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The investigation of the interaction between Tropicamide and bovine serum albumin by spectroscopic methods

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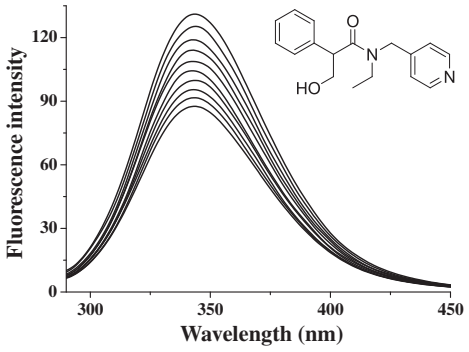
HIGHLIGHTS

- The interaction of bovine serum albumin (BSA) and Tropicamide (TA) was studied.
- The fluorescence quenching mechanism is static quenching.
- The binding constants and binding sites were calculated.
- Hydrogen binds and vander Waals interaction force played a major role in stabilizing the complex.
- The TA affects the conformation of BSA.

GRAPHICAL ABSTRACT



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ABSTRACT

The fluorescence and ultraviolet–visible (UV–Vis) spectroscopy were explored to study the interaction between Tropicamide (TA) and bovine serum albumin (BSA) at three different temperatures (292, 301 and 310 K) under imitated physiological conditions. The experimental results showed that the fluorescence quenching mechanism between TA and BSA was static quenching procedure. The binding constant (K_a), binding sites (n) were obtained. The corresponding thermodynamic parameters (ΔH, ΔS and ΔG) of the interaction system were calculated at different temperatures. The results revealed that the binding process is spontaneous, hydrogen binds and vander Waals were the main force to stabilize the complex. According to Förster non-radiation energy transfer theory, the binding distance between TA and BSA was calculated to be 4.90 nm. Synchronous fluorescence spectroscopy indicated the conformation of BSA changed in the presence of TA. Furthermore, the effect of some common metal ions (Mg^{2+}, Ca^{2+}, Cu^{2+}, and Ni^{2+}) on the binding constants between TA and BSA were examined.

Introduction

Fluorescence studies on protein–ligand interaction with characteristic fluorescence properties of the drug (ligand) have gained enormous interest in recent times [1,2]. It is well known that the serum albumin is a major soluble protein constituent in the circulatory system, which plays an important role in the transportation and deposition of many drug molecules in the blood [3–5]. In this work, bovine serum albumin (BSA) is selected as our protein model because of its medical importance, stability, low cost, unusual ligand-binding properties. It displays approximately 76% sequence homology, and the 3D structure of BSA is believed to be similar to that of HSA [6–8]. However, they differ in the number of tryptophans: BSA has two tryptophans, but HSA has only one [9–10].

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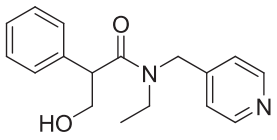

Scheme 1. Molecular structure of TA.

Tropicamide (TA) (Scheme 1) also known as mydriacyl, which is a nonselective muscarinic antagonist. It produces a short-lasting mydriasis and cycloplegia in the eye, helps to make the pupil of eyes larger and relax the muscles in eyes [11]. The peak Tropicamide concentrations varied between 1.3 and 5.2 ng/ml; The drug is easily absorbed from the eye, and then disappears rapidly from the systemic circulation [12]. Based on the effectiveness of dilation, duration of action, and low occurrence of side effects, it usually selected as eye drops and used to aid eye examinations [13]. Besides, it has a good effect on juvenile pseudo myopia and mild hyperopia, especially plays a significant role in the prevention of juvenile myopia.

Fluorescence spectroscopy is a probe technique to detect the local environment of the fluorophore, which has been widely used for drug–protein studies [14,15]. In this paper, the binding of TA to BSA was studied under imitated physiological conditions by fluorescence and ultraviolet spectroscopy. The binding constants were calculated and binding mechanism was investigated. In addition, the effect of TA on the conformational change of BSA was also studied. We hope this work will not only provide useful information for understanding of the TA, but also illustrate its binding mechanisms at a molecular level.

Materials and methods

Reagents

BSA (⩾99%) was obtained from Huamei Bioengineering Co. (Shanghai, China) and was dissolved in a Tris–HCl (0.05 mol L^{-1}, pH = 7.43) buffer to form the BSA solution with a concentration of 1.00×10^{-5} mol L^{-1}. A Tris–HCl buffer (0.05 mol L^{-1}, pH = 7.43) containing 0.10 mol L^{-1} NaCl was selected to keep the pH value constant and to maintain the ionic strength of the solution. TA was obtained from wujing Pharmaceutical Co. (Wuhan, China). The TA (8.79×10^{-3} mol L^{-1}) solution was prepared in double-distilled water. All other reagents were of analytical grade and double-distilled water was used during the experiment.

Apparatus

Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer (Tokyo, Japan) with a SB-11 water bath (Eyela) and 1.0 cm quartz cells. The emission and excitation slits were 10 and 5 nm, respectively. The synchronous fluorescence spectra were obtained by setting the excitation and emission wavelength interval ($\Delta\lambda$) at 15 nm and 60 nm. The absorption spectra were obtained from a Shimadzu UV-2501 spectrophotometer (Tokyo, Japan). The pH measurement was made with a Leici pHS-2 digital pH-meter (Shanghai, China) with a combinational glass calomel electrode.

Measurements of spectra

A 2.5 ml solution containing 1.00×10^{-5} mol L^{-1} BSA was titrated by successive additions of 8.79×10^{-3} mol L^{-1} TA solution and the concentration of TA varied from 0 to 3.16×10^{-4} mol L^{-1}. Titrations were done manually by using micro-injector. Fluorescence quenching spectra were measured in the range of 280–500 nm at the excitation wavelength of 280 nm. The fluorescence spectra were performed at three temperatures (292, 301 and 310 K).

The fluorescence spectra of BSA were also recorded in the presence of some metal ions, which contain Mg^{2+}, Ca^{2+}, Cu^{2+}, and Ni^{2+} at 310 K in the range of 280–500 nm at excitation wavelength of 280 nm. In the system, the overall concentration of BSA was fixed at 1.00×10^{-5} mol L^{-1}, and the common metal ion was maintained at 4.05×10^{-5} mol L^{-1}.

The UV–Vis absorption spectra of TA solution with the concentration of 1.00×10^{-5} mol L^{-1} was measured in the range of 200–500 nm at 292 K.

Results and discussion

The fluorescence quenching spectra

The fluorescence spectra of BSA in the presence of TA at different concentrations are shown in Fig. 1. It is obvious that BSA has a strong fluorescence emission peak at 343 nm after being excited at the wavelength 280 nm. The fluorescence intensity of BSA decreased gradually with the addition of TA and the maximum emission wavelength has occurred a small blue shift (from 345 to 342 nm) when a fixed concentration of BSA was titrated with different amounts of TA, which implying that the microenvironment around the chromophore of BSA was changed after adding TA.

The mechanism of quenching of BSA fluorescence by TA

Fluorescence quenching refers to any process that decreases the fluorescence intensity of a fluorophore. Before we starting analyze the mechanism, we need to solve an important problem "the inner-filter effect". The term can be illustrated as: when a standard 1 cm^2 cuvette is viewed at right angles to the exciting beam, only a small part of the solution is actually observed. If the sample solution has a sufficiently high optical density, an appreciable amount of the exciting light will be absorbed before it reaches the center of the cell. So correction must be made for this effective decrease in the light intensity in the region of observation. For a solution in which the absorbance does not exceed approximately 0.3, self-absorption and inner-filter effects can be corrected by the following equation [16–18]:

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

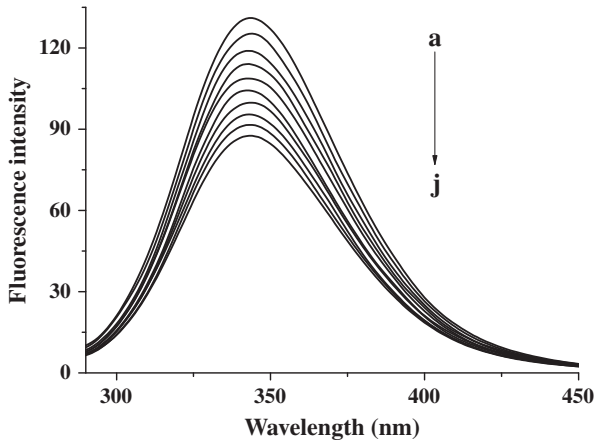

Fig. 1. The fluorescence quenching spectra of BSA by TA at 310 K. $\lambda_e x = 280$ nm. [BSA] = 1.00×10^{-5} mol L^{-1}: [TA] (*a–j*): 0, 3.52, 7.04, 10.56, 14.08, 17.60, 21.12, 24.64, 28.16, 31.68 ($\times 10^{-5}$ mol L^{-1}).