



Spectroscopic studies on the interaction of sodium benzoate, a food preservative, with calf thymus DNA



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ABSTRACT

The interaction between sodium benzoate (SB) and calf thymus DNA in simulated physiological buffer (pH 7.4) using acridine orange (AO) dye as a fluorescence probe, was investigated by UV–Vis absorption, fluorescence and circular dichroism (CD) spectroscopy along with DNA melting studies and viscosity measurements. An expanded UV–Vis spectral data matrix was resolved by multivariate curve resolution–alternating least squares (MCR–ALS) approach. The equilibrium concentration profiles and the pure spectra for SB, DNA and DNA–SB complex from the high overlapping composite response were simultaneously obtained. The results indicated that SB could bind to DNA, and hydrophobic interactions and hydrogen bonds played a vital role in the binding process. Moreover, SB was able to quench the fluorescence of DNA–AO complex through a static procedure. The quenching observed was indicative of an intercalative mode of interaction between SB and DNA, which was supported by melting studies, viscosity measurements and CD analysis.

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1. Introduction

It is well known that deoxyribonucleic acid (DNA) as the carrier of genetic information in organisms plays an important role in a broad range of vital biological processes, such as gene expression, gene transcription, mutagenesis, and carcinogenesis (Kathiravan & Renganathan, 2009). The study on the interaction between small molecules and DNA has been the focus of some recent research in the scope of life science, chemistry, clinical medicine and genetics. These studies are very useful for investigating the structure and biological function of DNA, and elucidating the damage mechanism of DNA. Generally, small molecules bind to DNA by three predominant non-covalent modes, including intercalation, groove binding, and electrostatic interaction (Grueso, López-Pérez, Castellano, & Prado-Gotor, 2012). Intercalative binding and groove binding are related to the grooves in the DNA double helix, while the electrostatic binding takes place out of the groove.

With the increase in the production of processed and convenience foods, food preservatives have become an increasingly important practise in modern food technology. The preservatives are substances which are deliberately added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods and to prolong the shelf life and quality of foods (Saad, Bari, Saleh, Ahmad, & Talib, 2005). Sodium benzoate (SB, structure shown in Fig. 1) is a sodium salt of benzoic acid, which

is generally effective to inhibit the growth of mold and yeast, and against a wide range of bacterial attack. It is commonly used as a preservative in a variety of foods, such as marinated fish, fruit-based fillings, jam, salad dressings, soft drinks and beer. The acceptable daily intake (ADI) level recommended by the joint FAO/WHO expert committee on food additives (JECFA) for SB is 0–5 mg/kg body weight (Mpountoukas, Vantarakis, Sivridis, & Lialiaris, 2008). However, excess intake of SB can induce childhood hyperactivity (Egger, Graham, Carter, Gumley, & Soothill, 1985) and provoke urticaria, angioedema and asthma (Michaels-son & Juhlin, 1973). Furthermore, it has been proved that certain food preservatives, especially antimicrobial agents, were genotoxic in different test systems. Yüzbaşıoğlu et al. have investigated the potential genotoxic effects of SB using chromosomal aberrations (CA), sister chromatid exchange (SCE) and micronuclei (MN) tests on human peripheral lymphocytes and the comet assay (alkaline single cell gel electrophoresis) on isolated human lymphocytes (Zengin, Yüzbaşıoğlu, Ünal, Yılmaz, & Aksoy, 2011). Their results indicated that SB is clastogenic, mutagenic and cytotoxic to human lymphocytes *in vitro*, and SB can increase significantly DNA damage. Taking the harmfulness of consumption of SB into consideration, more widespread safety assessment of SB should be given. Consequently, it is necessary to investigate the interaction between SB and DNA to further understand the binding mechanism at the molecular level and estimate the toxicology of the preservative.

In the present work, the binding of SB to calf thymus DNA under simulated physiological conditions (pH 7.4) was investigated with

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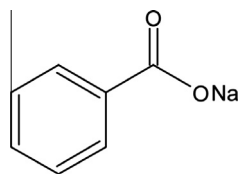


Fig. 1. Molecular structure of sodium benzoate (SB).

the aid of acridine orange (AO) dye as a fluorescence probe, using UV–Vis absorption, fluorescence and circular dichroism (CD) spectroscopy, coupled with DNA melting studies and viscosity measurements. The binding mode of SB to DNA was estimated. Furthermore, the chemometrics approach, multivariate curve resolution-alternating least squares (MCR–ALS) (Jaumot, Gargallo, Juan, & Tauler, 2005), was applied to resolve the expanded UV–Vis absorption spectral data collected from the SB–DNA mixtures so as to improve the understanding of complex kinetic processes. The equilibrium concentration profiles of the reacting species and the corresponding pure spectra were simultaneously extracted to quantitatively analyse the interaction between SB and DNA.

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. pH measurements were taken with a pH-3C digital pH-metre (Shanghai Exact Sciences Instrument Co. Ltd., Shanghai, China) with a combined glass-calomel electrode. The viscosity measurements were carried out using an NDJ-79 viscometer (Yinhua Flowmeter Co. Ltd., Hangzhou, China). An electronic thermostat water bath (Shanghai Yuejin Medical Instrument Company, Shanghai, China) was used for controlling the temperature. 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

The stock solution ($3.00 \times 10^{-3} \text{ mol L}^{-1}$) of SB (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) was prepared by dissolving its crystals in ultrapure water. Calf thymus DNA (Sigma Chem. Co., St. Louis, USA) was used without further purification, and dissolved in ultrapure water containing 0.1 mol L^{-1} NaCl. The purity of DNA was checked by monitoring the absorption ratio at 260/280 nm (A_{260}/A_{280}), and the ratio was observed as 1.98, indicating that DNA was sufficiently free from protein (Xu et al., 2009). The concentration of DNA in stock solution was determined to be $1.60 \times 10^{-3} \text{ mol L}^{-1}$ by UV absorption at 260 nm using a molar absorption coefficient $\epsilon_{260} = 6600 \text{ L mol}^{-1} \text{ cm}^{-1}$ (Saha & Kumar, 2011). Acridine orange (AO) stock solution ($1.00 \times 10^{-3} \text{ mol L}^{-1}$) 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 solution. 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

The interaction procedure of SB with DNA in pH 7.4 Tris–HCl buffer was characterised by UV–Vis absorption spectroscopy and two different UV spectral titration experiments were performed. Experiment 1: the concentration of SB was kept at $4.5 \times 10^{-5} \text{ mol L}^{-1}$, and the concentration of DNA added was varied from 0 to $8.00 \times 10^{-5} \text{ mol L}^{-1}$ (total 26 solutions). Experiment 2: the concentration of DNA was kept constant ($3.6 \times 10^{-5} \text{ mol L}^{-1}$), and different amounts of SB were added in the concentration range of 0 to $4.15 \times 10^{-5} \text{ mol L}^{-1}$. A total of 26 solutions were prepared.

Each solution sample of experiments 1 and 2 were allowed to stand for 6 min to equilibrate, and then the UV–Vis absorption spectra were recorded between 200 and 320 nm at 1 nm interval (total of 121 wavelengths) and room temperature. All observed absorption spectra were corrected for the buffer absorbance. Thus, two data matrices D^{SB} (26×121) and D^{DNA} (26×121) were obtained from these measurements, and column- and row-wise expanded data matrix was constructed.

2.3.2. DNA melting studies

DNA melting experiments were carried out by monitoring the absorbance intensities of the sample in the absence and presence of SB at different temperatures. The temperature of DNA and DNA–SB complex were continuously detected by a thermocouple attached. The absorbance intensities at 258 nm of the sample were then plotted as a function of temperature ranged from 20 to 100°C . The values of melting temperatures of DNA and DNA–SB complex 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, A_f is the final absorbance intensity (Zhang, Fu, Wang, & Hu, 2011).

2.3.3. Viscosity measurements

Viscometric titrations were performed using a viscometer, which was immersed in a thermostatic water bath at $25 \pm 0.1^\circ\text{C}$. The concentration of DNA was fixed at $3.6 \times 10^{-5} \text{ mol L}^{-1}$, and titrations were carried out by adding appropriate amounts of SB. After each addition, the flow time of the solution through the capillary was measured using a digital stopwatch with an accuracy of $\pm 0.20 \text{ s}$. The mean values of five replicated measurements were used to evaluate the average relative viscosity of the samples. The data was presented as $(\eta/\eta_0)^{1/3}$ versus the ratios of the concentration of SB to that of DNA ($[\text{SB}]/[\text{DNA}]$) (Sun et al., 2008), where η and η_0 represent the viscosity of DNA in the presence and absence of SB, respectively. Viscosity values were calculated from the observed flow time of DNA containing solutions (t) and corrected for buffer solution (t_0), $\eta = (t - t_0)/t_0$.

2.3.4. Circular dichroism (CD) studies

CD spectra of DNA in the absence and presence of different amounts of SB were measured at wavelengths between 225 and 325 nm. The optical chamber of the CD spectrometer was deoxygenated with dry nitrogen before use and kept in a nitrogen atmosphere during experiments. The concentration of DNA was kept at $6.0 \times 10^{-4} \text{ mol L}^{-1}$, and the molar ratio values of SB to DNA were 0, 1, and 2, respectively. All observed CD spectra were corrected for buffer signal and results were expressed as ellipticity in millidegree. The CD measurements were performed in pH 7.4 Tris–HCl buffer at room temperature.

2.3.5. Fluorescence measurements using AO as probe

The fluorescence measurements were conducted by successively adding an appropriate amount of SB solution into the quartz

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