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Research Article

Evaluation of the effect of Levetiracetam drug on BSA structure

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ABSTRACT

Levetiracetam (LV, [S]-alpha-ethyl-2-oxo-1-pyrrolidine acetamide) is a new antiepileptic that has been used as adjunctive therapy to treat patients with intractable epilepsy. Its interaction with bovine serum albumin (BSA) is of great use for the understanding of the pharmacokinetic and pharmacodynamic mechanisms of the drug. The effect of levetiracetam drug on native BSA structure has been studied by UV-visible and circular dichroism methods. In addition, fluorescence spectroscopy was used to investigate the effect of LV on F and B conformers of BSA. UV-visible results indicated that the interaction of LV with BSA may cause the conformational changes in BSA and change the polarity of the microenvironment around tyrosine and tryptophan residues of BSA. Analysis of CD spectrum displayed an increase in content of α-helical structure of BSA induced by LV and stabilized it. This study makes an important contribution to enhance our basic knowledge of the molecular basis of drug-protein interaction, which could be applicable for the development of more efficacious antiepileptic medicines.

Keywords: Aripiprazole drug; In vitro DNA interaction; Spectroscopic methods; Molecular docking; Groove binder

1. Introduction

Binding of drug with plasma proteins not only influences the distribution and elimination of medicine, but can also affects the pharmacological effect of the medicine [1]. Studying the molecular basis of drug-protein interaction is crucial for designing new therapeutic agents to improve drug activity [2]. A large number of researchers have paid attention to bovine serum albumin (BSA), particularly because of its structural homology with human serum albumin (HSA) [3-5]. In view of this, we planned to carry out the levetiracetam drug (Figure 1) interaction with BSA in vitro.

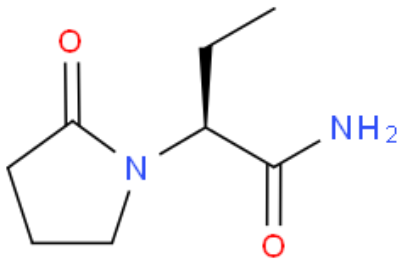

Fig. 1: The molecular structure of levetiracetam drug

Interactions involving biological molecules are best understood if described in physicochemical terms. There are a variety of techniques currently available for obtaining information about drug–protein interactions. Fluorescence and UV–visible absorption spectroscopy are effective techniques to study the small molecules-proteins interactions, because of their sensitivity, reproducibility and convenience. Also, circular dichroism (CD) is reliable method for analysing the contents of secondary conformation forms of proteins, which can explain the conformational changes of proteins induced by ligands [6, 7].

2. Experimental

2.1. Materials

All materials used were of analytical reagent grade and used without further purification. All stock solutions were prepared in the buffer solution adjusted to pH 7.4 with 0.1 M Na_2HPO_4 and NaH_2PO_4 in pure aqueous medium. BSA stock solution (1×10^{-3} M, based on its molecular weight of 66,000 g mol^{-1}) was prepared in 0.1 M phosphate buffer of pH 7.4 and was kept in the dark at 277 k. Triple distilled water was used throughout the experiment.

2.2. UV–visible absorption studies

Absorbance spectra were recorded using a HP spectrophotometer (Agilent 8453), equipped with a thermostat bath. Absorption experiments were carried out by keeping the concentration of BSA constant (1×10^{-5} M) while varying the LV concentration from 0.0 to 0.7×10^{-5} M (ri= [LV]/[BSA]= 0.0, 0.2, 0.4, 0.6 and 0.7). Absorbance values were recorded after each successive addition of BSA solution and equilibration (ca. 5 min).

2.3. Circular dichroism studies

Circular dichroism (CD) measurements were recorded on a JASCO (J-810) spectropolarimeter (200-250 nm and cell length path was 0.1 cm) by keeping the concentration of BSA constant (3×10^{-6} M) while varying the LV concentration from 0.0 to 3×10^{-6} M (ri=[LV]/[BSA])= 0.0, 0.4, and 1).

2.4. Fluorescence studies with different conformers of BSA

BSA exits in different isomeric forms as native (N), basic (B), fast moving (F). The N, B and F conformations were prepared by mixing 100 µl of BSA monomer stock solution (5.0×10^{-6} M) with 1900 µl of pH 7.4 (0.1 M phosphate), pH 9 (0.1 M phosphate) and pH 3 (0.1 M phosphate) buffers, respectively. The existence of different isomers in the

experimental preparations was confirmed with various fluorescence properties of different forms. A 2 mL solution of different conformers of BSA was titrated by different aliquots of LV solution. Then the fluorescence emission spectra were measured in the wavelength range of 300-450 nm with exciting wavelength at 295 nm.

3. Result and discussion

3.1. UV-visible spectroscopy

UV–visible absorption spectroscopy is a simple but effective method in detecting the conformational changes of proteins and the complex formation. BSA has two absorption peaks. The strong absorption of BSA at 210 nm resulted from the $\pi \rightarrow \pi^*$ transition of BSA's characteristic polypeptide backbone structure C=O and was related to the changes in the conformation of peptide backbone associated with helix-coil transformation in the difference spectra of proteins. The weak absorption peak at 280 nm was concerned with the polarity of the microenvironment around tyrosine and tryptophan residues of BSA [8].

In order to obtain more information on LV–BSA interaction, the absorption spectra of BSA in the absence and presence of LV were recorded and presented in Figure 2. It is clear from the Figure 2 that with increasing amounts of LV added to the BSA solution, the intensity of the absorption peak of BSA at 280 nm increased. These observations indicated that the interaction of LV with BSA may cause the conformational changes in BSA and change the polarity of the microenvironment around tyrosine and tryptophan residues of BSA.

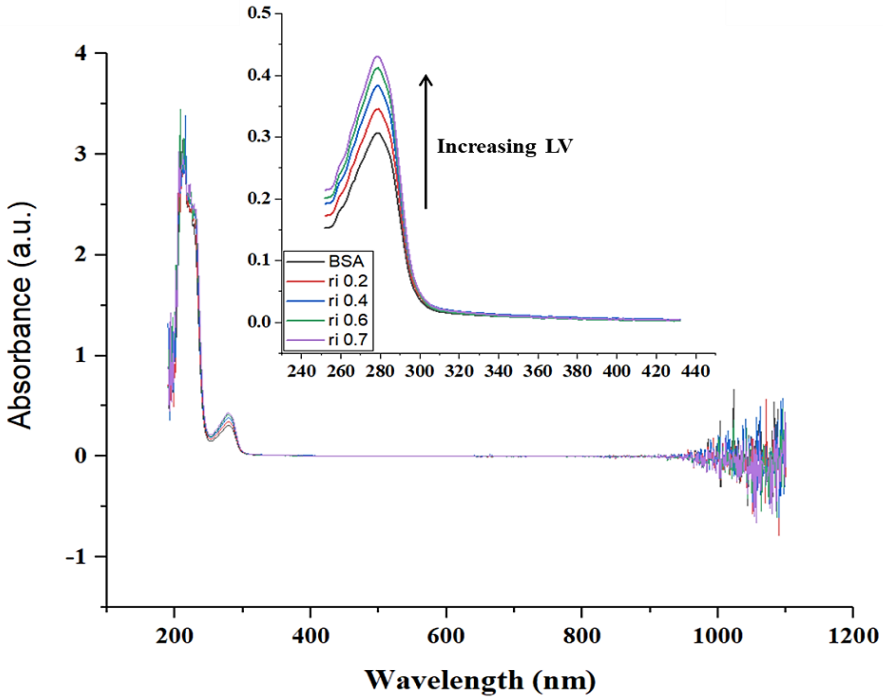

Fig. 2: UV–visible spectra of native BSA in the absence and the presence of LV; [BSA] = 1×10^{-5} M and [LV] = 0.0, 2, 4, 6 and 7×10^{-6} M, at room temperature and pH=7.4

3.2. Circular dichroism (CD) studies

CD spectroscopy is a sensitive technique and is commonly used to investigate secondary structure of protein in solution. In order to obtain better understanding about the conformational behavior of BSA in the presence of LV, further experiments were performed on the CD spectroscopy. The CD spectra of BSA exhibit two negative bands in the UV region at 208 and 220 nm, which are characteristic of α-helix of protein and both contributed to n→π* transfer for the peptide bond of α-helical structure [9]. Figure 3 shows the CD spectra of BSA with various amounts of LV. As shown in Figure 3 the CD signal of BSA increased without any significant shift of the peaks upon increasing the concentration of LV.

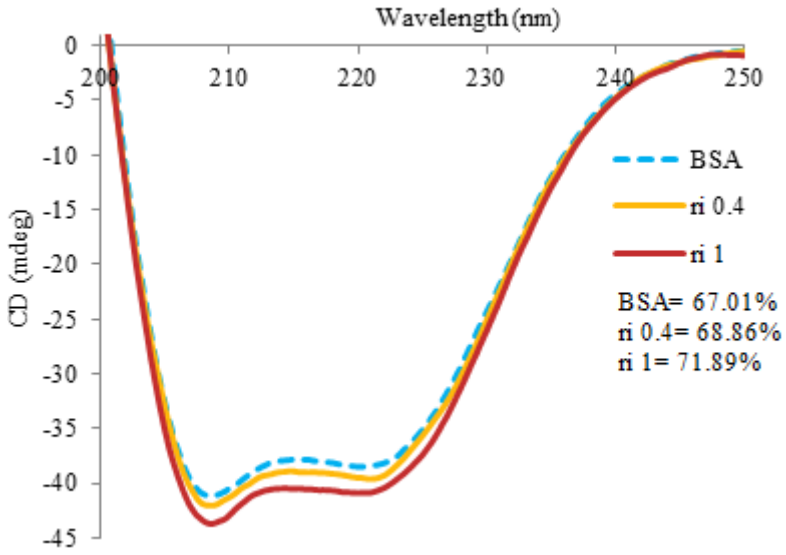

Fig. 3: CD spectra of native BSA in the absence and the presence of LV; [BSA] = 3×10^{-6} M and [LV] = 0.0, 0.4 and 3×10^{-6} M, at room temperature and pH=7.4

The CD results were expressed in terms of mean residue ellipticity (MRE) in deg cm^2 $dmol^{-1}$ according to the following equation (Eq. 1):

$$MRE = \frac{ObservedCD(m\,deg)}{C_p nl \times 10} \quad (1)$$

Where Cp is the molar concentration of the protein, n is the number of amino acid residues (583 for BSA) and l the path-length (0.1 cm). The α-helical contents of free and combined BSA were calculated from MRE values at 208 nm using the following equation (Eq. 2):

$$\alpha - helix\,(\%) = \frac{-MRE_{208} - 4000}{33000 - 4000} \quad (2)$$

The percentage of helicity of BSA is 67.01% in pure BSA (Figure 3) and in the presence of LV it becomes 71.89% at a molar ratio 1:1 which shows that binding of LV to BSA may induce some conformational changes. It can be deduced that the α-helical structure is affected probably due to insertion of some of LV molecules into the hydrophobic surface of BSA. So

when LV bounds to BSA the α-helicity increases. This observation strongly indicates that the binding of LV to BSA induces some conformational changes in BSA but the protein retains its secondary structure and helicity when interact with LV. It means that LV stabilized the secondary structure of BSA. This phenomenon is important for biomedical applications. Similar changes in the conformation of protein's secondary structure by ligands upon were observed in recent papers [10].

3.3. Interaction of LV with N, F and B conformers of BSA

The fluorescence emission spectra of different conformers of BSA were recorded in the absence and the presence of LV. At physiological pH (7.4), BSA assumes the native form (N) which changes to fast migrating form (F) at pH < 4.3 and at pH < 2.7 it changes to the fully extended form (E), whereas on the basic side at pH > 8 the N form changes to basic form (B) [11]. The fluorescence intensity of BSA alone at pH 3 and 9 were lower than the fluorescence intensity at pH 7.4. For N and B conformers, the λ_{max} were found to be almost the same, whereas for the F form the value of was blue shifted. According to Burstein et al., [12] the maximum emission wavelength of Trp residues is extremely sensitive to its environment, and the maximum fluorescence emission wavelength will be 350–353 nm if they are in the aqueous phase; and a blue shift will appear if the protein is exposed to a hydrophobic environment. The fluorescence emissions spectra of BSA in the absence and presence of LV are shown in Figure 4. As shown in Figure 4, BSA exhibited a strong fluorescence emission peak, while LV did not show intrinsic fluorescence under the same experimental conditions. Furthermore, the fluorescence intensity of BSA remarkably decreased with increase in concentration of LV. This indicated that LV interacted with BSA and quenched its fluorescence intensity.

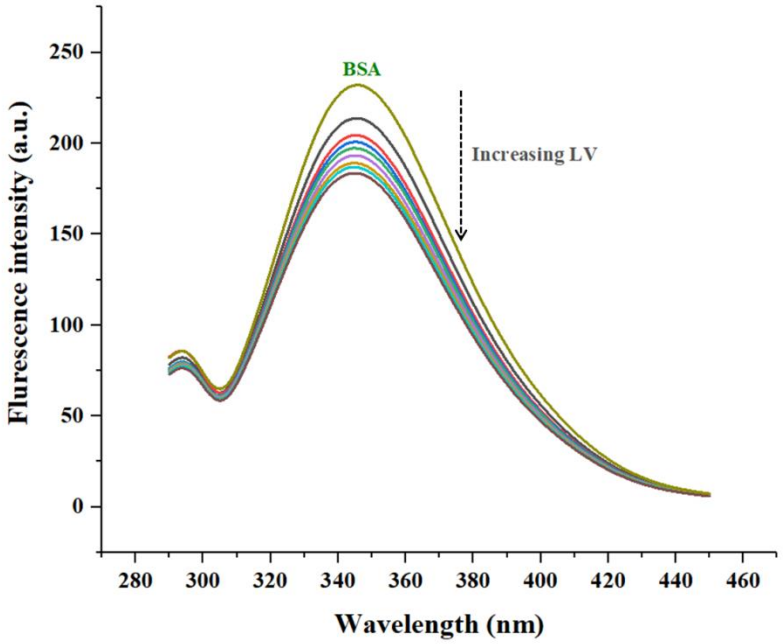

Fig. 4: Fluorescence spectra of native BSA in the absence and the presence of LV, [BSA] = 5×10^{-6} M and [LV] = 0.0 to 4.76×10^{-5} M (λ_{ex} = 295 nm, T = 310 k and pH7.4)

With increasing concentration of LV, the fluorescence intensity of B form decreased with a slight blue shift (Figure 5). Blue shift suggested that the polarities around tryptophan residues were decreased, the hydrophobicity was increased and the amino acid residues were less exposed to the solvent with the addition of LV and the quenching of fluorescence intensity might be due to the formation of non-fluorescence complex between LV and BSA.

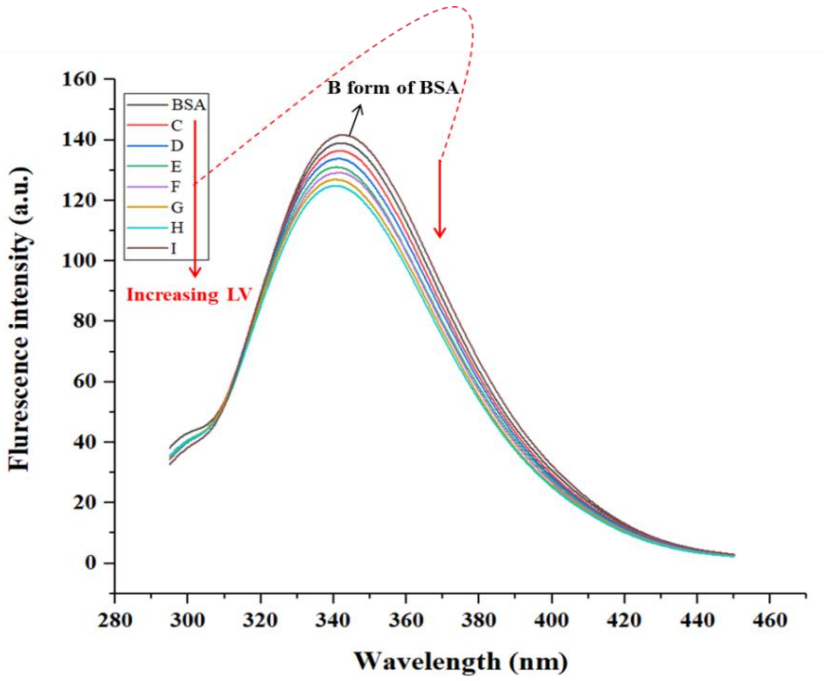

Fig. 5: Fluorescence spectra of B form of BSA in the absence and the presence of LV, [BSA] = 5×10^{-6} M and [LV] = from 0.0 to 4.76×10^{-5} M (λ_{ex} = 295 nm, T = 283 k and pH 9)

Compared to B form the fluorescence intensity of F form enhanced dramatically with a strong red shift from 336 nm to 347 nm (Figure 6), which indicates that LV could interact with BSA and might form a fluorescence complex between them with increasing concentration of LV. The red shift indicates that the interaction between them leads to the loosening and unfolding of the protein backbone and decrease the hydrophobicity of the microenvironment of BSA.

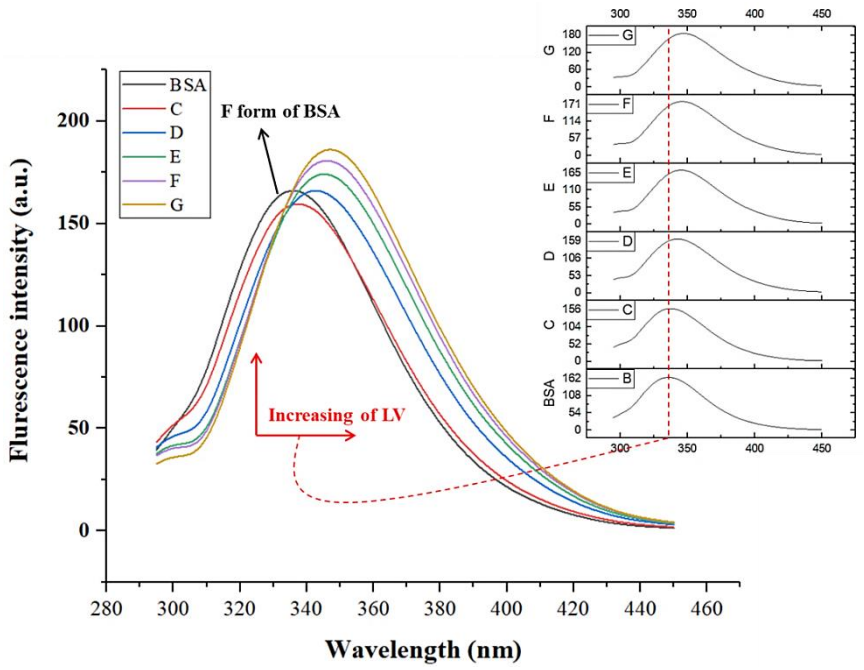

Fig. 6: Fluorescence spectra of F form of BSA in the absence and the presence of LV, [BSA] = 5×10^{-6} M and [LV] = from 0.0 to 4.76×10^{-5} M (λ_{ex} = 295 nm, T = 283 k and pH 3)

4. Conclusion

In summary, the effect of LV on BSA structure has been investigated in vitro using different spectroscopic techniques. A noticeable increase in absorbance was noted in the peak range of 200–400 nm, which may be attributed to BSA structural changes as a result of the formation of a new LV-BSA complex. Analysis of CD spectra indicated that the formation of LV–BSA complex induced changes in the secondary structure of protein with an increase in the α-helix content. The fluorescence spectroscopy studies on F and B forms of BSA showed that the microenvironment around Trp and Tyr residues may be changed during interaction with LV. We hope that, this work provides useful information for understanding the structure–activity relationship of the levetiracetam and BSA for design new improved antiepileptic drugs.

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