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Binding of Valsartan to Bovine Serum Albumin: an *in vitro* Study

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Abstract

The binding of valsartan, an anti-hypertensive drug, to bovine serum albumin (BSA) has been studied by equilibrium dialysis (ED) method at pH 7.4 and 27^{o}C (room temperature) in order to explore the affinity of this dug to BSA. The binding of valsartan has been characterized by two sets of association constants: high affinity association constant (k_1) with low capacity (n_1) and low affinity association (k_2) constant with high capacity (n_2). Different analyses of binding to BSA suggested the presence of four high affinity binding sites and eleven low affinity sites for this drug on BSA at pH 7.4 and 27°C. High affinity association constant (K_1) of valsartan to BSA at pH 7.4 was found to be $175x10^5$ M^{-1} and the low affinity association constant (k_2) was $18x10^5$ M^{-1}. Site specific probe displacement data showed that valsartan primarily binds to site II (the benzodiazepine site), while the low affinity site of this drug is site I (the warfarin site) on BSA.

Key words: Albumin, valsartan, protein binding.

Introduction

Serum albumin, the most abundant protein in blood, plays a very important role in the binding phenomenon and serves as a depot protein and transport protein for numerous endogenous and exogenous compounds (Krag–Hansen, 1981). Plasma protein binding properties are primary determinants of the pharmacokinetic properties of most of the drugs, such as plasma clearance, half-life, apparent volume of distribution and the duration & intensity of pharmacologic effect (Jiunn *et al*., 1987). The early work of Klotz *et al.* (1949) and Scatchard (1949) formed the basis for investigation of drug protein binding that has been carried out during subsequent decades. To understand the nature of drug protein interaction, the affinity of the drug for protein and number of binding sites are important. The binding affinity is quantified in terms of association constant. The pH of the medium and temperature are two important factors that affect association constant (Gani *et al*., 2002).

From different investigations, it has been suggested that human serum albumin (HSA) has limited number of binding sites (Fehske *et al*., 1979; Hansen, 1981). On the basis of probe displacement method, it has been detected that there exist at least three relatively high affinity binding sites on HSA. These sites are commonly called the warfarin, the benzodiazepine and the digoxin binding sites which are also denoted as site I, site II and site III, respectively (Rahman, 1994; Fehske *et al*., 1981; Sudlow *et al*., 1975, 1976).

The study of drug protein interaction is significant both with respect to pharmacokinetics and pharmacodynamics. The present study has centered mainly on the binding chemistry of valsartan to BSA. Valsartan is widely used for clinical purposes such as to treat high blood pressure and heart failure. To observe the binding of valsartan, the association constants as well as the number of sites for binding of valsartan to BSA were calculated by Scatchard analysis (Scatchard, 1949) at pH 7.4 and 27°C (room temperature). In this study BSA in lieu of HSA, was used because of its structural similarity with HSA, low cost and easy availability.

Materials and Methods

Valsartan was kindly supplied by Navana Pharmaceuticals Ltd. Dhaka. Probes namely ranitidine hydrochloride and diazepam were supplied by Drug

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International Ltd. Dhaka and Essential Drugs Co. Ltd., Dhaka respectively. Dialysis membrane was purchased from Medicell International Ltd., Liverpool, London and BSA was obtained from the Sigma Chemical Co. Ltd.

Estimation of binding parameters: The association constants and the number of corresponding binding sites of valsartan for BSA were studied by Scatchard method (Scatchard, 1949) of analysis using equilibrium dialysis technique (Singlas, 1987).

Valsartan solution (0.01M) was added with increasing concentrations into 6 out of 7 test tubes containing 5 ml of 2×10^{-5} M BSA solution in each to have the final concentrations ranging from 2×10^{-5} M to 20×10^{-5} M. The seventh test tube containing only BSA solution was taken as 'control'. After proper mixing 3.5 ml of solution was taken from each test tube and poured into 7 different semipermeable membrane tubes. The tubes were then immersed in separate 50-ml conical flasks containing 20 ml of phosphate buffer solution (pH 7.4). All the conical flasks were placed in a metabolic shaker for dialysis at 27°C and 20 rpm. After proper shaking in a metabolic shaker for 12 hours the concentration of free valsartan in different sample was measured by UV spectrophotometer (SP8-400 UV/VIS spectrophotometer, Pye Unicum, England) at 276 nm.

Characterization of binding sites of valsartan using ranitidine as site I specific probe and diazepam as site II specific probe: Ranitidine as ranitidine hydrochloride solution was added to 7 out of 8 test tubes containing $2x10^{-5}$ M BSA solution to have the final ratio between the protein and ranitidine at 1:1 ($2x10^{-5}$ M : $2x10^{-5}$ M). The eighth test tube containing only BSA solution was marked as "control". Valsartan solution was added with increasing concentrations into six out of seven test tubes containing 1:1 mixture of BSA-ranitidine, so that the final ratios between valsartan and the protein were 1:1, 2:1, 4:1, 5:1, 6:1 and 8:1. Valsartan was not added into the seventh test tube containing protein and ranitidine mixture (1:1). After proper mixing 3.5 ml solution was taken from each test tube and poured into eight different semipermeable membrane bags. The bags were then immersed in separate eight 50-m1 conical flasks containing 20 ml of phosphate buffer solution (pH 7.4). All the conical flasks were placed in a metabolic shaker for dialysis at 27°C and 20 rpm. After proper shaking for 12 hours the concentration of free ranitidine was measured by a UV spectrophotometer at 318 nm. Similar method was followed for diazepam and the concentration of free diazepam was measured by UV spectrophotometric analysis at 235 nm.

Results and Discussion

Estimation of binding parameters: Scatchard plot of valsartan at pH 7.4 and at 27°C is shown in Figure 1. Scatchard analysis of the equilibrium dialysis data showed a non-linear curve, suggesting the presence of at least two classes of binding sites for the binding of valsartan to BSA. As can be seen in Figure 1, the number of high affinity binding site (n_1) for valsartan was approximately four (low capacity) and the number of low affinity binding site (n_2) was approximately eleven (high capacity). The high affinity association constant (k_1) for the valsartan binding to BSA at pH 7.4 was quite high ($175x10^5M^{-1}$), while the low affinity association constant (k_2) for this drug to BSA was about 9.7 fold lower ($18x10^5M^{-1}$) than that of the primary association constant (Table 1).

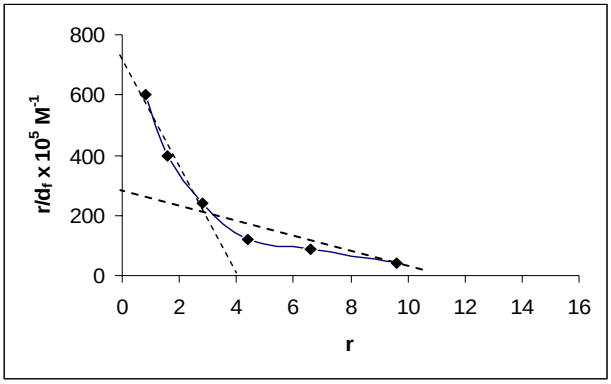

Figure 1. Scatcherd plot for the binding of valsartan to BSA by ED at 27^0C and pH 7.4

Table 1. Association constant for valsartan bound to BSA at 27°C at pH 7.4

Association constant	
k_1 (high affinity) $\times 10^5 M^{-1}$	k_2 (low affinity) $\times 10^5 M^{-1}$
175 ± 0.07	18 ± 0.03

Each value represents the average value from three experiments ± SD

Identification and characterization of binding sites: Binding sites of drugs are determined by studying their ability to displace the site specific probes. In this study

ranitidine hydrochloride and diazepam were used as site I and site II specific probes respectively. Figure 2 shows the change in free concentration of ranitidine (♦) and diazepam (▲) by valsartan. From figure 2 it is seen that the free concentration of ranitidine increased from 100% (as % of initial) to 305%, whereas, the free concentration of diazepam was increased from 100% (as % of initial) to 453% by the same drug. From this observation it can be said that valsartan displaced diazepam to a greater extent as compared to ranitidine, so valsartan has greater affinity for site II than for site I on the BSA molecule. This implies the fact that at a lower drug to BSA ratio, valsartan binds to its high affinity site i.e., site II or the benzodiazepine site, whereas at higher ratio it not only binds to its high affinity site but also to its low affinity site i.e., site I or the warfarin site on the BSA molecule.

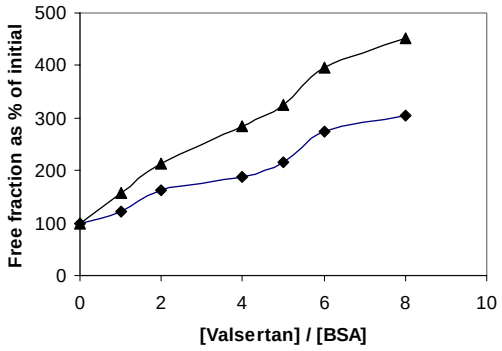

Figure 2. Free fraction of ranitidine (♦) and diazepam (▲) bound to BSA (1:1) as % of initial upon the addition of valsartan by ED at 28^0C and pH 7.4.

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