNA; AO, acridine orange; EB, ethidium bromide; KI, potassium iodide; CD, circular dichroism.

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2. Material and methods

2.1. Materials

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

2.2. Sample preparation

Stock solution of Ct-DNA was prepared by dissolving 60 mg of Ct-DNA in 30 mL of 10 mM Tris–HCl buffer (pH 7.4) overnight at 4 °C with gentle mixing to make a homogenous solution. Final concentration of the stock solution of Ct-DNA was measured by spectrophotometer using molar excitation coefficient of 6600 M^{-1} cm^{-1} [16]. The purity of the Ct-DNA solution was determined by calculating the absorbance ratio A_{260}/A_{280}which was found to be 1.83, showing that the DNA was free from protein and pure for experimental use. Stock solution was stored at −20 °C for further use. 3 mM stock solution of capsaicin was made in absolute ethanol and diluted in 10 mM Tris–HCl buffer (pH 7.4) for further use.

2.3. UV–vis spectroscopic study

UV–vis spectrophotometric studies were performed on UV–vis Shimadzu 1800 UV–vis Spectrophotometer. The UV–vis spectra of free capsaicin and Ct-DNA-capsaicin complex were recorded in the wavelength range 200–500 nm. The experiment was carried out in the presence of fixed concentration of capsaicin (30 μM) and by titrating varying concentration (10–100 μM) of Ct-DNA. Base line correction was carried out using blank solution containing 10 mM Tris-HCl buffer (pH 7.4).

2.4. Steady state fluorescence

Fluorescence studies were performed on RF-5301PC Spectrofluorophotometer, Shimadzu Scientific Instruments, Japan having xenon flash lamp and using 1.0 cm quartz cells. Fluorescence emission spectra of capsaicin were recorded in the range of 290–600 nm after excitation at 280 nm. The change in fluorescence intensity was observed by titrating the fixed concentration of capsaicin (30 μM) with varying concentration of Ct-DNA (10–100 μM). The steady state fluorescence experiment was performed at different temperatures (298 K, 303 K and 310 K) for the evaluation of various thermodynamic parameters involved in the formation of capsaicin–DNA complex.

2.5. Potassium iodide quenching

This experiment was performed in two sets. In one set capsaicin alone (30 μM) was excited at 280 nm and emission spectra was recorded in presence of increasing concentration of KI (10–100 mM) in the wavelength range of 290–600 nm. In another set, experiment was performed in presence of Ct-DNA (30 μM) and capsaicin-DNA complex was excited and emission spectra were recorded at increasing concentration of KI in 10 mM Tris-HCl (pH 7.4). K_{sv} values were calculated in both the cases using Stern-Volmer equation.

2.6. Continuous variation analysis (Job's plot)

The continuous variation method also called as Job's plot was employed for the evaluation of binding stoichiometry of the capsaicin-DNA complex [17,18]. All the measurements were done at 25 °C. The fluorescence emissions for all the solutions were recorded. The concentration of capsaicin and Ct-DNA was varied while sum of their concentration was kept constant at 30 μM. The difference in the fluorescence emission intensity (ΔF) of capsaicin in absence and presence of DNA was plotted as a function of mole fraction of capsaicin. The break point of the above mentioned plot yielded the mole fraction of the bound capsaicin in the complex. The stoichiometry was obtained in terms of DNA-capsaicin $[(1-\chi_{capsaicin})/\chi_{capsaicin}]$, where $\chi_{capsaicin}$ denotes the mole fraction of capsaicin.

2.7. DNA melting experiment

In DNA melting experiment, the absorbance of Ct-DNA (50 μM) was monitored at varying intervals of temperature from 25 to 100 °C at 260 nm. The same experiment was repeated for capsaicin-DNA complex (50 μM each) to determine the change in melting temperature (T_m) of capsaicin-DNA complex as compared to DNA alone. EB-DNA complex was taken as positive control in which the concentration of EB and DNA was 10 μM and 50 μM respectively. The absorbance recorded in each case was plotted as function of temperature. The transition midpoint of the curve was used for determining the T_m.

2.8. Effect of urea

The mode of interaction between Ct-DNA and capsaicin was further studies studying urea induced denaturation. A fixed concentration of capsaicin-DNA complex (30 μM each) was titrated with increasing concentration of urea (0–1 M). The capsaicin-DNA complex was excited at 280 nm and the emission spectra was recorded from 290 to 500 nm.

2.9. Circular dichroism studies

CD spectral studies of DNA was recorded at 25 °C using JASCO spectropolarimeter (J-815) equipped with a Peltier temperature controller with accuracy of ±0.1 °C to maintain a constant temperature. Far-UV CD spectra of Ct-DNA in absence and presence capsaicin were monitored with speed of 200 nm per min. The concentration of DNA was 100 μM capsaicin was varied to maintain the molar ratios of 1:0, 1:0.5 and 1:1. The spectra of buffer solution (10 mM Tris–HCl, pH 7.4) were subtracted from the spectra of DNA and capsaicin-DNA complex for the base line correction.

2.10. Effect of ionic strength

This experiment was performed to evaluate the role of ionic strength in the formation of capsaicin-DNA complex. Briefly, capsaicin-DNA complex (30 μM each) was titrated with varying concentration of NaCl (10–100 mM). The excitation wavelength was 280 nm and emission was recorded form 290–600 nm in 10 mM Tris-HCl (pH 7.4).

2.11. Viscosity measurements

In order to further elucidate the binding mode of capsaicin, viscosity measurement was carried out in absence and presence of Ct-DNA using an Ubbelohde viscometer suspended in water bath at 25 °C. To a fixed concentration of Ct-DNA (100 μM), varying concentration of capsaicin (20–100 μM) was added and the flow time of each concentration was measured thrice using a digital stopwatch. The data was plotted as$(\eta/\eta_0)^{1/3}$ versus ratio of capsaicin to Ct-DNA