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Binding of the neuroleptic drug, gabapentin, to bovine serum albumin: Insights from experimental and computational studies

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ABSTRACT

The interaction between antiepileptic drug, gabapentin (GP), and bovin serum albumin (BSA) was studied by spectroscopic and computational methods. The native fluorescence of BSA was quenched by GP. Stern–Volmer quenching constant was calculated at different temperatures which suggested a static mechanism. The association constant (K_a) was calculated from fluorescence quenching studies, which increased with temperature rising. GP competed well with warfarine for hydrophobic subdomain IIA (Sudlow's site I) on the protein. Enthalpy and entropy changes during the interaction of GP with BSA were obtained using van't Hoff plot, which showed an entropy-driven process and involvement of hydrophobic forces ($\Delta H > 0$ and $\Delta S > 0$). Synchronous fluorescence measurements of BSA solution in the presence of GP showed a considerable blue shift when $\Delta\lambda = 15$ nm, therefore, GP interacts with tyrosine-rich sites on BSA. Optimized docked model of BSA–GP mixture confirmed the experimental results.

1. Introduction

GABA is the chief inhibitory transmitter in the mammalian central nervous system. It plays a role in regulating neuronal excitability throughout the nervous system. In human, GABA is also directly responsible for the regulation of muscle tone.

Gabapentin (GP), 2-[1-aminomethyl]cyclohexyl]acetic acid (Scheme 1) is a cyclic analogue of GABA, which may alter GABA transmission in the central nervous system [1]. The molecule incorporates a lipophilic cyclohexane ring into its structure, which allows GP, unlike GABA, to cross the blood–brain barrier. Pharmacokinetic and pharmacodynamic investigations have largely relied on study of animals. The drug was initially developed as spasmolytic but demonstrated effective anticonvulsant properties, and thus entered the pharmaceutical market as an anti-epileptic drug [2]. Its binding site has been recently identified as a subunit of a calcium channel on neuronal cells surfaces [3,4]. GP increases the rate of synthesis and accumulation of GABA, decreases the release of monoamines (dopamine, norepinephrine, and serotonin), and weakly inhibits GABA-transaminase, an enzyme which degrades GABA into other amino acids [3,5].

Serum albumins are the major soluble protein constituents of the circulatory system, and include 50–60% of the total amount of plasma proteins [6]. They bind metabolites, endogenous molecules, hormones, drugs, and so on [7,8]. The most important property of this group of proteins is that they serve as transporters for a variety of compounds such as drugs, fatty acids, and so on [9,10]. Knowledge of interaction mechanism between drugs and plasma proteins is of crucial importance in the pharmacodynamics and pharmacokinetics of drugs [11–15].

Bovine serum albumin (BSA) is one of the most extensively studied of this group of proteins, particularly because of its structural homology with human serum albumin (HSA). BSA molecule is made up of three homologous domains (I–III) that are divided into nine loops (L1–L9) by 17 disulfide bonds. Each domain in turn is the product of two subdomains (IA, IB, etc.). X-ray crystallographic data show that the albumin structure is predominantly α-helical, with the remaining polypeptide occurring in turns and in extended or flexible regions between subdomains [16,17].

In our previous study, the interaction between GP and ct-DNA was reported [18]. In this work, interaction of GP with BSA is investigated by spectroscopic and molecular modeling methods.

2. Material and methods

2.1. Reagents

BSA (> 96%, essentially free from fatty acid, lyophilized powder) was purchased from Sigma (USA). BSA solution was prepared in sodium phosphate buffer (PBS, 0.1 M, pH 7.4) and was kept in

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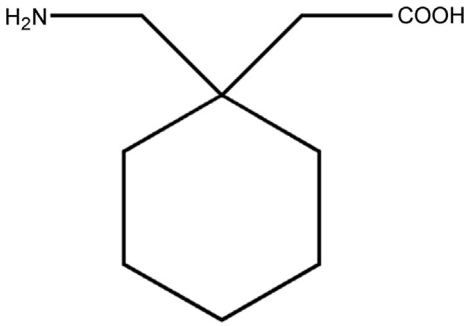

Scheme 1. Chemical structure of gabapentin.

the dark at 4 °C and used soon after preparation. GP powder was obtained from Bakhtar Bioshimi Pharmaceutical Company (Kermanshah, Iran). All other chemicals were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Fluorescence spectroscopy

Fluorescence emission spectra of BSA were recorded from 290 to 450 nm (λ_{ex}=295 nm) on a FP-6200 JASCO spectrofluorimeter (Tokyo, Japan) with slit widths of 5 nm. Synchronous fluorescence spectra were collected in the synchronous scan mode with an offset of 15 or 60 nm ($\Delta\lambda=\lambda_{em}-\lambda_{ex}$=15 or 60 nm).

In each assay, a sample solution (2.0 mL) containing BSA (30.0 μM) and small aliquots (μL) of a stock solution (1.0 M) of GP was added into a 1 cm quartz cuvette at intervals of 5 min.

2.2.2. Site marker competitive experiments

Binding location of GP in BSA was studied in the presence of two site markers (ibuprofen and warfarin) using the fluorescence titration method. The concentration of BSA and GP were fixed at 3.0×10^{-5} M, and 3.4×10^{-5} M, respectively (pH 7.4). Ibuprofen or warfarin was gradually added to BSA–GP mixtures. The fluorescence spectra were recorded over a wavelength range of 290–450 nm (λ_{ex}=295 nm). In another experiment, GP was gradually added to a mixture of BSA and site markers held in equimolar concentrations (3.0×10^{-5} M).

2.2.3. Absorption spectroscopy

Absorption spectra were recorded on an Agilent 8453 spectrophotometer (Germany, Waldbornn) using quartz cuvettes of 1 cm. The absorption spectra were recorded for free BSA and for its mixture with GP.

2.2.4. Circular dichroism (CD)

CD spectra of BSA and its GP complex were recorded using a JASCO (J-810) spectropolarimeter. For measurements in far-UV region (200–250 nm), a quartz cell with a path length of 0.1 cm was used in a nitrogen atmosphere. BSA concentration was kept constant (5 μM) while varying GP concentration (3, 6 and 9 μM).

2.2.5. Molecular docking study

For docking calculations, the crystal structure of BSA (PDB code 3V03) was downloaded from the protein data bank (PDB) (www.pdb.org). Atomic missed data of the protein was modeled by MODELLER version 9.10 [19] using HSA (PDB code 1AO6) as a template. Atomic coordinates of GP were built using Hyperchem program (version 8.0) in PDB format. The GP structure built with this manner was geometrically optimized by Polak–Ribiere's conjugate gradient method implemented in Hyperchem program. The modeled protein was selected for docking process to study the interactions with GP. The optimized structure of GP was used as input of AutoDockTools (ADT) and the partial charges of atoms were calculated using Gasteiger–Marsili procedure. Using AutoGrid tools, the grid maps were generated adequately large to include regions of the BSA where, according to experimental data, estimated as binding sites.

In all cases, a grid of $46\times74\times56$ points in the Cartesian space (x, y, z) and a grid spacing of 0.375 Å was used. The most suitable structure for GP was optimized by rotation of all single bonds in the molecule. The grid parameter and the docking parameter files were set up by the AutoDock Tools program. Population size was 256 and a maximum number of 2,500,000 were used for energy evaluations. Default settings were used for all other parameters. Docking calculations were carried out with the rigid BSA and flexible GP using a Lamarckian genetic algorithm implemented in AutoDock version 4.2.3 [20].The most suitable conformations of BSA–GP complexes, in terms of energy and cluster population, were selected for molecular dynamics studies. Molecular graphics were prepared by VMD version 1.8.9 [19].

3. Results and discussion

3.1. Fluorescence quenching of BSA in the presence of GP

Fluorescence spectrum of BSA contains a single peak with the maximum intensity at 340 nm (λ_{max}) as was shown in Fig. 1. The emission is due to the three amino acids in BSA: tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe) [21,22]. GP did not emit at the near-UV and visible wavelengths. Incubation of BSA solution with different concentrations of GP (final volumes of 2 mL) causes a gradual reduction in fluorescence intensity of the mixture. The fluorescence data were introduced to Stern–Volmer equation (Eq. (1)):

$$F_0/F = 1+K_q\tau_0[Q] = 1+K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities of BSA in the absence and presence of GP. τ_0 is the average fluorescence lifetime of the biomolecule and equals to 10^{-8} s; K_q is the apparent bimolecular quenching rate constant. K_{SV} is the Stern–Volmer quenching constant and [Q] is the concentration of quencher (GP).

The plot of Stern–Volmer equation for BSA–GP mixture at 25 °C is shown in Fig. 2A. Linear Stern–Volmer plot indicates a single quenching mechanism, either static (due to complex formation) or dynamic (due to collision). Moreover, as is shown in Table 1, K_{SV} decreases with rising temperature, which indicates that the fluorescence quenching of BSA is static (in the case of dynamic quenching, an increase in K_{SV} is observed with rising temperature) [23].

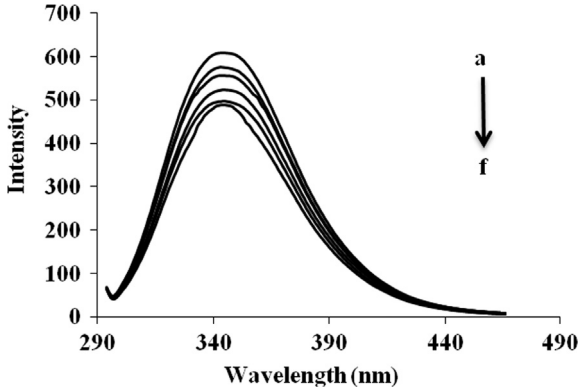

Fig. 1. Fluorescence emission spectra of BSA in the presence of GP in phosphate buffer (0.1 M, pH 7.4), λ_{ex}=295 nm, T=298 K. [BSA]=30 μM; [GP]=(a) 0; (b) 220 μM; (c) 277 μM; (d) 368 μM; (e) 469 μM; (f) and 520 μM.