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Influences of urea and guanidine hydrochloride on the interaction of 6-thioguanine with bovine serum albumin

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ABSTRACT

The interaction of 6-thioguanine (6-TG) and bovine serum albumin (BSA) in the absence and presence of denaturant (urea and Guanidine hydrochloride) was investigated by fluorescence spectroscopic techniques. Changes of fluorescence intensity both in F_{304} and in F_{348} of BSA reflected increasing participation of tyrosine fluorescence in the total emission with increasing denaturant, which indicated that energy transfer from tyrosyl residues to tryptophanyl residues became less efficient in the denatured tertiary protein structure. The quenching effects of 6-TG were shown not only on the native but also on the unfolded form of BSA. The quenching constants and binding constants were calculated from the fluorescence spectra of the BSA/6-TG complex both in the absence and presence of the denaturant. The data suggested that the quenching constants and binding constants of 6-TG for BSA decreased with increasing concentration of denaturant. The spectroscopic analysis also showed antidenaturant properties of 6-TG under both denaturant conditions.

1. Introduction

The interactions between bio-macromolecules and drugs have attracted increasing research interest in recent years [1–4]. Among various bio-macromolecules, serum albumins are the most abundant soluble proteins in the circulatory system of a wide variety of organisms and have many indispensable physiological functions. The most outstanding function of serum albumins is that they serve as a depot protein and a transport protein for many exogenous compounds [5,6]. Moreover, they can hold some ligands in a strained orientation and render potential toxins harmless by transporting them to disposal sites. In pathogenous states such as chronic renal and liver diseases the transporting function of albumin can be disturbed. Defective binding of albumin can cause the inhibition of the metabolism of amphipathic hormones. In this pathogenic state of albumin, weaker drug–protein interaction can result in the increase of drug concentration in the blood serum. Sometimes this effect can cause toxic poisoning or even lead to death [7]. Therefore, studies on the binding of drug with protein in the absence and presence of chemical denaturant will increase interpretation of the metabolism and transporting process of drug and will help to explain the relationship between the structure and the function of the protein. As one kind of model proteins, BSA has been studied extensively due to its structural homology with human serum albumin (HSA) [8–11].

The field of protein folding has seen essential advances in recent years because of growing interest in diseases that results from protein misfolding and aggregation [12–14]. It has been reported that the structural alteration in either plasma or tissue protein in patients, induced by unfolding process, will be reflected in unbound drug concentration [15].

2-amino-6-purinethiol or 6-thioguanine (6-TG), a sulfur derivative of guanine (see Fig. 1), is one of the oldest anticancer drugs and immunosuppressive agents [16]. It has been established that 6-TG and its metabolites exerted their primary cytotoxic function through incorporation of deoxythioguanosine into DNA, and inhibit the function of RNase H in DNA–RNA heteroduplex molecules [17,18].

Spectroscopic methods including fluorescence spectroscopy techniques are popular techniques to investigate the interaction between some small molecule ligands and bio-macromolecules [19–22]. Bovine serum albumin is a globular protein (molecular mass 67,000) consisting of a single chain of 585 amino acids residues. Its tertiary structure is characterized by a repetitive pattern of three α-helical homologous domains. Each domain is divided into two subdomains A and B composed of six and four α-helices, respectively. All the subdomains are linked by paired 17 disulfide bonds [7]. BSA contains two tryptophans, Trp 135 and Trp 214, which are located in hydrophobic cavities in IB and IIA subdomains, respectively. Additionally, some tyrosyl residues are

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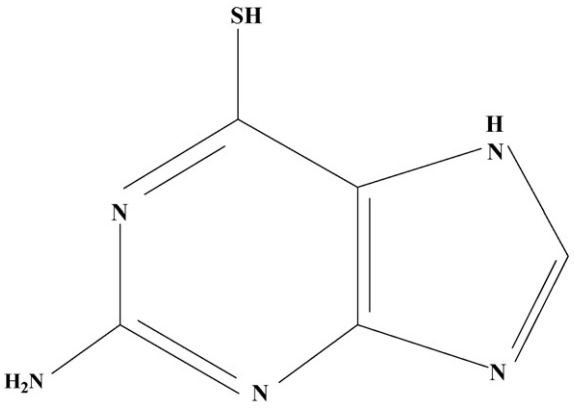

Fig. 1. The structure of 6-TG.

located in subdomain IA (Tyr 30, Tyr 84), IB (Tyr 140, Tyr 148, Tyr 150, Tyr 156, Tyr 157) and IIA (Tyr 263) [23]. By measurement and analysis of maximum fluorescence intensity, maximum emission wavelength, quenching efficiency, binding constants, etc., in the presence or absence of denaturant, the changes of microenvironment surrounding tryptophanyl and tyrosyl residues in BSA can be acquired [24–27]. Previously, the interactions between serum albumin and some pyrimidine drugs, such as uracil [28,29], mercaptopyrimidine [28,29], 6-propyl-2-thiouracil [7], and sulfasalazine drug [30,31] in the presence of urea or guanidine hydrochloride have been reported. No attempts had been made so far to investigate the interaction of 6-TG with BSA in the presence of urea or guanidine hydrochloride by fluorescence spectroscopy based on the literature survey.

Herein, we presented the interaction studies in BSA/6-TG system with or without denaturant and clarified one kind of cooperation mechanism of 6-TG and denaturant for the structural changes of BSA. Additionally, we aim to show the alteration of the binding site of 6-TG in BSA in the presence of urea and guanidine hydrochloride. For a safe and effective therapy, it is significant to estimate the binding ability of the changed albumin in uremic and diabetic patients because the conformational disorder of transporting protein-serum albumin usually occurs for those patients suffering from chronic renal and liver diseases.

2. Experimental

2.1. Apparatus and reagents

Fluorescence measurements were performed on a LS 55 luminescence spectrometer (PerkinElmer, USA). The pH measurements were carried out on a PB-10 Exact digital pH meter (Sartorius, Germany), which was calibrated with standard pH buffer solution before use.

BSA was purchased from Amresco Company (USA) and the purity was checked for monomer by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The stock solution of BSA (2.5×10^{-5} mol/L) was prepared in 0.2 mol/L phosphate buffer (pH 7.4) containing 0.05 mol/L NaCl. BSA solution was prepared based on its molecular weight of 67,000 and stored in the 4 °C refrigerator prior to use. The concentration of native proteins in 0.2 mol/L sodium phosphate buffer was determined spectrophotometrically from the extinction coefficient reported at 280 nm, pH 7.4. 6-TG was obtained commercially from Acros Organics (USA). A standard solution of 6-TG (5.0×10^{-3} mol/L) was prepared by dissolving 6-TG in 0.4 % NaOH and then allow it to be dissolved in distilled water. All other chemicals used in this study were of analytical grade. All the measurements were carried out at room temperature.

2.2. Fluorescence spectra

The mixtures of 6-TG solution and BSA solution with appropriate ratio were added into the different 15 mL flasks and then appropriate quantities of 0.2 mol/L phosphate buffer (pH 7.4), in the presence or absence of denaturant and appropriate ratio of 1.0 mol/L NaCl were transferred into the above flasks. The resultant mixture was incubated at 37 °C for 30 min before the determination of fluorescence. Steady-state fluorescence experiments were carried out by spectrofluorimeter (PerkinElmer, USA) in a 10 mm path length quartz cell. Fluorescence was excited at 280 nm and the emission intensity was recorded ranging from 280 to 400 nm. The quenching effect of 6-TG on protein was monitored at λ_{max} (304–306 nm) and λ_{max} (338 nm or 348 nm), responding to Tyr and Trp emission.

To quantitatively estimate the unfolding progress in BSA, the coefficient D was calculated for each sample:

$$D = \frac{F_{Trp}}{F_{Tyr}} \quad (1)$$

where, F_{Tyr} were relative fluorescence intensities at 304 nm and F_{Trp} were relative fluorescence intensities at 338 nm or 348 nm, respectively.

2.3. Quenching constants and binding constants

The quenching constants were calculated from equation:

$$\frac{F_0}{F} = 1 + K_q \tau_0 Q = 1 + K_{sv} Q \quad (2)$$

where F_0 and F represent the fluorescence intensities in the absence and in the presence of quencher, K_q is the quenching rate constants of the bimolecular, K_{sv} is the dynamic quenching constants, τ_0 is the average lifetime of the molecule without quencher, 10^{-8} s [32] and Q is the concentration of the quencher.

The binding constants for 6-TG/protein complex were calculated from the Scatchard equation:

$$lg\left[\frac{(F_0 - F)}{F}\right] = lgK_b + nlgQ \quad (3)$$

where Q is the concentration of the quencher, 6-TG, K_b is binding constants, F_0 and F represent the fluorescence intensities in the absence and presence of quencher. A plot of $lg[(F_0-F)/F]$ versus lgQ will give a straight line with a slope of n and y-axis intercept of lgK_b [33].

3. Results and discussion

3.1. Characteristics of the fluorescence spectra

Fluorescent emission spectra of BSA showed an emission maximum at 338 nm (Fig. 2-spectrum 1 and Fig. 3-spectrum 1) obtained with 280 nm excitation. In the presence of 1.0×10^{-6} mol/L 6-TG the BSA emission spectrum did not change in the maximum emission wavelength but decreased in the fluorescence intensity (Fig. 2-spectrum 2 and Fig. 3-spectrum 2). This suggested that the tertiary structure of BSA remained unaltered with the addition of 6-TG. In the presence of 6.5 mol/L urea or 2.5 mol/L GdmCl the fluorescence spectra of BSA display two maxima, respectively at ~304 nm and ~348 nm (Fig. 2-spectrum 3 and Fig. 3–spectrum 3). This indicated the fluorescence from tyrosine appeared and the tryptophan peak red-shifted from 338 to 348 nm in the unfolded protein. The fluorescence intensity of two above-mentioned systems (in the presence of 6.5 mol/L urea or 2.5 mol/L GdmCl), with addition of 1.0×10^{-6} mol/L 6-TG also showed significant decrease