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Fluorometric investigation of the interaction of bovine serum albumin with surfactants and 6-mercaptopurine

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Abstract

Fluorescence quenching in solutions of bovine serum albumin has been investigated in the presence of 6-mercaptopurine and ionic surfactants. Spectroscopic analysis of the emission quenching at different temperatures revealed that the quenching mechanism of bovine serum albumin by 6-mercaptopurine was dynamic quenching mechanism. The Stern–Volmer quenching model has been successfully applied, and the activation energy of the interaction between 6-mercaptopurine and bovine serum albumin as much as 4.26 kJ mol^{-1} was calculated. The distance r between donor (bovine serum albumin) and acceptor (6-mercaptopurine) was obtained according to fluorescence resonance energy transfer (FRET). The result of synchronous fluorescence spectra shows that the conformation of bovine serum albumin has been changed at the present of 6-mercaptopurine.

Keywords: 6-Mercaptopurine; Bovine serum albumin; Fluorescence spectroscopy; UV–Visible spectroscopy; Ionic surfactant; Fluorescence resonance energy transfer

1. Introduction

6-Mercaptopurine (6-MP) is an antiviral agent of purine series, which has been used extensively since the 1950s in remission maintenance therapy for patients suffering from acute lymphoblastic leukemia [1–4]. It is a yellow solid with a molecular weight of 152.18 (Molecular structure: Scheme 1; Formula: $C_5H_4N_4S$), which is stable at room temperature (decomposes on heating above 308 °C), and minimally toxic [5]. 6-MP is used as an oral medicine in the treatment of acute lymphoblastic leukemia, acute myoblastic leukemia, chronic myelocystic leukemia, trophoblastic neoplasia, ulcerative colitis and Crohn's disease [5–11]. It was also found that 6-MP is a potent anticancer agent with a reasonable anti-HIV activity [11–13]. Recent research indicated that oral absorption of 6-MP is relatively low, and about 50% of the drug appears in the plasma in 1–2 h [10,11].

Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms, being the major macromolecule contributing to the osmotic blood pressure [14]. In addition to blood plasma, serum albumins are also found in tissues and bodily secretions throughout the body; the extravascular protein comprises 60% of the total albumin [15]. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells in vivo and in vitro; they can play a dominant role in drug

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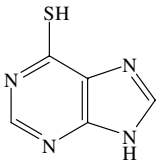

Scheme 1. Molecular structure of 6-mercaptopurine.

disposition and efficacy [16]. Consequently, it is important to study the interactions of drugs with this protein. However, detailed investigations of the interaction of bovine serum albumin (BSA) with 6-MP are scanty. In this work, BSA is selected as our protein model because of its medically important, low cost, ready availability, unusual ligand-binding properties [17], and the results of all the studies are consistent with the fact that human and bovine serum albumins are homologous proteins [16–18].

Fluorescence and UV–Vis absorption spectroscopies are powerful tools for the study of the reactivity of chemical and biological systems. Protein-drug binding greatly influences absorption, distribution, metabolism, and excretion properties of typical drugs, and there are many correlated studies based on spectroscopic methods [19–22]. In the present investigation, studies of the interaction of BSA with 6-MP and ionic surfactants such as sodium dodecyl sulfate (SDS) and cetytrimethyl ammonium bromide (CTAB) are reported, using spectroscopic technique including fluorescence spectra and UV–Visible absorption spectra to characterize the different species present in solution as function of surfactant, the conformation of BSA was also investigated at the present of 6-MP.

2. Experimental

2.1. Materials

6-MP, BSA, SDS and Tris (hydroxymethyl) aminomethane (Tris) were obtained from Sigma, CTAB was obtained from Amresco. The samples were dissolved in Tris–HCl buffer solution (0.05 mol L^{-1} Tris, pH = 7.4). All other reagents were of analytical reagent grade and doubly distilled water was used throughout.

2.2. Apparatus

All fluorescence spectra were recorded with a F-2500 Spectrofluorimeter (Hitachi, Japan), equipped with 1.0 cm quartz cells and a thermostat bath.; TU-1901 spectrophotometer (Puxi Ltd. of Beijing, China) was used for scanning UV–Visible spectra; sample masses were accurately weighted using a microbalance (Sartorius, ME215S) with a resolution of 0.1 mg.

2.3. Spectroscopic measurements

The absorption spectra of BSA, 6-MP and their mixture were performed at room temperature.

The fluorescence measurements were performed at different temperatures (298, 301, 304, 307 and 310 K). Excitation wavelength was 280 nm. The widths of both the excitation slit and the emission slit were set to 2.5 nm. Appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background.

3. Results and discussions

3.1. Fluorescence characteristics of bovine serum albumin

Fluorescence quenching refers to any process which decreases the fluorescence intensity of a sample [23]. A variety of molecular interactions can result in quenching, including excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching.

When the concentrations of BSA were stabilized at 10^{-5} mol L^{-1}, and the content of 6-MP varied from 0 to 6.4×10^{-5} mol L^{-1} at increments of 0.4×10^{-5} mol L^{-1}. The effect of 6-MP on BSA fluorescence intensity is shown in Fig. 1. As the data show, the fluorescence intensity of BSA decreased regularly with the increasing of 6-MP concentration.

The fluorescence quenching data are usually analysed by the Stern–Volmer equation [24]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q], \tag{1}$$

where F_0 and F are the steady-state fluorescence intensities in the absence and in the presence of quencher

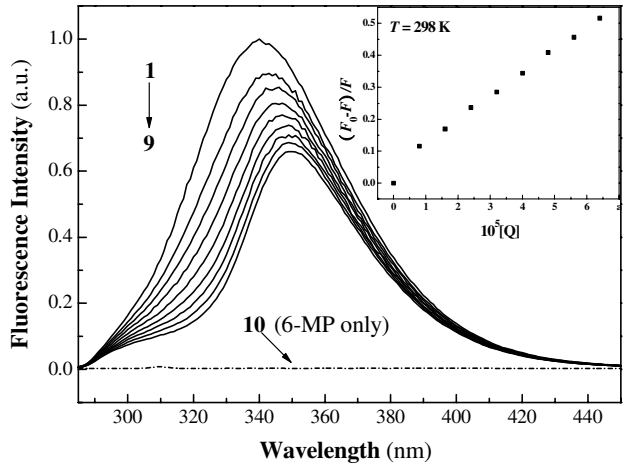

Fig. 1. Emission spectra of BSA in the presence of various concentrations of 6-MP. The inset corresponds to the Stern–Volmer plot. c (BSA) = 1.0×10^{-5} mol L^{-1}; c (6-MP)/(10^{-5} mol L^{-1}), 1–9: 0; 0.8; 1.6; 2.4; 3.2; 4.0; 4.8; 5.6; 6.4.