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Characterization of the interaction between furosemide and bovine serum albumin

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Abstract

The interaction of furosemide (FU), one kind of potent diuretic, with bovine serum albumin (BSA) has been investigated at physiological acidity (pH 7.40) by fluorescent technique. Displacement experiment with site markers and Synchronous fluorescence clearly reveal that there are non-specific binding sites of FU with BSA. This conclusion was supported by the binding studies in the presence of the hydrophobic probe 1-anilinonaphthalene-8-sulfonic acid (ANS) and in different ionic strength. The binding sites number n and binding constant K were measured. The thermodynamic parameters ΔH°, ΔG°, ΔS° at different temperatures were calculated. The effects of other four diuretics, some common metal ions and bioactive components from herbal medicine on the binding are also considered. The results show only bumetanide has strong effect on FU's binding. Moreover, several data processing methods presently used were evaluated with Data of the same set. Quite different results were obtained from these methods suggesting more attention should be paid to the data processing methods.

Keywords: Furosemide; Serum albumin–drug interactions; Fluorescence quenching; Bovine serum albumin; Non-specific binding

1. Introduction

Serum albumin is the most abundant protein in animal's including human circulatory system. It is in charge of the transport of a variety of endogenous and exogenous substances in body and plays an important role in the distribution and deposition of these substances [1]. When drugs are absorbed, they enter into the circulatory system and extensively and reversibly bind to serum albumin [2]. An important aspect of a drug's biodisposition profile is the extent to which it binds to plasma proteins [3]. Drug–protein interaction has significance in pharmacology. It can affect the biological activity [4,5] and toxicity [6–8] of drug. The binding parameters are helpful in the study of pharmacokinetics and the design of dosage forms [9,10]. BSA consists of 583 amino acid resides in a single polypeptide chain. It possesses a high helical content (about 67%). It is built from three structurally homologous domains (I, II and III). Each domain is the product of two sub-domains (A and B) [11,12]. Serum albumin is postulated to have a heart-shaped structure with dimensions of $80 \times 80 \times 30$ Å [13]. There are two famous drug-binding sites in albumin, namely Sudlow's binding sites I and II [14]. Later some researchers [15] found digitoxin binds to albumin at site different from sites I and II and it was so-called site III. X-ray studies [16] suggested that sites I and II are located in subdomain IIA and IIIA of albumin, respectively.

Different methods have been developed to evaluate drug–protein interactions, such as the traditional equilibrium dialysis [17–19], ultrafiltration [20] and chromatographic methods (HPLC [21,22] and CE [23,24]). All these approaches provide useful binding information.

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However, these methods are constrained by one or more of the factors such as Donnan effect, Sieve effect, nonspecific adsorption, leakage through membranes, long analysis time and large-sample size requirements. Owing to its exceptional sensitivity, selectivity, convenience and abundant theoretical foundation [25], fluorescence spectroscopy is a popular method for studying the drug-protein interaction [26]. It can provide the binding information and reflect the conformation changes of proteins in various environments.

FU is a potent, promptly acting diuretic used for diverse treatments in mankind, as well as in veterinary medicine [27]. Diverse experiments have been done to investigate the binding properties of FU to human serum albumin (HSA) or plasma; little concern was placed on the binding of FU to bovine serum albumin (BSA). Moreover, distinct binding parameters, such as binding constant and sites, were got by different methods and sometimes even by the same method. Table 1 shows some results about the binding of FU with HAS. As can be seen from Table 1, the results are quite different. In this paper, interaction between FU and BSA was studied using fluorescence quenching method. The binding sites number n, apparent binding constant K and thermodynamic parameters were measured. The effects of other four diuretics: cyclopenthiazide, triamterene, bumetanide and hydrochlorothiazide; some common metal ions and bioactive components from herbal medicine on the binding were also investigated. The results show that only bumetanide has strong effect on FU's binding. Displacement of FU with phenylbutazone (PB) and flufenamic acid (FA) showed the binding of FU with BSA is non-specific. This result is consistent with the synchronous fluorescence research. This conclusion was supported by the binding studies in the presence of the hydrophobic probe ANS and in different ionic strength. In addition, several data processing methods commonly used at present were evaluated with data of the same set and quite different results were obtained. Suggesting more attentions should be paid to the data processing methods. Ultrafiltration and capillary electrophoresis method revealed a high binding percentage of FU with BSA.

2. Experimental

2.1. Materials

BSA ($\geqslant$98%, Roche) was used without further purification and its molecular weight was assumed to be 66,500. BSA stock solution (2×10^{-5} mol L^{-1}) was prepared with doubly distilled water and was kept in the dark at 4 °C. Furosemide, 8-anilino-1-naphthalenesulfonic acid (ANS) (Sigma–Aldrich, Inc., USA). 18β-Glycyrrhetinic acid (GA) ($\geqslant$97%, Fluka, product of Spain); Glycyrrhizic acid ammonium salt (GL) ($\geqslant$95%, Fluka, product of Japan); phenybutazon (PB) (99%), fluofenamic acid (FA) (97%) and hydrochlorothiazide (HD) (98%) were products of Alfa Aesar (Lancaster); cyclopenthiazide (CY), triamterene (TR) and bumetanide (BU) (analytical grade) were obtained from the National Institute for Control of Pharmaceutical and Products, China. FU was prepared in 90% ethanol to get a 8×10^{-4} mol L^{-1} solution. ANS was dissolved in a little thin sodium hydroxide and then added buffer to the scale. Stock solutions of GA, GL, PB, FA, HD, CY, TR and BU were prepared in 90% ethanol and their final concentration in titration were equal to BSA's, namely 4×10^{-6} mol L^{-1}. NaCl (0.5 mol L^{-1}) solution was used to maintain the ionic strength. Buffer (pH 7.40) consists of 2-amino-2-(hydroxymethyl) propane-1, 3-diol (Tris 0.1 mol L^{-1}) and the pH was adjusted to 7.40 by adding HCl (0.1 mol L^{-1}) monitored by pH-meter. Solutions of common metal ions (2.0×10^{-2} mol L^{-1}) were prepared by $MgCl_2$, $AlCl_3$, $CoCl_2$, $ZnSO_4$ and $CuSO_4$, respectively. The final of concentration common metal ions in fluorescence titration were 1.0×10^{-4} mol L^{-1}. All reagents are of analytical grade unless specialized. Water is doubly distilled water.

2.2. Apparatus and methods

All fluorescent measurements were carried out on an F-4500 fluorescence spectrophotometer (Hitachi, Kyoto, Japan) equipped with a xenon lamp source and 1.0 cm cell. All pH measurements were made with a pHS-3 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass–calomel electrode. The mass of sam-

Table 1
Binding constant of FU to HAS by diverse methods

Binding constant		Temperature (°C)	Method	Literature
K_1 ($\times10^{-5}$)	K_2 ($\times10^{-4}$)			
0.31	0.83	Ambient	Equilibrium dialysis	[17]
0.69	–	37	Equilibrium dialysis	[18]
0.51	1.58	37	Equilibrium dialysis	[19]
3	–	Ambient	Ultrafiltration	[20]
1.68	0.96	37	HPLC	[21]
0.94	0.55	35	CZE	[23]
0.27	–	–	–	[28]
137	–	Ambient	Fluorescence quenching	[29]
0.19	–	25	Fluorescence quenching	[30]