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Characterization of 6-mercaptopurine binding to bovine serum albumin and its displacement from the binding sites by quercetin and rutin

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ABSTRACT

Binding of a drug to the serum albumins as major serum transport proteins can be influenced by other ligands leading to alteration of its pharmacological properties. In the present study, binding characteristics of 6-mercaptopurine (6-MP) with bovine serum albumin (BSA) together with its displacement from its binding site by quercetin and rutin have been investigated by the spectroscopic method. According to the binding parameters, a static quenching component in overall dynamic quenching process is operative in the interaction between 6-MP and BSA. The binding of 6-MP to BSA occurred spontaneously due to entropy-driven hydrophobic interactions. The synchronous fluorescence spectroscopy study revealed that the secondary structure of BSA is changed in the presence of 6-MP and both Tyr and Trp residues participate in the interaction between 6-MP and BSA with the later one being more dominant. The binding constant value of 6-MP–BSA in the presence of quercetin and rutin increased. 6-MP was displaced by ibuprofen indicating that the binding site of 6-MP on albumin is site II. Therefore, the change of the pharmacokinetic and pharmacodynamic properties of 6-MP by quercetin and rutin through alteration of binding capacity of 6-MP to the serum albumin cannot be ruled out. In addition, the displacement study showed that 6-MP is located in site II of BSA.

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1. Introduction

6-Mercaptopurine (6-MP) (Fig. 1) is a purine analog with immunosuppressant and antineoplastic properties. It is used in the treatment of childhood acute lymphocytic leukemia and considered as an efficient maintenance therapy for Crohn's disease. The oral absorption of 6-MP is incomplete and variable and only 16%–50% of the administered dose reaches the blood. After absorption, the drug is bound to the serum albumin with the protein binding ranging from 19% to 30% [1,2].

Serum albumins are the major soluble protein constituents of the circulatory system and act as a depot protein. They also play an important role in the transport and distribution of many endogenous and exogenous compounds including drugs [3]. The binding of a drug to the serum albumins can influence its pharmacokinetics and pharmacodynamic properties. As it is the unbound fraction of a drug that exhibits pharmacological activity, the displacement of the bound drug from the albumin by other ligands can alter the active free drug concentration and consequently its effect.

Bovine serum albumin (BSA) is the most extensively used protein for ligand–protein binding studies due to its low cost, wide availability and structural homology with human serum albumin [4]. BSA consists of a single chain of 582 amino acid residues with a molecular mass of 66,200 Da. The chain is made up of three homologous but structurally distinct domains (I–III); each domain in turn is composed of two subdomains of A and B. BSA possesses intrinsic fluorescence which makes it possible to study the interaction of different ligands with this important protein by the fluorescence spectroscopic methods. This fluorescence property of BSA arises mainly from two tryptophan residues: Trp-212 located within a hydrophobic binding pocket of domain II, and Trp-134 located on the surface of domain I [5–8]. The intensity of BSA fluorescence may be quenched following the interaction of a molecule with this macromolecule. Therefore, it would be possible to investigate the interaction of drugs with BSA and gain insight into the mechanisms and physic–chemical characterization of binding of drugs to the serum albumin.

The interaction of 6-MP with serum albumins has been investigated with varying results [9]. Like other drugs, the binding of 6-MP could be affected by other ligands. Flavonoids are one of the largest groups of naturally occurring compounds distributed in the plant kingdom. They are consumed in high content in our daily life either as our dietary program or in the form of

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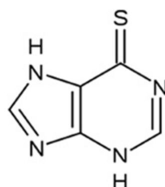

Fig. 1. 6-Mercaptopurine (3, 7-dihydropurine-6-thione).

dietary supplements and plant extract [10]. These compounds are able to inhibit several important enzymes including aldehyde oxidase (EC 1.2.3.1: aldehyde: oxygen oxidoreductase, AO) and anthine oxidase (EC 1.17.3.2: xanthine: oxygen oxidoreductase, XO) [11–13]. AO and XO which are collectively referred as "molybdenum hydroxylases", are two important cytosolic enzymes involved in the metabolism of numerous exogenous and endogenous substances [14–17]. 6-MP is one of these substances metabolized by AO and XO in its catabolic pathway. It implies the existence of a structural similarity between 6-MP and the flavonoids in binding to the target proteins and, therefore, a competition for the binding sites. In addition, flavonoids, like 6-MP, are able to bind to the serum albumin [18]. Therefore, it is likely that the flavonoids interfere with the binding of 6-MP to serum albumin and displace the bound drug from the carrier protein and, consequently, leading to alteration of the drug effects.

In the present study, after considering the inner filter effect, the binding characteristics between 6-MP and BSA, the effect of 6-MP on the microenvironment and conformation of BSA, and the thermodynamics of the interaction have been investigated using different spectroscopic methods. More important, for the first time, the ability of quercetin and rutin, as two important flavonoids, to displace 6-MP from its binding site on albumin serum has also been studied in detail.

2. Materials and methods

2.1. Apparatus

Both fluorescence and synchronous fluorescence spectra were recorded on a RF-5301 spectrofluorophotometer (Shimadzu, Japan). The widths of both the excitation and emission slits were set at 5 nm. The optimum excitation and emission wavelengths for BSA were obtained as 280 and 340 nm, respectively. To evaluate and correct the inner filter effect (IFE), the correction procedures based on absorbance measurements of solutions were performed at excitation and emission wavelengths of albumin.

2.2. Reagents

BSA, 6-MP, rutin and quercetin were purchased from Sigma-Aldrich (Poole, England). Double-distilled water was used throughout experiments. Solutions were prepared in 20 mM phosphate buffer (pH 7.4). A 1 mM stock solution of BSA was prepared daily at a pH of 7.4 buffer. Rutin and quercetin stock solutions (1 mM) were prepared in ethanol.

2.3. Procedure

A 3 mL solution of 0.01 mM BSA was titrated by addition of different concentrations of 6-MP. The mixture was allowed to stand for 5 min. Then, the fluorescence emission spectra were recorded in a wavelength range of 250–500 nm at 293, 300, 308 and 315 K. The displacement experiments were carried out in the presence of a certain concentration of quercetin or rutin (0.01 mM) in the mixture of 0.01 mM BSA and different concentrations of 6-MP. After 10 min, the fluorescence emission spectra were measured at 293, 300, 308 and 315 K. The absorption spectra were recorded on a Shimadzu UV-2550 spectrophotometer (Kyoto, Japan) equipped with 1.0 cm quartz cells.

3. Results and discussion

3.1. Fluorescence quenching spectra

Fluorescence has been widely used to investigate the interaction between ligands and proteins and can give some information about the quenching mechanism, binding constants and binding sites. The IFE would affect fluorescence measurements [19].To determine whether an IFE induced by the absorption of excitation and emission radiation is significant in this system, the absorbance of 6-MP at 280 nm (excitation wavelength) and 340 nm (fluorescence peak) were evaluated. It was found that at the highest added concentration of 6-MP (40 μM), there was about 0.5 and 0.8 absorbance unit (more than 0.1) at 280 and 340 nm, respectively (Fig. 2). Therefore, the IFE cannot be ignored in the system and should be corrected before further experiments. The fluorescence intensities were corrected for the absorption of the excitation light and reabsorption of the emitted light to decrease the inner filter using the following equation [20]:

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

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensities, respectively, A_{ex} and A_{em} are the absorbance values of 6-MP at excitation and emission wavelengths, respectively.

Under the Spectrofluorometric conditions described in Section 2, the fluorescence spectrum was recorded for BSA in the absence and presence of 6-MP. The excitation wavelength was kept constant at 280 nm. It was observed that the fluorescence intensity of BSA decreased and also a very slight red shift was shown for the emission wavelength with the concentration increase of 6-MP. The red shift of the bands also revealed that the tryptophan residue of BSA has been exposed to a more hydrophobic environment in the drug–protein system. Similar results have been reported by others [21–23]. In Fig. 3, the emission spectra of BSA in the presence and absence of various concentrations of 6-MP have been illustrated.

There are two major different mechanisms that occur in the fluorescence quenching: static quenching and dynamic quenching. Both of them can be described by the Stern–Volmer equation

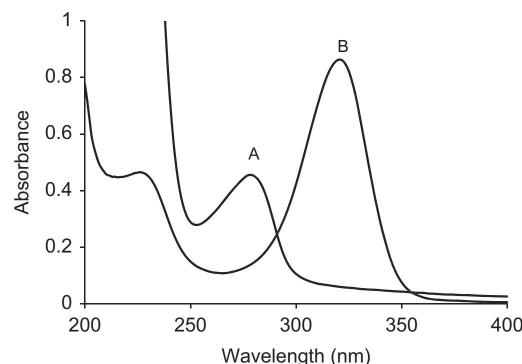

Fig. 2. UV spectra of (A) albumin (10^{-5} M) and (B) mercaptopurine (40 μM).

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