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Binding properties of butylated hydroxytoluene with calf thymus DNA in vitro

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ABSTRACT

The binding properties of butylated hydroxytoluene (BHT) with calf thymus DNA (ctDNA) in simulated physiological buffer (pH 7.4) were investigated using ethidium bromide (EB) dye as a fluorescence probe by various spectroscopic techniques including UV–vis absorption, fluorescence, circular dichroism (CD), and Fourier transform infrared (FT-IR) spectroscopy along with ctDNA melting studies and viscosity measurements. It was found that the binding of BHT to ctDNA could decrease the absorption intensity of ctDNA, significantly increase melting temperature and relative viscosity of ctDNA, and induce the changes in CD spectra. Moreover, the competitive binding studies showed that BHT was able to displace EB from the bound ctDNA–EB complex. All the experimental results indicated that the binding mode between BHT and ctDNA was an intercalation. The association constants between BHT and ctDNA were evaluated to be $(4.78 \pm 0.04) \times 10^3$, $(2.86 \pm 0.02) \times 10^3$ and $(1.80 \pm 0.04) \times 10^3$ L mol^{-1} at 298, 304, 310 K, respectively. Further, the FT-IR analysis revealed that BHT was more prone to interact with adenine and thymine base pairs, and no significant conformational transition of ctDNA occurred. Thermodynamic analysis of the binding data showed that the binding process was primarily driven by hydrogen bonds and van der Waals forces, as the values of the enthalpy change and the entropy change were calculated to be -62.47 ± 0.07 kJ mol^{-1} and -139.22 ± 0.22 J mol^{-1} K^{-1}, respectively.

1. Introduction

Lipid (oil and fat), an essential ingredient in daily diets, tends to encounter problems of oxidation and rancidity, which affects food quality and may endanger people's health. Consequently, prevention of lipid peroxidation has long become an important issue in food industry [1]. Among various modern methods to prevent lipid peroxidation, the addition of antioxidants to foods is preferentially considered. The primary antioxidants used in lipid processing are synthetic compounds such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ), due to their chemical stability, low cost, and availability [2]. They are commonly used, alone or together in commercial mixtures as additives to suppress the formation of free radicals via redox reactions and ultimately prevent lipid oxidation and food spoilage.

BHT (structure shown in Fig. 1) is a synthetic phenolic compound and has been used as an antioxidant since the 1950s to preserve and stabilize the freshness, nutritive value, flavor and color of some foods and animal feed products, especially lipid [3]. However, the safety of this synthetic antioxidant was questioned due to its potential risk. The previous studies revealed that excess intake of BHT can induce certain teratogenic and carcinogenic effects upon rodents [4]. BHT has been shown to act as a tumor initiator or a tumor promoter in a variety of tissues and organs [5]. Furthermore, Oikawa et al. [6] reported that BHT metabolites can generate peroxides in mice and rats and induce cellular DNA damage or have the capacity to be a cancer initiator. Because of the widespread uses of BHT, it is necessary to critically evaluate the safety of this antioxidant.

Deoxyribonucleic acid (DNA) is an important genetic substance in the organism, which carries most of hereditary information and facilitates the biological synthesis of proteins and enzymes through replication and transcription of this information [7]. It is one of the most important biological molecules targeted by natural and synthetic compounds. Investigation on the interactions of small molecules with DNA has been an intensive topic over the past years in the scope of life science, chemistry, clinic medicine and genetics. These studies are beneficial for screening new and more efficient drugs targeting to DNA, investigating the structure and biological function of DNA, and elucidating the damage mechanism of DNA [8]. Therefore, the study on the interaction of food antioxidant BHT with DNA is of great significance, which will be helpful to shed light on its toxicological action and its binding mechanism at a molecular level.

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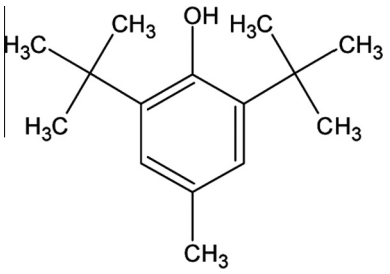

Fig. 1. Molecular structure of butylated hydroxytoluene (BHT).

In present work, the binding of BHT to calf thymus DNA (ctDNA) under simulated physiological conditions (pH 7.4) was investigated with the use of ethidium bromide (EB) dye as a fluorescence probe by multispectroscopic techniques including UV–vis absorption, fluorescence, circular dichroism (CD) and Fourier transform infrared (FT-IR) spectroscopy, coupled with ctDNA melting studies and viscosity measurements. The thermodynamic characteristic, binding mode and binding region between BHT and ctDNA were estimated. The results reported are expected to provide useful information for further investigating the mechanism of DNA damage by BHT.

2. Materials and methods

2.1. Apparatus

UV–vis absorption spectra were measured on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan) and a 1.0 cm quartz cell was used for the measurements. Fluorescence spectra were performed with a Hitachi spectrofluorimeter model F-4500 (Hitachi, Japan) equipped with a 150 W xenon lamp and a thermostat bath. CD spectra were recorded on a Bio-Logic MOS 450 CD spectrometer (Bio-Logic, France) using a 1.0 mm path length quartz cuvette. FT-IR spectra were measured on a Thermo Nicolet-5700 FT-IR spectrometer (USA) equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. pH measurements were taken with a pHS-3C digital pH-meter (Shanghai Exact Sciences Instrument Co., Ltd., Shanghai, China) with a combined glass-calomel electrode. The viscosity measurements were carried out using an Ubbelohde viscometer (⌀0.7–0.8 mm, Shanghai Qianfeng Rubber and Glass Company, Shanghai, China). An electronic thermostat water bath (Shanghai Yuejin Medical Instrument Company, Shanghai, China) was used for controlling the temperature. A Millipore Simplicity water purification system (Millipore, Molsheim, France) was applied to produce freshly ultrapure water. All experiments, unless specified otherwise, were carried out at room temperature.

2.2. Chemicals and reagents

BHT (purity ⩾99.0%) was obtained from Johnson Matthey Chemical Co., Ltd., (Shanghai, China), and its stock solution (4.90×10^{-3} mol L^{-1}) was prepared in absolute ethanol. ctDNA was purchased from Sigma–Aldrich Co., (St. Louis, MO) and dissolved in ultrapure water containing 0.1 mol L^{-1} NaCl. The purity of ctDNA was checked by monitoring the absorption ratio at 260/280 nm (A_{260}/A_{280}), and the ratio was determined to be 1.88, indicating that ctDNA was sufficiently free from protein [9]. The concentration of ctDNA in stock solution was determined to be 2.30×10^{-3} mol L^{-1} by UV absorption at 260 nm using a molar absorption coefficient ε_{260} = 6600 L mol^{-1} cm^{-1} [10,11]. The stock solution (1.00×10^{-3} mol L^{-1}) of EB (Sigma–Aldrich Co., St. Louis, MO) was made up by dissolving an appropriate amount of its crystals in ultrapure water. All stock solutions were diluted to the required concentrations with 0.05 mol L^{-1} pH 7.4 Tris–HCl buffer. All chemicals were of analytical reagent grade, and ultrapure water was used throughout the experiment.

2.3. Procedures

2.3.1. UV–vis absorption measurements

Absorption titration experiments were measured by keeping the concentration of ctDNA at 3.60×10^{-5} mol L^{-1}, while varying BHT concentration from 0 to 6.45×10^{-5} mol L^{-1}. The UV–vis absorption spectra of above samples and BHT solutions with the corresponding concentration (from 1.30 to 6.45×10^{-5} mol L^{-1}) were recorded over a wavelength range of 225–330 nm in the Tris–HCl buffer at room temperature. All observed absorption spectra were corrected for the buffer absorbance.

2.3.2. DNA melting studies

The melting experiments were carried out by monitoring the absorbance intensities of ctDNA in the absence and presence of BHT at different temperatures. The temperatures of ctDNA and the ctDNA–BHT complex were continuously detected by a thermocouple attached. The absorbance intensities of the solutions at 258 nm were then plotted as a function of temperature ranging from 20 to 100 °C. The values of melting temperatures were obtained from the transition midpoint of the melting curves based on f_{ss} versus temperature (T), where $f_{ss} = (A - A_0)/(A_f - A_0)$, A_0 is the initial absorbance intensity, A is the absorbance intensity corresponding to its temperature, and A_f is the final absorbance intensity [12].

2.3.3. Viscosity measurements

For viscosity measurements, the viscometer was thermostatted at 25 ± 0.1 °C in a thermostatic water bath. BHT concentrations ranging from 0 to 3.27×10^{-5} mol L^{-1} were added to the viscometer to give a certain molar ratio of BHT to ctDNA, and the concentration of ctDNA was fixed at 3.60×10^{-5} mol L^{-1}. After each addition, the solution was allowed to stand for 30 min to reach the thermal equilibrium, and then the flow time of the sample through the capillary was measured using a digital stopwatch with an accuracy of ±0.20 s. The mean values of five replicated measurements were used to evaluate the average relative viscosity of the samples. The relative viscosity values of ctDNA in the absence and presence of BHT were calculated from the following equation [13]:

$$\eta/\eta_0 = (t - t_0)/(t_{ctDNA} - t_0) \quad (1)$$

where t_0 and t_{ctDNA} are the observed flow time of the Tris–HCl buffer and ctDNA, respectively, while t is the flow time of the ctDNA–BHT mixture. The data have been presented as $(\eta/\eta_0)^{1/3}$ versus the [BHT]/[ctDNA] ratio, where η and η_0 represent the viscosity of ctDNA in the presence and absence of BHT, respectively.

2.3.4. Fluorescence measurements using EB as probe

A quantitative analysis of the potential interaction between BHT and ctDNA was carried out by fluorimetric titration. A 3.0 mL solution containing a fixed concentration of ctDNA–EB was added to a 1.0 cm quartz cuvette and then titrated by successive addition of 4.90×10^{-3} mol L^{-1} BHT solution (to give a final concentration of 1.27×10^{-4} mol L^{-1}). These solutions were allowed to stand for 6 min to equilibrate, and the fluorescence spectra were then measured at three temperatures (298, 304, and 310 K) in the wavelength range of 550–750 nm with exciting wavelength at 525 nm. The widths of both the excitation and emission slits were set at