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Binding characteristics of salbutamol with DNA by spectral methods

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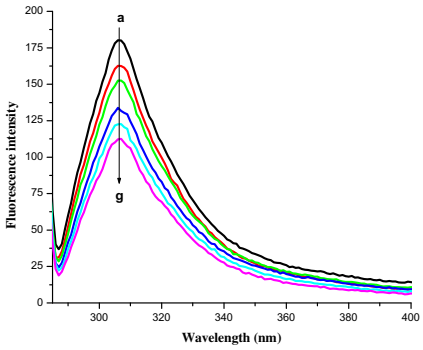
HIGHLIGHTS

- DNA quenching the fluorescence of salbutamol was studied for the first time.
- Binding constant, binding force and binding distance were determined.
- Binding mode of salbutamol to DNA was groove binding.

GRAPHICAL ABSTRACT



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ABSTRACT

Salbutamol interacting with deoxyribonucleic acid (DNA) was examined by fluorescence, UV absorption, viscosity measurements, and DNA melting techniques. The binding constants and binding sites were obtained at different temperatures by fluorescence quenching. The Stern–Volmer plots showed that the quenching of fluorescence of salbutamol by DNA was a static quenching. To probe the binding mode, various analytical methods were performed and the results were as follows: hyperchromic effect was shown in the absorption spectra of salbutamol upon addition of DNA; there was no appreciable increase in melting temperature of DNA when salbutamol was presented in DNA solution; the fluorescence intensity of salbutamol–DNA decrease with the increasing ionic strength; the relative viscosity of DNA did not change in the presence of salbutamol; the binding constant of salbutamol with double strand DNA (dsDNA) was much higher than that of it with single strand DNA (ssDNA). All these results indicated that the binding mode of salbutamol to DNA should be groove binding. The thermodynamic parameters suggested that hydrogen bond or van der Waals force might play an important role in salbutamol binding to DNA. According to the Förster energy transference theory, the binding distance between the acceptor and donor was 3.70 nm.

Introduction

Studies of drug–DNA bindings continue to be a vibrant area of research. As we know, deoxyribonucleic acid (DNA) is a molecule of great biological significance, which is a hereditary material in humans and almost all other organisms. DNA as a carrier of genetic information is a major target for drug interaction because of the ability to interfere with transcription and DNA replication. So investigating drugs binding to DNA can be of great benefit to understanding the action mechanism of the drugs and designing new drugs. There are three main binding modes of small molecules to DNA: intercalation into the base pairs, in the grooves (major or minor) and outside the helix by electrostatic binding [1]. Small molecules are stabilized on groove binding and intercalation with DNA through a series of interactions such as π-stacking, hydrogen bond, van der Waals and hydrophobic interaction [2]. Intercalative binding and groove binding are the two most likely binding modes of drug and DNA [3,4].

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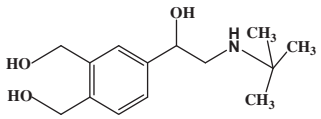

Fig. 1. Structure of salbutamol.

Natural fluorescence intensity of DNA is so weak that it has little practical use [5]. Many researchers have reported on the interaction mechanisms between small molecules and DNA with the help of fluorescence probes of DNA [4–13]. In this work, it was found that salbutamol (Fig. 1) can emit strong fluorescence. Salbutamol is a member of the β_2-agonists, which is widely used for effective relief from asthmatic disorders and chronic obstructive lung diseases in human [14]. It is also possible to improve growth rate and reduce carcass fat when fed to animals, which will be harmful to people's health [15]. Although salbutamol has been prohibited in food-producing animals in the most countries [16,17], the illegal use of salbutamol still exists. For a better understand the action of salbutamol in body, we demonstrated for the first time the interaction of salbutamol with DNA. Because the fluorescence quantum yield of salbutamol (the value of the fluorescence quantum yield cannot be found in previous references to the best of our knowledge) shown in this study is higher, the binding characteristics of the drug to DNA can be studied by fluorescence quenching, and the binding constant and binding site of salbutamol with DNA were obtained accordingly. Various available techniques including absorption, viscosity measurements, melting experiments and ionic strength effect tests were used to probe binding mode in the absence of any expensive and intricate instrument. The subject of this study is novel and interesting. The present work will be of importance and the knowledge gained can be used in the rational design process to enhance the specificity and therapeutic effectiveness of other drugs.

Experimental

Reagents

Salmon sperm DNA was purchased from Sigma Chem. Co., and its stock solution was prepared by dissolving an appropriate amount of solid DNA in double distilled water overnight and stored at 4 °C in the dark for no longer than a week. The stock solution of DNA was determined by UV absorption at 260 nm using extinction coefficients of ε_{260} (25 °C) = 6600 L mol^{-1} cm^{-1} [18,19]. The purity of DNA preparation was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of $A_{260}/A_{280} > 1.8$, indicating that DNA was sufficiently free from protein [20]. Salbutamol bought from Chinese Drug Biological Products Qualifying Institute was made into a 1.00×10^{-3} mol L^{-1} aqueous solution. A Tris–HCl buffer (0.05 mol L^{-1}, pH 7.40) containing 0.1 mol L^{-1} NaCl was used to control the acidity throughout.

DNA in this work is double stranded DNA (dsDNA) unless it is especially noted clearly.

All chemicals used were of analytical reagent grade and double distilled water was used throughout.

Apparatus

The fluorescence measurements were performed on RF-5301PC spectrofluorometer (Shimadzu, Japan) equipped with a xenon lamp source, using a quartz cell of 1 cm, and a thermostatic controller was used to control the temperature. The UV spectra were recorded on TU-1901 UV-Spectrometer (Beijing Purkinje General Instrument Co., Ltd.). Viscosity experiments were conducted on an Ubbelohde viscometer [21]. pH measurements were carried out with a pH-3S digital pH-meter (Nanjing Sangli electronic equipment factory, Nanjing, China). An electronic thermostat water-bath (Shandong Juancheng Instrument Company) was used for controlling the temperature.

Procedures

Fluorescence measurements

Fluorescence spectra of salbutamol were recorded from 280 to 450 nm when it was excited at 278 nm. Both emission and excitation slits widths were 5 nm. All the fluorescence data are corrected for absorption of exciting light (278 nm) and emitted light (306 nm) according to the relationship [22]

$$F_{corr} = F_{obs} e^{(A_{ex}+A_{em})/2} \quad (1)$$

F_{corr} and F_{obs} are the corrected and observed fluorescence intensities, respectively. A_{ex} and A_{em} are the absorbance of DNA at the excitation and emission wavelengths.

Absorption measurements

The UV absorption spectra of DNA, salbutamol and the mixture of DNA and salbutamol were measured. The slit width was 1.5 nm, the scan rate was 442 nm min^{-1} and scan wavelengths were from 220 to 600 nm.

Melting studies

The melting temperature (T_m) of DNA in the absence and presence of salbutamol were determined by monitoring the absorbance at 260 nm as a function of temperature ranging from 20 to 100 °C. The absorbance was recorded every 2–5 °C. About every 1.5 min, the temperature of the water increase 1 °C. T_m of DNA sample was determined as the transition midpoint [23].

Effect of ionic strength

A series of assay solutions containing fixed amount of salbutamol–DNA and various amounts of NaCl ranging from 0 to 0.4 mol L^{-1} in steps of 0.1 mol L^{-1} were prepared to measure the fluorescence intensity.

Viscosity measurements

Viscosity measurements were performed by using an Ubbelohde viscometer at 18.0 ± 0.5 °C. A series of solutions containing various concentrations of salbutamol (ranging from 0.0 to 0.6×10^{-5} mol L^{-1} in steps of 0.1×10^{-5} mol L^{-1}) and a certain

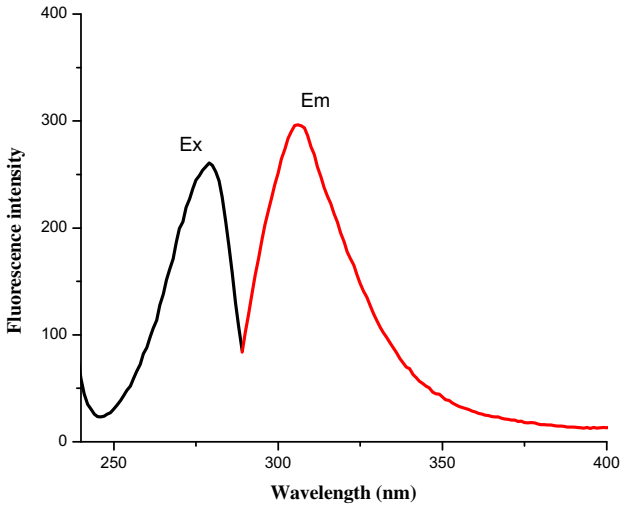

Fig. 2. Excitation (E_x) and emission (E_m) spectra of salbutamol.