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Characterization of the binding of sulfonylurea drugs to HSA by high-performance affinity chromatography

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ABSTRACT

Sulfonylurea drugs are often prescribed as a treatment for type II diabetes to help lower blood sugar levels by stimulating insulin secretion. These drugs are believed to primarily bind in blood to human serum albumin (HSA). This study used high-performance affinity chromatography (HPAC) to examine the binding of sulfonylureas to HSA. Frontal analysis with an immobilized HSA column was used to determine the association equilibrium constants (K_a) and number of binding sites on HSA for the sulfonylurea drugs acetohexamide and tolbutamide. The results from frontal analysis indicated HSA had a group of relatively high-affinity binding regions and weaker binding sites for each drug, with average K_a values of 1.3 ($\pm$0.2) $\times$ 10^5 and 3.5 ($\pm$3.0) $\times$ 10^2 M^{-1} for acetohexamide and values of 8.7 ($\pm$0.6) $\times$ 10^4 and 8.1 ($\pm$1.7) $\times$ 10^3 M^{-1} for tolbutamide. Zonal elution and competition studies with site-specific probes were used to further examine the relatively high-affinity interactions of these drugs by looking directly at the interactions that were occurring at Sudlow sites I and II of HSA (i.e., the major drug-binding sites on this protein). It was found that acetohexamide was able to bind at both Sudlow sites I and II, with K_a values of 1.3 ($\pm$0.1) $\times$ 10^5 and 4.3 ($\pm$0.3) $\times$ 10^4 M^{-1}, respectively, at 37 °C. Tolbutamide also appeared to interact with both Sudlow sites I and II, with K_a values of 5.5 ($\pm$0.2) $\times$ 10^4 and 5.3 ($\pm$0.2) $\times$ 10^4 M^{-1}, respectively. The results provide a more quantitative picture of how these drugs bind with HSA and illustrate how HPAC and related tools can be used to examine relatively complex drug–protein interactions.

1. Introduction

Sulfonylureas are a group of drugs used to treat type II diabetes (i.e., adult onset or non-insulin dependent diabetes). These drugs stimulate acute insulin release from the beta cells of pancreatic islet tissue [1]. Tolbutamide and acetohexamide are two common "first-generation" sulfonylurea drugs (see Fig. 1) [1–3]. These agents have been widely used since the introduction of tolbutamide in 1956 [2,4]. All sulfonylureas bind tightly to serum proteins, with human serum albumin (HSA) being the main protein that is believed to be involved in these interactions [2].

HSA is the most prevalent plasma protein [5,6]. This protein is composed of a single peptide chain and has a typical concentration in blood of 35–50 mg/ml (i.e., 0.6–0.7 mM) [5–9]. HSA is known to act as a transport protein that binds to a wide variety of compounds, including many drugs, hormones, bilirubin, and fatty acids [5–8]. In this role, HSA and its interactions with drugs can have a strong influence on the free concentrations of drugs in plasma [6,7,10] and the pharmacologic and pharmacokinetic properties of a drug [5–8,11]. For instance, this binding can affect drug adsorption, distribution, metabolism and excretion [5,12].

Previous studies have been conducted to investigate the binding of both acetohexamide [4,13–15] and tolbutamide [3,13–19] to HSA using equilibrium dialysis, dynamic dialysis, equilibrium gel filtration, fluorescence quenching, ultrafiltration, isothermal titration calorimetry, heteronuclear 2-D NMR, and reversed-phase liquid chromatography. However, the binding constants that have been obtained in these studies have ranged by almost 10-fold for both acetohexamide (i.e., 0.4–4.1 $\times$ 10^5 M^{-1}) [4,15] and tolbutamide (0.4–3.0 $\times$ 10^5 M^{-1}) [15–20]. It is also not yet apparent as to whether one or several major sites on HSA are involved in these interactions [4,16–18].

This current report will use the method of high-performance affinity chromatography (HPAC) to obtain more detailed information on the identity and strength of the binding sites on HSA for acetohexamide and tolbutamide. This method has previously been used to examine the binding of HSA to many other drugs and small solutes, such as coumarins [20–22], indoles [23], carbamazepine [20,24,25], ibuprofen and benzodiazepines [22,26]. Immobilized proteins used in HPAC have also been shown to give good qualitative and quantitative agreement with proteins in solution. For instance, the binding constants, displacement and allosteric inter-

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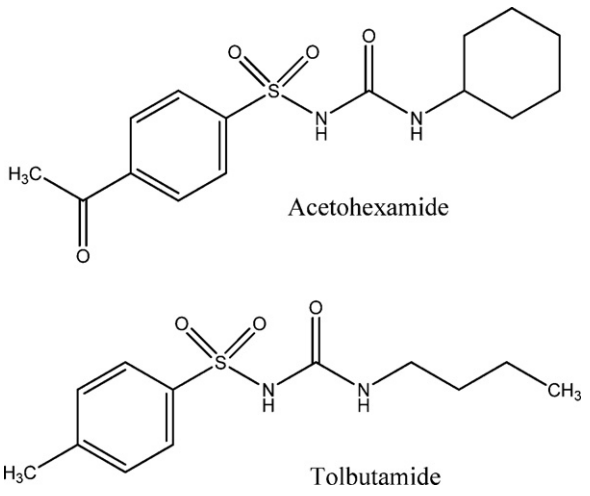

Fig. 1. Structures of acetohexamide and tolbutamide.

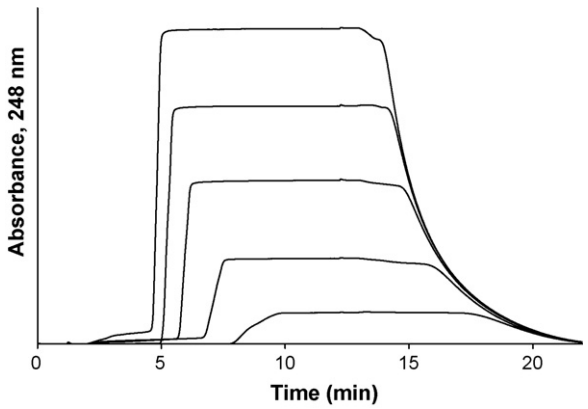

Fig. 2. Breakthrough curves for acetohexamide on an immobilized HSA column at applied concentrations (from left to right) of 10, 7.5, 5, 2.5, and 1 μM. Alternative detection wavelengths were used for some of the higher concentrations of analyte to maintain a linear response in absorbance versus concentration during these studies, as described in Section 3. Other conditions are given in the text.

actions seen between various solutes on immobilized HSA columns have been shown in numerous studies to be good models for the behavior observed for soluble HSA (e.g., see review in Ref. [27] and references cited therein). The benefits of HPAC over traditional methods like ultrafiltration and equilibrium dialysis include its use of smaller amounts of sample, its speed, its better reproducibility and precision, and its ease of automation [27–29].

The combined use of HPAC with frontal analysis (i.e., frontal affinity chromatography) and immobilized HSA columns will first be used in this study to estimate the total number of binding sites and association equilibrium constants of acetohexamide and tolbutamide with HSA. Zonal elution and competition with site-selective probe compounds for HSA will then be used to examine the binding of these two sulfonylurea drugs at the major binding regions for drugs on this protein (i.e., Sudlow sites I and II) [30,31]. The results will be compared to previous observations made in the literature and should provide a more complete picture of how these drugs bind with HSA and are transported by this protein in the circulation. This work will also be used to illustrate how HPAC and several tools available in this method (e.g., equations for examining multi-site interactions or allosteric effects) [26] can be utilized to examine relatively complex drug–protein interactions.

2. Theory

2.1. Frontal analysis

In these studies, frontal analysis was used to estimate the association equilibrium constants (K_a) and the number of binding sites of each drug with HSA by using HPAC and columns that contained immobilized HSA. This was done by measuring the binding capacity of this column (m_L) as the concentration of drug that was applied to the column was varied. Some typical breakthrough curves that were obtained in these experiments are shown in Fig. 2. If fast association/dissociation kinetics are present for the binding of the applied analyte with the immobilized protein (i.e., as is typically present during drug–HSA interactions), the central position of the resulting breakthrough curve can be related to K_a, m_L, and the applied concentration of the analyte [A] [27,28]. For an analyte that binds to only a single type of site within the column, the following equations can be used to describe this relationship [24,27].

$$\frac{1}{m_{Lapp}} = \frac{1}{(K_a m_L[\mathrm{A}])} + \frac{1}{m_L} \quad (1)$$

or

$$m_{Lapp} = \frac{m_L K_a[\mathrm{A}]}{(1 + K_a[\mathrm{A}])} \quad (2)$$

In these equations, m_{Lapp} is the apparent moles of analyte required to saturate the column at a particular concentration. Eq. (1) indicates for a system with a single type of binding site that a plot of $1/m_{Lapp}$ versus 1/[A] should provide a linear relationship from which the values of K_a and m_L can be determined from the slope and intercept. If multi-site binding is present, such a plot should approach a linear response at low concentrations (i.e., high values for 1/[A]) and give a curved response and negative deviations at high analyte concentrations (i.e., low values for 1/[A]), as illustrated in Fig. 3.

In the case of multi-site binding, Eq. (1) can be expanded to allow for more than one class of binding sites. For example, a system containing two binding sites would have the following relationship

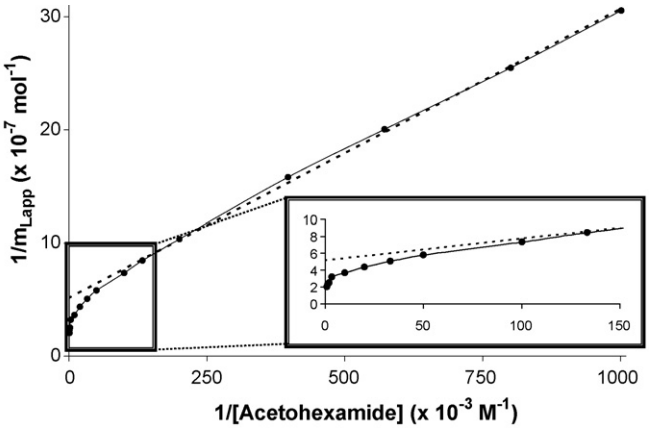

Fig. 3. A double-reciprocal plot for frontal analysis studies examining the binding of acetohexamide to an immobilized HSA column. When comparing this response to the linear relationship that is predicted by Eq. (1), it was apparent that negative deviations occurred at high analyte concentrations (i.e., low values of 1/[A]), indicating that multiple binding regions for acetohexamide were present. The dashed line shows the linear response that was obtained for the data at relatively low analyte concentrations (i.e., high 1/[A] values), which can still be used in such a case to estimate the association equilibrium constant for the highest affinity binding sites in such a system.