

Investigation on the Interaction of Gabapentin with Bovine Serum Albumin by Spectroscopic Techniques

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Spectroscopic investigations on the interaction of gabapentin (GBP) with bovine serum albumin (BSA) were reported. The association constant of GBP-BSA system was determined at different temperatures (298, 302, 306 and 311 K) based on the fluorescence quenching results. The GBP was found to quench the fluorescence of BSA through static mechanism. Thermodynamic parameters, the standard enthalpy change, (ΔH°) and the standard entropy change (ΔS°) were observed to be $-9.61 \pm 0.008 \text{ kJ mol}^{-1}$ and $3.58 \pm 0.011 \text{ Jmol}^{-1} \text{ K}^{-1}$ respectively. These indicated that the hydrophobic and electrostatic forces played a role in the interaction of GBP with BSA. The negative value of ΔG° revealed that the binding reaction is spontaneous. The circular dichroism studies indicated the conformational changes in BSA upon interaction with GBP. The effect of some metal ions on the binding constant was also investigated.

keywords: Bovine serum albumin, gabapentin, fluorescence quenching

INTRODUCTION

Reversible attachment to serum proteins plays a significant role in pharmacokinetics and pharmacodynamics, and a clear understanding of this process is fundamental in the development of the rational use of many therapeutic agents [1]. Serum albumin (SA), the important protein in the circulatory system, is one of the most extensively studied of all proteins [2]. It is synthesized in the liver, exported as a non-glycosylated protein, and is present in the blood at around 40 mgmL^{-1} [3]. It is the major transport protein for unesterified fatty acids, but is also capable of binding an extraordinarily diverse range of metabolites, drugs and organic compounds. The remarkable binding properties of albumin accounts for the vital role it can play in both the efficacy and rate of delivery of drugs. Many drugs, including anticoagulants, tranquilizers and general anesthetics, are transported in the blood while bound to albumin [2]. This has stimulated much interest on the nature of the drug binding sites and investigations of whether fatty acids, natural metabolites and drugs compete with one another for binding to the protein. These studies provide information on the structural features that determine the therapeutic effectiveness of drugs and have become an important research field in the life science, chemistry and clinical medicine [4].

Gabapentin (GBP), chemically, (Fig. 1) is an anticonvulsant that is chemically unrelated to any other anticonvulsant or mood regulating medication. It is also widely used to treat

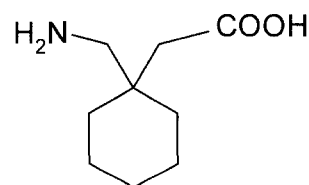


Figure 1. Molecular structure of gabapentin.

individuals suffering from many kinds of pain problems, tremors, restless legs syndrome, hot flashes associated with menopause, and various psychiatric disorders. GBP, one of the relatively new antiepileptic drugs, has a novel mechanism of action that is not fully understood and is effective in the treatment of partial seizures with and without secondary generalization. Evidence suggests that it may have mood-stabilizing and possibly antidepressant properties in bipolar depression [5].

MATERIAL AND METHODS

Fluorescence measurements were performed on a Hitachi spectrofluorimeter Model F-2000 equipped with a 150W Xenon lamp and slit width of 10 nm. The circular dichroism (CD) measurements were made on a JASCO-J-715 spectropolarimeter using a 0.1 cm cell at 0.2 nm intervals, with 3 scans averaged for each CD spectrum.

The absorption spectra were recorded on a double beam CARY 50-BIO UV-visible spectrophotometer equipped with a 150W Xenon lamp and slit width of 10 nm. A quartz cell of 1.00 cm was used for measurements of both absorbance and fluorescence.

BSA was obtained from Sigma Chemical Company, St

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Louis, USA. GBP was obtained as gift sample from Parke Davis India Ltd., India. The solutions of GBP and BSA were prepared in 0.1M phosphate buffer of pH 7.4 containing 0.15 M NaCl. BSA solution was prepared based on its molecular weight of 65,000. All other materials were of analytical reagent grade and double distilled water was used throughout.

GBP-BSA interactions

On the basis of preliminary experiments, BSA concentration was kept fixed at $5 \mu\text{M}$ and drug concentration was varied from 1000 to 6000 μM . Fluorescence spectra were recorded at 298, 302, 306 and 311 K in the range of 300-500 nm upon excitation at 296 nm in each case. The absorbances of drug-protein mixtures in the concentration range employed for the experiment did not exceed 0.05 at the excitation wavelength to avoid inner filter effect.

Circular dichroism (CD) measurements

The CD measurements of BSA in the absence and presence of GBP (1:2, 1:6, 1:12 and 1:20) were made in the range of 200-250 nm. A stock solution of 150 μM BSA was prepared.

Effects of some common ions

The fluorescence spectra of GBP (1000-6000 μM)-BSA (5 μM) system was recorded in the absence and presence of various metal ions (each of 5 μM) viz., K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Co^{2+} , Mn^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} and Pb^{2+} in the range of 300-500 nm upon excitation at 296 nm.

RESULTS AND DISCUSSION

Fluorescence Measurements

For macromolecules, the fluorescence measurements can give some information of the binding of small molecules to protein, such as the binding mechanism, binding mode, binding constants, binding sites etc. Fluorescence intensity of a compound can be decreased by a variety of molecular interactions viz, excited-state reactions, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. Such decrease in intensity is called quenching. The fluorescence spectra of BSA in presence of different amounts of GBP were recorded (Fig. 2). GBP causes a concentration dependent quenching of the intrinsic fluorescence of BSA without changing the emission maximum thereby indicating that the GBP binds to BSA and does not alter the local dielectric environment. The interaction

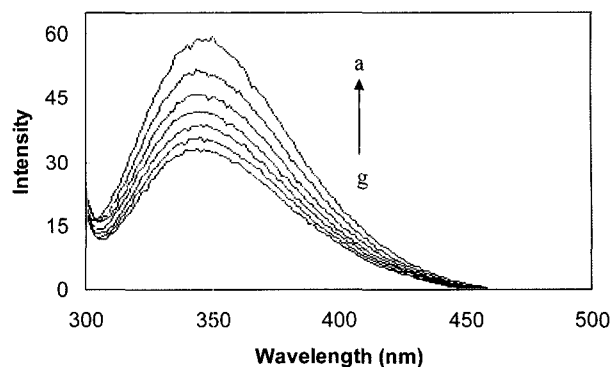


Figure 2. Fluorescence spectra of BSA in the presence of GBP. BSA concentration was fixed at $5 \mu\text{M}$ (a). GBPH concentration was varied: 1000 (b), 2000 (c), 3000 (d), 4000 (e), 5000 (f), 6000 μM (g).

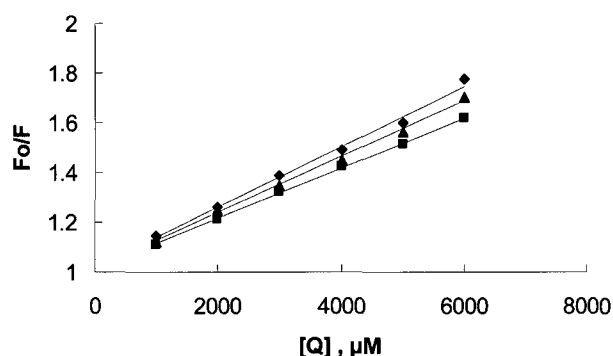


Figure 3. The Stern-Volmer curves for the binding of GBP with BSA at 298 (◆), 302 (▲) and 306 K (■). Excitation wavelength was 296 nm. Concentration of BSA was fixed at $5 \mu\text{M}$.

of GBP to BSA was further confirmed by absorption and CD techniques.

The fluorescence quenching data are analyzed by the Stern-Volmer equation, $F_0/F = 1 + K_{SV} [Q]$ ----- (1) where F_0 and F are the steady state fluorescence intensities in the absence and presence of quencher, respectively, K_{SV} is the Stern-Volmer quenching constant and $[Q]$ is the concentration of quencher (GBP). From the values of K_{SV} (Table 1) it can be deduced that the GBP quenched the BSA through static quenching procedure since the K_{SV} values decreased with increase in temperature (Fig. 3).

Analysis of binding equilibria

When small molecules bind independently to a set of

Table 1. Thermodynamic and binding parameters of GBP-BSA system

Temp (K)	K_{sv} ($10^2, \text{M}^{-1}$)	Association constant, K (10^2)	ΔG^0 (kJmol^{-1})	ΔH^0 (kJmol^{-1})	ΔS^0 ($\text{Jmol}^{-1}\text{K}^{-1}$)
298	1.21 ± 0.032	0.74 ± 0.042	-10.68		
302	1.12 ± 0.028	0.70 ± 0.021	-10.66	-9.61 ± 0.008	3.58 ± 0.011
306	1.01 ± 0.033	0.67 ± 0.034	-10.72		
311	0.99 ± 0.021	0.63 ± 0.019	-10.71		

equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the following equation [6]

$$\log (F_o-F)/F = \log K + n \log [Q] \quad (2)$$

where K and n are the binding constant and the number of binding sites, respectively. Thus, a plot of $\log (F_o-F)/F$ versus $\log [Q]$ yielded the values of K and n . The values of K obtained at 298, 302, 306 and 311 K are given in the Table 1. The values of n were found to be in the range of 0.92-0.98. These values are approximately equal to 1 indicating that there is one class of binding sites to the GBP in BSA. In BSA, the tryptophan residues involved in binding could be either Trp 135 or Trp 214. Of both tryptophans in BSA, Trp 135 is more exposed to a hydrophilic environment, whereas Trp 214 is deeply buried in the hydrophobic loop [7]. So, from the value of n it is proposed that GBP most likely binds to the hydrophobic pocket located in subdomain II A; that is to say Trp 214 is near or within the binding site [8].

Type of interaction force between GBP and BSA

Considering the dependence of binding constant on temperature, a thermodynamic process was considered to be responsible for the formation of the complex. Hence, the thermodynamic parameters depend on the temperatures and were analyzed to characterize the acting forces between GBP and BSA. The interaction forces between a drug and biomolecule may include hydrogen bond, van der Waals force, electrostatic force, hydrophobic interaction force, and so on. The thermodynamic parameters were evaluated using the following equations:

$$\log K = - \Delta H^o / 2.303RT + \Delta S^o / 2.303 R \quad (3)$$

$$\Delta G^o = - RT \ln K \quad (4)$$

where ΔH^o , ΔG^o and ΔS^o are respectively enthalpy change, free energy change and entropy change. The binding studies were carried out at 298, 302, 306 and 311 K. At these temperatures, the BSA doesn't undergo any structural degradation. The values of ΔH^o , ΔS^o and ΔG^o are calculated and are shown in Table 1. The characteristic sign of the thermodynamic parameters associated with molecular interactions can provide clues on the types of interaction forces. From the point of view of water structure, a positive entropy value is usually considered as the evidence for hydrophobic interaction. In addition, specific electrostatic interaction between ionic species in aqueous solution is also characterized by a positive ΔS^o and a negative ΔH^o [9]. Generally, ΔG^o mainly comes from the large contribution of ΔS^o with little contribution from ΔH^o for electrostatic interaction. However, in this work, ΔG^o was mainly from the contribution of ΔH^o but not from the ΔS^o . Therefore we inferred that hydrophobic interaction might play a major role in the interaction of GBP with BSA in addition to electrostatic

Table 2. Effects of some cations on the binding constant of BSA-GBP system at 298 K

System	Association constant, M ⁻¹
BSA + GBP	0.74 ± 0.021×10 ²
BSA + GBP + K ⁺	8.66 ± 0.034×10 ³
BSA + GBP + Ca ²⁺	1.33 ± 0.046×10 ²
BSA + GBP + Mg ²⁺	0.44 ± 0.070×10 ²
BSA + GBP + Cu ²⁺	0.31 ± 0.054×10 ²
BSA + GBP + Co ²⁺	1.88 ± 0.041×10 ²
BSA + GBP + Mn ²⁺	1.25 ± 0.039×10 ²
BSA + GBP + Zn ²⁺	2.31 ± 0.067×10 ²
BSA + GBP + Pb ²⁺	0.71 ± 0.011×10 ²
BSA + GBP + Hg ²⁺	0.41 ± 0.060×10 ²
BSA + GBP + Cd ²⁺	0.65 ± 0.037×10 ²

forces [10]. However, the possibility of hydrogen bonding may also not be ruled out in the binding process.

The effect of some cations on the association constant of GBP-BSA

In plasma, there are some ions, which can affect the reactions of the drugs and the serum albumins. Hence, we have investigated the effects of some cations *viz.*, K⁺, Ca²⁺, Mg²⁺, Cu²⁺, Co²⁺, Mn²⁺, Zn²⁺, Cd²⁺, Hg²⁺ and Pb²⁺ on the binding constant of GBP-BSA system at 298 K. The results are shown in Table 2. It is evident that the binding constants of GBP-BSA increased in presence of K⁺, Ca²⁺, Co²⁺, Mn²⁺ and Zn²⁺ implying the stronger binding between GBP and BSA. The higher binding constant values might be resulted from the interaction of metal ion with the drug to form a complex, which in turn interacted with protein [11]. Thus, prolonging the storage time of the drug in blood plasma and enhancing the maximum effectiveness of the drug [11]. Hence, GBP can be stored and removed better by the proteins in the presence of above cations. However, Mg²⁺, Cu²⁺, Cd²⁺, Hg²⁺ and Pb²⁺ reduced the binding of GBP with BSA, there by making GBP to be quickly cleared from the blood [9]. This may lead to the need for more doses of GBP to achieve the desired therapeutic effect.

UV-Visible absorption spectroscopy

UV-visible absorption measurement is a very simple method and applicable to explore the structural change [12]. To explore the structural changes of BSA upon the addition of GBP, we have recorded the absorbances of BSA in presence of different amounts of GBP (Fig. 4). It is evident that the absorbance of BSA increased regularly with the variation of the concentration of GBP. The maximum peak position of GBP-BSA was shifted slightly towards lower wavelength region indicating the structural change of BSA upon interaction with GBP.

Circular dichroism

When a drug binds to a globular protein, the intermolecular

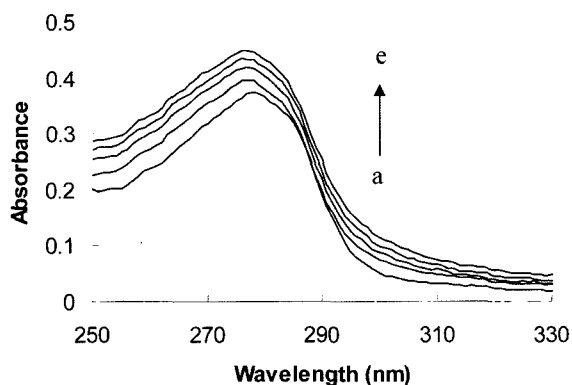


Figure 4. Absorption spectra of BSA and BSA-GBP system. BSA concentration was fixed at $5 \mu\text{M}$ (a). GBP concentration for GBP-BSA system was at 1000 (b) 2000 (c) 3000 (d) and 4000 μM (e).

forces responsible for maintaining the secondary and tertiary structure can be altered, resulting in a conformational change of protein [13]. To obtain an insight into the structure of BSA, CD spectra of BSA in presence and absence of GBP were recorded (Fig. 5). The CD spectra of BSA exhibited two negative bands in the UV region at 208 and 222 nm, characteristic of an α -helical structure of protein [14]. The CD results were expressed in terms of mean residue ellipticity (MRE) in $\text{deg cm}^2 \text{dmol}^{-1}$ according to the following equation [11]

$$\text{MRE} = \frac{\text{Observed CD (mdeg)}}{C_p n l \times 10} \quad (5)$$

where C_p is the molar concentration of the protein, n is the number of amino acid residues and l is the path length. The α -helical contents of free and combined BSA were calculated from MRE value at 208 nm using the following equation [15]

$$\alpha\text{-Helix(\%)} = \frac{[-\text{MRE}_{208} - 4000]}{[33000 - 4000]} \times 100 \quad (6)$$

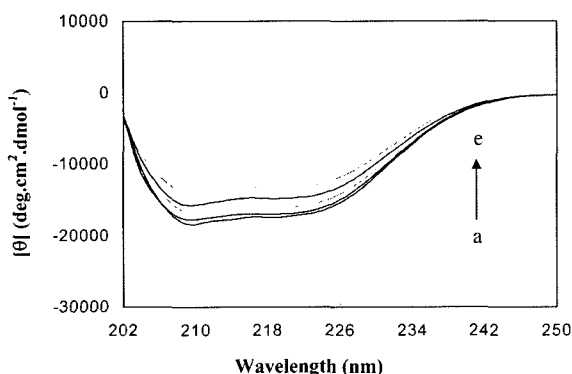


Figure 5. The CD spectra of BSA-GBP in 0.1M phosphate buffer of pH 7.4 at 298 K. BSA concentration was fixed at $5 \mu\text{M}$ (a). In BSA-GBP, the GBP concentration was 10 (b) 30 (c) 60 (d) and 100 μM (e).

where MRE_{208} is the observed MRE value at 208 nm, 4000 is the MRE of the β -form and random coil conformation cross at 208 nm, and 33000 is the MRE value of a pure α -helix at 208 nm. From the above equation, the α -helicity in the secondary structure of BSA was determined. The α -helicity decreased from 71.1 % in free BSA to 60.91% in bound form. The loss of α -helicity signified that the drug bound with the amino acid residue of the main polypeptide chain of protein and destroy its hydrogen bonding networks [16] which resulted in a conformation change of the protein. Further, the CD spectra of BSA in presence and absence of GBP are similar in shape and there is no significant shift of the peaks, indicating that the structure of BSA after GBP binding to it is also predominantly α -helical [17].

CONCLUSIONS

This paper provided an approach for studying the interactions of fluorescent protein with GBP using absorption, fluorescence and CD techniques. The results showed that BSA fluorescence was quenched by GBP through static quenching mechanism. GBP interacted with BSA by hydrophobic interaction. The biological significance of this work is evident since albumin serves as a carrier molecule for multiple drugs and the interaction of GBP with albumin was not characterized earlier. This report is believed to be significant in pharmacology and clinical medicine.

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