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Application of capillary electrophoresis-frontal analysis for comparative evaluation of the binding interaction of captopril with human serum albumin in the absence and presence of hydrochlorothiazide

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ABSTRACT

The application of capillary electrophoresis-frontal analysis for comparative evaluation of the binding interaction between antihypertensive drug captopril and human serum albumin in the absence and presence of diuretic drug hydrochlorothiazide was presented in this work. At near-physiological conditions (67 mM phosphate buffer, pH 7.4, $I = 0.17$, 37 °C), the individual solution of 100 μM captopril and the co-binding solution with 60 μM hydrochlorothiazide added were pre-equilibrated with series concentrations of HSA (10–475 μM) respectively, introducing hydrodynamically into an uncoated fused silica capillary (35 cm × 50 μm I.D. with 26.5 cm effective length). The values of number of binding sites, the binding constant for captopril and hydrochlorothiazide binding to HSA were obtained, respectively. It can be found that both drugs exhibit moderate binding properties towards HSA and there does not exist significant competitive binding effects between them.

1. Introduction

Plasma protein binding, along with the properties of ionization and lipophilicity, plays a critical role in drug discovery and development. These properties determine whether a compound has a suitable pharmacokinetic absorption, distribution, potency, excretion, and toxicity profile, thus eventually influence the processing of the compound [1–4]. When being absorbed and distributed throughout the blood circulation, most drugs bind to plasma proteins in varying degree. It is considered that only free drug can penetrate the blood vessel membranes, reach the target, and exert pharmaceutical activities, while bound drug cannot cross the barriers which usually serving as a drug reservoir which can prolong the duration of the drug actions in vivo [5–7]. Therefore, extensive knowledge of the binding characteristics of the drug is essential for understanding the pharmacological efficacy and its clinical safety concerning, as well.

Human serum albumin (HSA) is the most abundant protein in plasma at a concentration level of about 500 - 750 μM [1,8]. It is regarded that most drugs bind to HSA noncovalently and there are two major binding sites, termed as the Sudlow site I (abbreviated as site I) and the Sudlow site II (abbreviated as site II) [9,10], respectively. Those high affinity compounds at the specific site with the binding constant (K) at the range of 10^5 to 10^6 M^{-1} can be regarded as a site marker, for example, phenylbutazone (Fig 1a) as the binding marker of site I and flurbiprofen (Fig 1b) as the binding marker of site II, therefore competitive binding studies should then be designed to identify the binding sites for a specific drug on HSA [9–11].

Different strategies have been developed to investigate the drug-protein interactions, including equilibrium dialysis, ultrafiltration, and ultracentrifugation [4,5,7]. However, some disadvantages of these analytical techniques limit their applications, such as long equilibrium time and large sample consumption. Capillary electrophoresis (CE) has been proved to be more convenient and cost-effective for the interaction study [5,12–17]. There are several modes of CE have been proposed for the quantitative assessment of drug-protein interactions, such as affinity capillary electrophoresis, the Hummel-Dreyer method, vacancy peak method, vacancy affinity capillary electrophoresis, and capillary electrophoresis-frontal analysis (CE-FA) [11,18]. CE-FA has been a desirable method for drug-protein binding studies with its obvious

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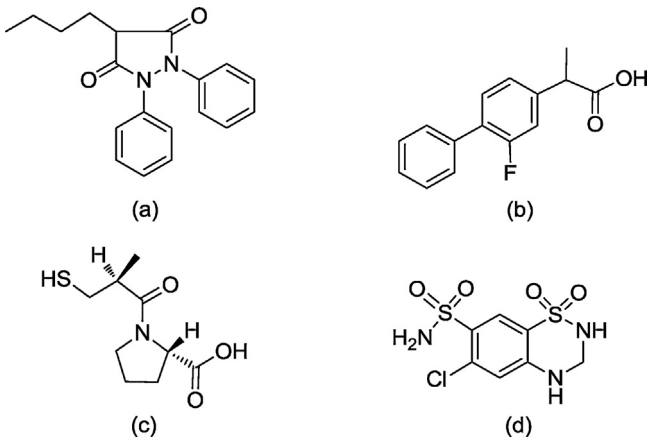

Fig. 1. Chemical structures of (a) phenylbutazone, (b) flurbiprofen, (c) captopril and (d) hydrochlorothiazide.

advantages, such as high efficiency and separation selectivity, low sample and reagent consumption, and ability to work under physiological relevant conditions with the specific requirements for pH, ionic strength and temperature [19].

Captopril (Fig. 1c), an oral inhibitor of angiotensin converting enzyme (ACE), exhibited antihypertension effects by blocking the conversion of angiotensin I to angiotensin II [20,21]. In clinical practice, captopril is often co-administrated with hydrochlorothiazide (Fig. 1d) which is a kind of diuretic drug acting as inhibiting the kidneys' ability to retain water. It was estimated that about 75% of hypertensive patients had a satisfactory effect of blood pressure reduction when they were treated with the co-administration of captopril and hydrochlorothiazide [20,22–24]. The combination dosage form of captopril and hydrochlorothiazide now is available on the markets of the US, Canada and China. Therefore, it is necessary to evaluate the binding behavior of captopril with HSA with the co-administrated hydrochlorothiazide. To the best of the authors' knowledge, there has been no publication for the purpose study. In this study, a CE-FA method was developed to locate the binding site on HSA for captopril and a comparative binding study was evaluated in the presence of hydrochlorothiazide.

2. Material and methods

2.1. Instrumentation

Capillary electrophoresis was performed on an Agilent CE G1600A (Agilent Technologies, Waldbronn, Germany) equipped with a diode array UV/vis detector (DAD). The electropherograms were recorded and analyzed with Agilent 3D-CE ChemStation software (Version A.10.02). Uncoated fused-silica capillary of 35 cm × 50 μm I.D. with 26.5 cm effective length (Yongnian Optical Fiber Corporation, Heibei, China) was used. The capillary cassette temperature was controlled at 37 °C and the detection wavelength was set at 210 nm for the sensitive detection of captopril. A Sartorius PB-10 pH meter (Beijing, China) was employed to adjust the pH of the following buffer. Water with a resistivity of 18.2 MΩ•cm was purified by a Milli-Q plus system from Millipore AS (Bedford, MA, USA).

2.2. Chemicals and samples

HSA lyophilized powder (fatty acid free, globulin free, ≥99%), flurbiprofen, phenylbutazone, methanol, and dimethylsulfoxide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Captopril and hydrochlorothiazide was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Sodium hydroxide, sodium dihydrogen phosphate, and acetone were of analytical reagent grade from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

The running buffer containing 67 mM sodium dihydrogen phosphate, adjusted to pH 7.4 by 1 M sodium hydroxide, was used as background electrolyte (BGE) for CE performance. It was also utilized for preparing the stock solutions of HSA, captopril, hydrochlorothiazide, phenylbutazone, and flurbiprofen, each at 1 mM concentration. Working solutions for each component were prepared by diluting the corresponding stock solution to 100 μM with BGE, except for HSA working solutions ranged between 10 and 475 μM. The working solutions were all filtered through 0.45 μm membrane filters (Xingya Purification Materials Inc., Shanghai, China) prior to injection.

2.3. Procedures

New capillary was rinsed with 1 M NaOH, water, and BGE, successively for 30 min at 940 mbar. Between measurements, the capillary was flushed with 0.1 M NaOH for 2 min, water for 2 min, then BGE for 2 min at 940 mbar. Samples were injected into the capillary by employing a pressure of 50 mbar for 20 s at the anode. A voltage of +8 kV in the normal polarity mode was applied.

Series solutions of fixed concentration (100 μM) of captopril with an increasing protein concentration (10–475 μM) were prepared. In the co-binding experiment, for simulating the concentration of the compound tablets administrated, the mixture solutions consisted of 100 μM captopril, 60 μM hydrochlorothiazide and 10–475 μM HSA. The binding solutions were equilibrated in 37 °C water bath at least 2 h before the injection. The free captopril concentration was determined from a linear relationship obtained between the plateau height and captopril concentration (5–100 μM) in the absence of HSA.

All the measurements were performed at least in triplicate. The Origin software (Version 8.5.0, Northampton, MA, USA) was used for the data processing.

3. Results and discussion

3.1. CE-FA method development

It is required that the mobility of drug differs from that of protein or the drug–protein complex for the interaction study using CE-FA [11,18]. At near physiological conditions (67 mM phosphate buffer, pH 7.4, $I = 0.17$, 37 °C), the electrophoretic mobility of captopril, hydrochlorothiazide, and HSA were determined respectively, by injecting the corresponding working solution into the capillary filled with BGE. The mobility of captopril–HSA complex was obtained by injecting 100 μM HSA working solution into the capillary filled with captopril working solution as running electrolyte, while for the mobility of hydrochlorothiazide-HSA complex determination, the capillary was filled with hydrochlorothiazide working solution as running electrolyte. The apparent electrophoretic mobility (μ_{app}) was calculated according to Eq. (1):

$$\mu_{app} = \frac{Ll}{Vt} \quad (1)$$

where L, l are the total length and effective length of the capillary, respectively, V is the separation voltage, and t is the migration time of analyte concerned.

As the apparent electrophoretic mobility (μ_{app}) usually varies with the separation voltage, length of the capillary, and even vis-