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Interactions of cephalexin with bovine serum albumin: displacement reaction and molecular docking

Hamed Hamishehkar[1], Soheila Hosseini[2], Abdolhossein Naseri[3], Azam Safarnejad[2], Farzaneh Rasoulzadeh[1*]

[1] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Biotechnology Research Center and School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Department of Analytical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran

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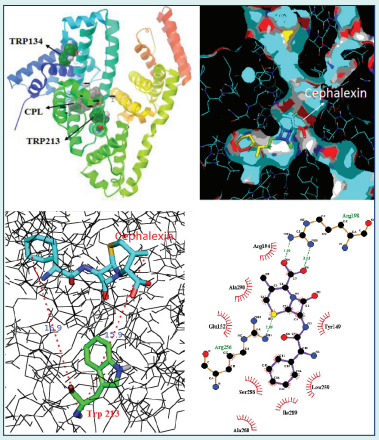
Abstract

Introduction: The drug-plasma protein interaction is a fundamental issue in guessing and checking the serious drug side effects related with other drugs. The purpose of this research was to study the interaction of cephalexin with bovine serum albumin (BSA) and displacement reaction using site probes.

Methods: The interaction mechanism concerning cephalexin (CPL) with BSA was investigated using various spectroscopic methods and molecular modeling method. The binding sites number, n, apparent binding constant, K, and thermodynamic parameters, ΔG^0, ΔH^0, and ΔS^0 were considered at different temperatures. To evaluate the experimental results, molecular docking modeling was calculated.



Results: The distance, r=1.156 nm between BSA and CPL were found in accordance with the Forster theory of non-radiation energy transfer (FRET) indicating energy transfer occurs between BSA and CPL. According to the binding parameters and ΔG^0= negative values and ΔS^0= 28.275 j $mol^{-1}K^{-1}$, a static quenching process is effective in the CPL-BSA interaction spontaneously. ΔG^0 for the CPL-BSA complex obtained from the docking simulation is -28.99 kj mol^{-1}, which is close to experimental ΔG of binding, -21.349 kj mol^{-1} that indicates a good agreement between the results of docking methods and experimental data.

Conclusion: The outcomes of spectroscopic methods revealed that the conformation of BSA changed during drug-BSA interaction. The results of FRET propose that CPL quenches the fluorescence of BSA by static quenching and FRET. The displacement study showed that phenylbutazon and ketoprofen displaced CPL, indicating that its binding site on albumin is site I and Gentamicin cannot be displaced from the binding site of CPL. All results of molecular docking method agreed with the results of experimental data.

Introduction

The drug–protein interaction may establish a protein–drug complex, which has an important effect on the drug tissue distribution and metabolic rate that aids in understanding the drug pharmacokinetics and pharmacodynamics.[1] Therefore, the information of the interactions among plasma bio-macromolecules and drugs is a crucial issue in biological and the medical sciences. Albumin is the most important protein of plasma for carrying many exogenous and endogenous compounds.[3] It rises the apparent solubility of hydrophobic drugs in the plasma and moderates their transport to cells. Subsequently, the drug protein binding and probable interactions are routinely investigated by this protein.[4] Cephalexin (CPL) (Fig. 1) is a first-generation cephalosporin antibiotic that has a broad spectrum antibiotic and is extensively administered therapeutically for the most common and uncomplicated infections including upper breathing infections, ear infections, skin infections, and urinary organs infections with an acceptable level of side effects. Antibiotics are also a regularly prescribed medication for in-patient use in hospitals, particularly the intensive care units (ICUs).[5] The probability for pharmacokinetic properties changes by β-lactam antibiotics is increased with administration

***Corresponding author:** Farzaneh Rasoulzadeh, Email, Rasoulzadeh91@gmail.com

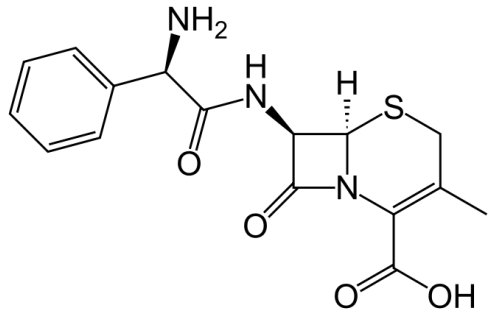


Fig. 1. Molecular structure of cephalexin.

of a combination of multiple medications, particularly in long suffering diagnosed with kidney issues and variable plasma albumin levels. The knowledge of drug-plasma protein interaction is a crucial issue in predicting and preventing serious drug side effects associated with other drugs, which contain a high protein binding.[6-10] One of the medicines that can interact with CPL is gentamicin. These interactions can affect the metabolism of the drugs and cause kidney damage or other things. For this purpose, it is necessary to define the bond connection of CPL to bovine serum albumin (BSA) and displacement interaction of BSA-CPL with other drugs such as gentamicin and study the thermodynamic parameters of CPL- BSA interaction. Fluorescence and UV–Vis absorption spectroscopy techniques, Fourier transform infrared spectroscopy (FT-IR) and circular dichroism (CD) are powerful tools for investigating the reactivity of chemical and biological systems. To access this function, the spectroscopic properties of CPL in the presence of BSA were investigated. The interactions of β-lactam with other antibiotics which affect their protein binding characteristics, have been reported in a number of earlier studies.[11] In this work, we use spectroscopic methods to study the binding of CPL to BSA in aqueous solution under biological conditions. We use also molecular modeling to approve the outcomes of spectroscopic methods. Gaining interaction evidence concerning quenching mechanisms, thermodynamic parameters, binding parameters, probing the binding site, binding modes, effect of metal ions, and intermolecular binding distance were our purposes. Moreover, the possibilities of gentamicin interaction with protein binding characteristics of CPL were investigated.

Materials and methods

Materials

Drug and BSA purchased from Sigma-Aldrich Co. (Poole, England) was daily used to make the stock solution of 10^{-4}M. Standard stock solutions of CPL was set by dissolving a suitable amount of the pure drug (98%) in distilled water as required to 10^{-3}M. All solutions in this study were diluted with 0.1M phosphate buffer (pH 7.4).

Apparatus

All fluorescence spectra were studied on a RF-5301 spectrofluorophotometer (Shimadzu, Kyoto Japan) and a 10 mm quartz cuvette. The widths of both the excitation and emission slits were set at 5 nm. The optimum excitation and emission wavelengths for BSA were obtained as 280 and 340 nm, respectively. To estimate and eliminate the inner filter effect (IFE), the correction ways were performed based on the absorbance amounts of solutions at excitation and emission wavelengths of albumin. UV spectra measurements were performed on a Shimadzu 2550 UV– spectrometer using a 10 mm cell at 20 nm intervals. FTIR spectra were acquired on a Shimadzu FTIR–8400 S (Shimadzu, Kyoto, Japan).

Circular Dichroism (CD) studies were made on an Aviv, model 215, spectrophotometer (Aviv Biomedical, Inc., Lakewood, NJ, USA) using 1 mm path length at room temperature with a scan rate of 500 nm/min and a reaction time of 0.5 s. The scans were repeated three times for each spectrum.

Spectroscopic investigations

All solutions must be relaxed at least 10 min before measuring the spectrum. To correct the fluorescence or absorption background, proper blanks corresponding to the buffer were deduced. The following measurable analysis was obtained by using the corrected fluorescence intensities at λ_{em}=340 nm at three temperatures.

Fluorescence quenching spectra of BSA

Fluorescence quenching spectra of BSA with CPL were recorded at excitation wavelength (280 nm) and emission wavelength (300–450 nm) at three different temperatures (299, 305 and 311 K) and pH=7.4. Fluorescence spectra of these solutions were investigated upon addition of Ibuprofen, Phenylbutazon, Ketoprofen, Gentamicin, and some metal ions at the same conditions. The synchronous fluorescence spectra were also scanned from 280 to 400 nm at Δλ=15 and 60 nm, respectively.

UV–Visible absorption spectra

The absorption spectra of BSA in the presence of different concentrations of CPL were determined in the range of 200–400 nm at room temperature. The concentration of BSA was set at 3.33 ×10^{-6}M while that of CPL was changed from 0 to 1.30×10^{-4}M.

FT-IR spectroscopic measurements

The FTIR spectrum of BSA in the absence and presence of CPL in phosphate buffer was found in the range of 4000–500 cm^{-1} with a small resolution of 2 cm^{-1} and 100 scans. The concentrations of the CPL in the complex mixtures were 20 ×10^{-6}M with a final BSA concentration of 3.33×10^{-6}M. Spectra were gathered after 10 min of incubation of BSA with CPL solution at room temperature.

Circular dichroism measurements

CD measurements were studied at room temperature in the wavelength range of 200–240 nm. These measurements were carried out by mixing a fixed concentration of BSA (3.33×10^{-6}M) with varying concentration of CPL (0, 3.33, 10)×10^{-6}M. All scans were measured under continuous nitrogen atmosphere.

Molecular docking studies

The three-dimensional (3D) structure of CPL was drawn by ChemBioDraw Ultra and energy optimized using HyperChem software by AM1 semi-empirical method with RMS gradient of 0.001 kcalmol^{-1}. The crystal structure of BSA (pdb id: 4f5s) was downloaded from Protein Data Bank. CPL was docked to 6 subdomains of BSA by Autodock Vina with grid map of (22×22×22) and maps were located on each subdomain to determine the binding site of BSA, then the best docking was further considered by AutoDock. Molecular docking was finished in model1 and model2 by AutoDock Tools. Water molecules were removed and polar hydrogen atoms were added to BSA and then charged using kollman charges. The grid maps to (60×60×60Å) and a grid spacing of 0.375A^0. Center of the grid box (the x-, y, z-axes) was set to 0.527 Å, 28.13 Å, and 102.81Å with 100 docking runs. Population size was set to 150 with 2500000 energy evaluations (medium). Conformational searching was made using the Lamarckian genetic algorithm (LGA), analysis made by Autodock tools, and VDW scaling factor was 1.

Results

Analysis of fluorescence quenching data

By using fluorescence spectroscopy, the interaction between these drugs and BSA was investigated to collect the necessary information regarding its quenching mechanism, binding constants and binding sites. Some drug molecules absorb light at the excitation and emission wavelength of BSA that influences the fluorescence intensity. It is known as the IFE. To estimate the impact of IFE in this method, the absorbance of CPL at 280 nm, excitation wavelength of BSA, and 340 nm emission wavelength of BSA were determined. The absorbance of concentrated solutions (C_{CPL}= 1.30×10^{-4}M) was higher than 0.1, approximately about 0.25, at 2800 nm (Fig. 2). In such cases the effect of IFE should be corrected using Eq. 1.[12,13]

$$F_{cor} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad \text{(Eq. 1)}$$

Where F_{cor} and F_{obs} are the corrected and observed fluorescence, respectively. A_{ex} and A_{em} are the absorbance of the drug at excitation wavelength and emission wavelength, respectively.

Tryptophan residues (Trp) and tyrosine residues (Tyr) are two types of fluorophores in BSA that can be excited at 280 nm. As demonstrated and noted in Fig. 3 with the increasing amounts of CPL, the fluorescence intensities decreased without any shifts in λ_{max}.[14, 15] If the conditions of pH, temperature, and ionic strength are fixed, two different mechanisms can occur in the fluorescence quenching, static quenching, and dynamic quenching. Stern Volmer equation can describe both of them. Normally, increasing temperature causes the increase of quenching rate constants for dynamic quenching and the reverse effect was observed for the static quenching. To configure which mechanism has an important role in the interaction, fluorescence quenching data were studied by the stern–Volmer equation (Eq. 2)[16]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + K_q\tau_0[Q] \quad \text{(Eq. 2)}$$

Where F_0 and F are the steady state fluorescence intensities of BSA in the absence and presence of quencher, respectively. K_{SV} and [Q] are the Stern–Volmer quenching constant and the concentration of the quencher, respectively. K_q is the quenching rate constant and τ_0 is the average lifetime of the BSA in the absence of any quencher and is generally equal to 10^{-8} s.[17] Fig. 4 shows the Stern–Volmer graphs at three different temperatures.

It demonstrated the plots are linear for three temperatures and the slopes decrease with increasing temperature. The values of K_{SV} for the interaction of CPL with BSA at three temperatures are shown in Table 1.

This study found that the K_{sv} values are related reversely to temperature indicating a static quenching mechanism for the interaction of BSA with CPL.[18]

Determination of the binding constant and the number of binding sites

There are n equivalent binding sites on the BSA and there

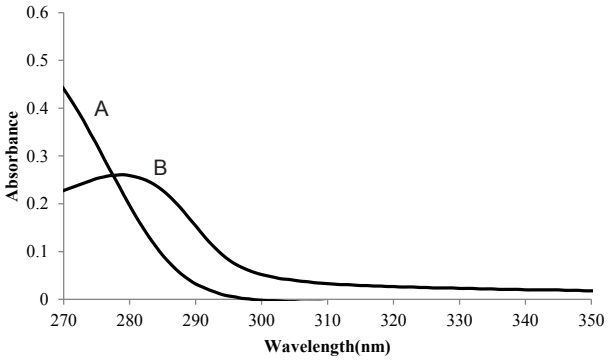


Fig. 2. UV spectra of A: CPL (130×10^{-6}M) and B: albumin (3.33×10^{-6}M).

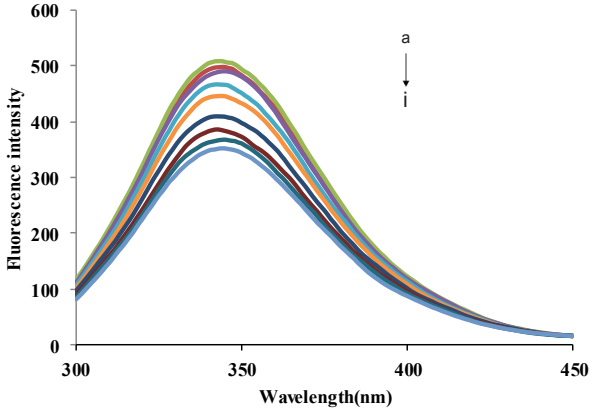


Fig. 3. Fluorescence emission spectra of BSA in the presence of different concentrations of CPL; [BSA] = 3.33×10^{-6}M; [CPL] = (0,10,30,50,70,90,110,120 ,130)×10^{-6}M (λ_{ex} = 280 nm, T = 293 K, pH =7.4).

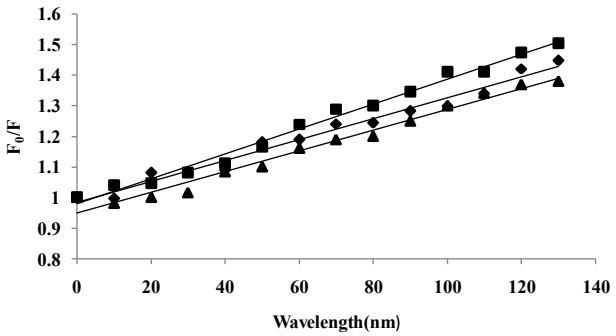


Fig. 4. The Stern–Volmer plots for the quenching of BSA by CPL at different temperatures, 299 K (■), 305 K (♦) and 311 K (▲) λ_{ex} = 280 nm, λ_{em} = 340 nm and pH= 7.40.

are many methods and equations for the investigation of the binding constant, K, and the number of binding sites, n, for the reaction, P + nD→D_nP. But, only in Eq. 3, the total drug concentrations can be used[19]:

$$\log\frac{F_0-F}{F}=n\log k-n\log\left(\frac{1}{[D_t]-(F_0-F)/[P_t]F_0}\right) \quad \text{(Eq. 3)}$$

Where, F_0 and F are the fluorescence intensities in the absence and presence of the quencher, $[D_t]$ and $[P_t]$ are the total quencher concentration and the total protein concentration, respectively. Therefore, the number of binding sites, n, and binding constant, K, can be estimated by the plots of log $[(F_0-F)/F]$ versus log $(1/([D_t]-(F_0-F)[P_t]/F_0))$, from the slopes and intercepts appropriately (Table 2). The correlation coefficients (larger than 0.98) indicates a good agreement in the interaction of CPL and BSA with Eq. 3. There are about two classes of binding site for CPL at the boundary of BSA. As demonstrated in Table 2, the K values between BSA and CPL decreased with increasing the temperature that shows the complex formation between CPL and BSA supporting the fluorescence quenching mechanism is a static process.

UV–Vis absorption spectra

The study of UV–Vis absorption spectra is a helpful method and appropriate to discover the structural change and know the formation of the complex.[20] For validation of the fluorescence quenching mechanism of BSA by CPL, UV–Vis absorption spectra of BSA were recorded before and after adding CPL (Fig. 5). As shown in Fig. 5, not only the absorbance intensity increased by the addition of CPL, but also the absorption spectra maximally shifted to a shorter wavelength region (279→263 nm).

Table 1. Stern–Volmer quenching constants of CPL –BSA at different temperatures

pH	T(K)	$K_{svx}(10^{-3})$	n	R
7.4	299	5.450	1.246	0.984
	305	4.793	1.795	0.976
	311	4.484	1.525	0.979

FT-IR spectroscopy

FTIR spectroscopy is a simple instrument for providing structural and conformational changes of proteins. IR spectra of proteins display a number of amide bands representing diverse vibrations of the peptide moiety. Both the amide I (ranging from 1600 to 1700 cm^{-1}) and amide II (around 1548 cm^{-1}) bands of the protein are related with secondary structure of protein.

However, the change of protein secondary structure affects the amide I band more than amide II.[21] Fig. 6 shows the FT-IR spectra of free BSA and CPL–BSA in phosphate buffer. As can be noted, there is a shift in the peak position of amide I of BSA from 1652 to 1656 cm^{-1} after addition of CPL demonstrating a slight change in the secondary structure of BSA.[22]

Fluorescence resonance energy transfer from BSA to CPL

Fluorescence resonance energy transfer (FRET) is a powerful model that helps to evaluate donor-acceptor interactions by calculating the distance between two fluorophores, donor and acceptor.[23] The distance between two fluorophores should be shorter than 8 nm.[24] The energy transfer efficiency, E, is well-defined by the following Eq. (4), where r_0 is the distance from the drug to the tryptophan residue of BSA, and R_0 is the Forster critical distance, at which 50% of the excitation energy is transferred to the drug.[12] R_0 can be estimated from donor emission, BSA, and acceptor absorption spectra, drug, using the Forster equation (Eq. 5):

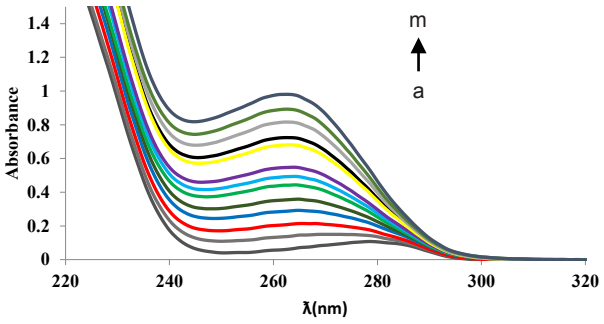


Fig. 5. Absorption spectra of BSA in the presence of CPL [BSA] = 3.33×10^{-6}M; [CPL] = (0,10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110)×10^{-6}M, from cure a to l), the absorption spectrum of CPL only(m).

Table 2. Modified Stern–Volmer association constant K_a and relative thermodynamic parameters of CPL–BSA

T	$K_{ax}(10^{-3})$	R	ΔH(Kj mol^{-1})	ΔG(Kj mol^{-1})	ΔS(j $mol^{-1}K^{-1}$)
299	5.451	0.991	-12.895	-21.349	28.275
305	4.793	0.981	-	-21.519	-
311	4.484	0.983	-	-21.688	-

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r_0^6} \quad \text{(Eq. 4)}$$

$$R_0^6 = 8.79 \times 10^{-25} K^2 \varphi N^{-4} j \quad \text{(Eq. 5)}$$

$$j = \frac{\int_0^{\alpha} F(\lambda)\varepsilon(\lambda)\lambda^4 \, d\lambda}{\int_0^{\alpha} F(\lambda) d\lambda} \quad \text{(Eq. 6)}$$

In Eq. 5, K^2 is the random orientation factor associated to the geometry of the donor and acceptor and K^2 = 2/3 for fluid solution; N the average refractive index of medium in the wavelength range where spectral overlap is significant; Φ the fluorescence quantum yield of the donor; *j* the effect of the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor, which could be determined by Eq. 6. Here, F(λ) is the corrected fluorescence intensity of the donor in the wavelength range λ to λ+Δλ; ε (λ) is the extinction coefficient of the acceptor at λ. FRET is an important method for studying the diversity of biological systems including energy transfer processes.

The spectral overlap between UV-Vis absorption spectrum of CPL and the fluorescence emission spectrum of free BSA is illustrated in Fig. 7. Because the fluorescence emission of BSA was affected by the excitation light around 288 nm, the spectrum ranging from 300 to 400 nm was selected to analyze the overlapping integral.

In this study, N= 1.36 and Φ= 0.15 according to Eqs. 4, 5 and 6. It was calculated that J = 1.97×10^{-15} cm^3 L mol^{-1}, E = 0.0462, R_0 = 0.698 nm, and r = 1.156 nm. The average distances between BSA and CPL is less than 8 nm suggesting that energy transfer happens between BSA and CPL. Furthermore, since r is larger than R_0, it recommends that CPL quench the fluorescence of BSA by non-radiative energy transferring and static quenching.

Synchronous fluorescence spectra analysis

Synchronous fluorescence spectroscopy is a method to find evidence about the molecular environment close the chromosphere molecules. Since tryptophan (Trp) and tyrosine (Tyr) residues of BSA show the fluorescence property, we can find the specific evidence of tyrosine and tryptophan residues in BSA by setting Δλ at 15 nm and 60 nm, respectively and determining the probable shift in wavelength emission maximum λ_{max}. There is no significant shift (299-301) in synchronous fluorescence spectra of BSA by addition of CPL enhancement at λ=15 nm demonstrating that CPL has slight effect on the environment of the Tyr residues in BSA. By contrast, when Δλ=60 nm (Fig. 8), the emission maximum wavelength shows a red shift (from 342 to 335 nm) representing that the environment of the tryptophan residues were altered and hydrophobicity near this residue decreased resulting the interaction between BSA and CPL.[25-27]

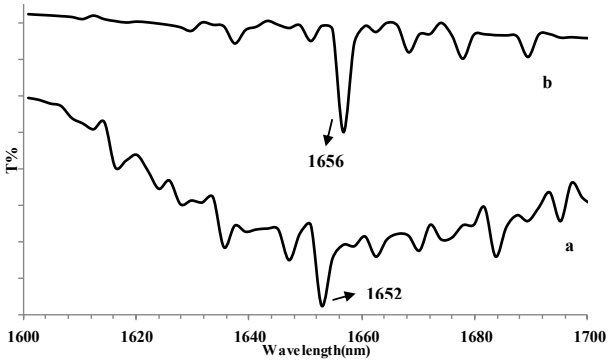


Fig. 6. Transmittance of the buffer solution from the spectrum of the (a) BSA solution, (b) the difference spectra of BSA (subtracting the absorption of the CPL–freeform from that of CPL–BSA boundform).

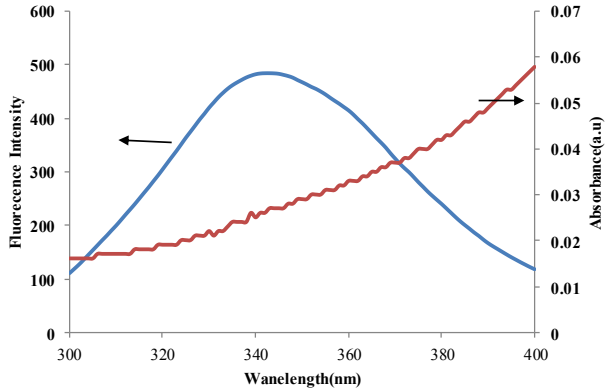


Fig. 7. The spectral overlap between UV/vis absorption spectrum of CPL (10×10^{-6}M) and the fluorescence emission spectrum of free BSA (10×10^{-6}M).

Circular dichroism studies

CD spectroscopy investigates the spectrum of a protein for secondary structure with highly reliability.[28] To study the structural change of BSA by the addition of CPL, the CD spectra of BSA was measured before and after the addition of CPL (Fig. 9). There are two negative bands in the far-UV region at ~208 and ~222 nm which are characteristic of the helical structure of proteins. As estimated, the CD spectrum displays a strong negative ellipticity at 208 nm and 222 nm.

The negative ellipticity in the region of far-UV CD was increased by the addition of the CPL to BSA (1:1, 1:3, and 1:4), without any substantial shift of peaks. This showed that the binding of CPL to BSA made an intense increase in the content of α-helical structure of the BSA clarifying the stabilization of the BSA, secondary structure as a consequence of the BSA–CPL interaction in the binary and ternary system.[29] This was supposed to be the result of the complex formation of BSA-CPL. However, the similarity between the shapes of the CD spectra relating to BSA before and after addition of the CPL in all relating systems proposed that the structure of BSA was still mainly α –helical.[30]

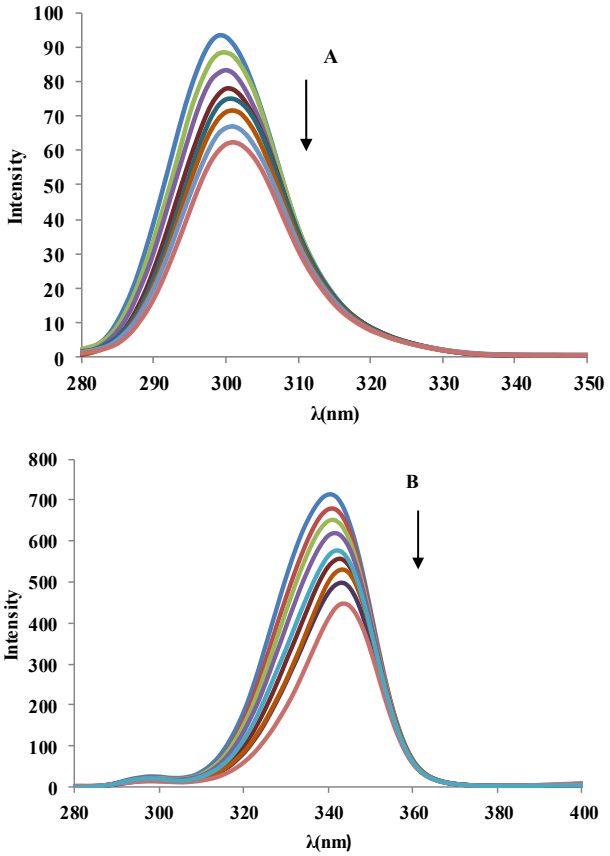


Fig. 8. The synchronous fluorescence spectra of BSA in the presence of CPL (T=293K, pH=7.4). [BSA]=3.33×10^{-6}M; [CPL]= (0, 10, 30, 50, 70, 90, 110, 120,130) × 10^{-6}M. (A) Δλ=15 nm, (B) Δλ = 60 nm.

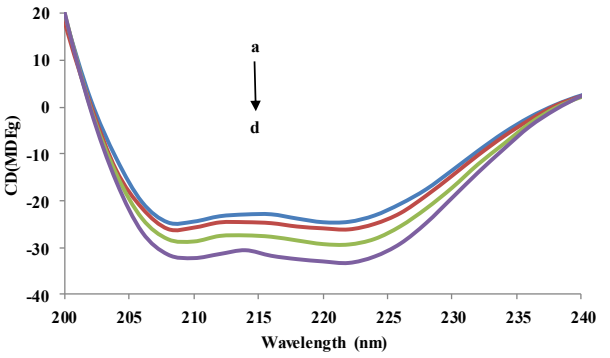


Fig. 9. Circular dichroic spectra in the 200–240 nm range; (a) BSA, 3.33×10^{-6}M; (b) BSA: CPL = 1:1; (c) 1:3; (d)1:4.

Discussion

Thermodynamic analysis for binding mode between CPL and BSA

Small particles are bound to macromolecule typically by four acting forces containing hydrogen bond, Vander Waals force, electrostatic force, and hydrophobic interaction force.

There are thermodynamic parameters dependent to temperature, enthalpy change (ΔH), entropy change (ΔS), and free energy change (ΔG) of the reaction that are significant for characterizing the binding type. Therefore, the thermodynamic parameters were investigated to describe the acting forces between CPL and BSA.

The enthalpy change (ΔH°) did not differ meaningfully with the temperature range considered, and the thermodynamic factor of ΔH°, entropy change (ΔS°), and free energy (ΔG°) were calculated by the Van 't Hoff equation:

$$\ln K = -\frac{\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} \qquad \text{(Eq. 7)}$$

Where K is the binding constant and R is the gas constant. The values of H° and ΔS° were evaluated using Eq. 7 by the plot of ln K versus 1/T.[10] The value of ΔG° was calculated using the following equation[31]:

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ} \qquad \text{(Eq. 8)}$$

The calculated thermodynamic parameters for CPL-BSA interaction are illustrated in Table 2. The values of ΔG are negative indicating that the binding procedure is spontaneous. The enthalpy (ΔH) and entropy (ΔS) of the interaction of CPL and BSA are negative and positive, respectively. According to the report of Ross and Subramanian,[32] the positive ΔH and ΔS value shows that the binding is mainly entropy-driven and a hydrophobic effect contributes in the interaction between CPL and BSA. The negative ΔH and ΔS values are related with hydrogen bonding and Van der Waals force. As a final point, low positive or negative ΔH and positive ΔS values are categorized by electrostatic interactions. Consequently, the interaction of CPL with BSA might contain the electrostatic interaction.

Displacement experiments using site probes and Gentamicin

BSA consists of amino acid chains making a single polypeptide, which has three homologous-helices in domains (I–III). Each domain is divided into anti-parallel six helices and four sub-domains (A and B). A cluster of two sub-domains with their grooves to each other forms a domain, and three of such domains form an albumin molecule. Sites I and II are two main definite drug-binding sites in serum albumin, which are situated in particular holes in sub-domains IIA and IIIA, respectively.[33] The majority of small molecules, which are identified to combine with BSA, form a complex at site I, and only a few at site II. Though, it is challenging to find the real site from the structure of the small molecule involved. Therefore, it is proposed that site I of serum albumin demonstrated an attraction for Ketoprofen and Phenylbutazone (PB), and site II for Ibuprofen (IB) and others. To recognize the position of the CPL binding site on BSA, the displacement experiments were performed by the site probes Ibuprofen, ketoprofen, and phenylbutazon.

The percentage of fluorescence probe displaced by the drug was calculated by determining the variations in fluorescence intensity according to the method suggested

by Sudlow *et al.*[34] Relative fluorescence (RF) can be used to display the alterations in fluorescence intensity in the presence of probes as Eq. 9:

$$RF = \frac{F}{F_0} \times 100\% \quad \text{(Eq. 9)}$$

Where, F_0 and F represent the fluorescence of CPL plus BSA in the absence and presence of the probe, respectively. According to the spectral data determined in the displacement studies, the plots of F/F_0 against site probe concentration were found and are shown in Fig. 10. The comparative fluorescence intensity meaningfully reduced after adding the phenylbutazon and ketoprofen, whereas the addition of Ibuprofen produced no observed changes, which shows that phenylbutazon and ketoprofen can displace the CPL, however, Ibuprofen has little effect on the binding of CPL to BSA. In all displacement tests, the implication was that CPL fixes to the site I in subdomain IIA of BSA. These findings are in agreement with the results reported previously.[35,36]

The reasonable procedure was examined for gentamicin, the importance of these studies is that gentamicin is classified to the aminoglycozide and are drugs typically prescribed in combination with CPL (illustrated in Fig. 10). The fluorescence demonstrated no significant change with the addition of gentamicin to the same solution, which indicates that gentamicin cannot be displaced from the binding site of CPL. Then it can be decided that gentamicin was bound to site II of BSA.

The effect of common metal ions on the binding constant

If there are metal ions present in the solution, the binding properties between CPL and BSA may be affected. A number of trace metal ions exist within the human body. Therefore, this aspect is particularly important and necessary in this research.

The effect of common ions such as Fe^{3+}, Zn^{2+}, K^+ and Na^+ on the CPL– BSA binding was studied at 293 K by investigating the fluorescence intensity of CPL–BSA compound in the presence of each ion, distinctly in the range of 300–500 nm with the excitation wavelength at 280 nm. The studied cations in the phosphate buffer have no effect on the CPL–BSA interaction under the experimental conditions. The effect of properties of such cations on the interaction between a drug and BSA has been described in the former works.[37] The fluorescence emission spectrum of CPL in the presence of common ions (Table 3) shows no interaction between the ions and CPL. However, there is a binding reaction between the common ion and protein and thus the presence of common ion directly affects the binding between CPL and BSA.

The CPL–BSA binding constant was increased in the presence of studied ions. Therefore, the binding force between BSA and CPL was improved which extended the serum-level of CPL in the blood plasma and improved the extreme efficiency of CPL.

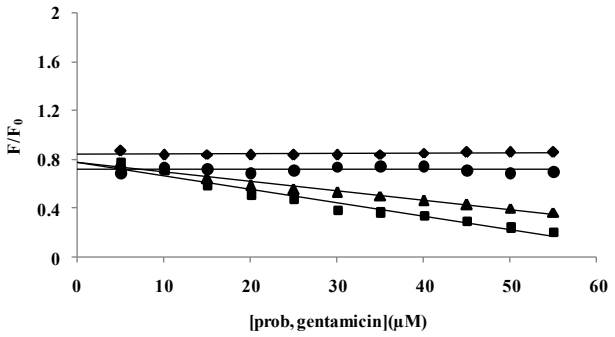


Fig. 10. Effect of site marker probes (Ketoprofen ■, Phenylbutazone▲, Ibuprofen ♦) and Gentamicin (●) on the fluorescence of CPL–BSA; [BSA] = 3.33×10^{-6}M; [CPL]=60×10^{-6}M; [prob, Gentamicin]=(0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50) × 10^{-6}M.

Molecular docking results

The lowest energy in AutoDock Vina outcome (Table 4) specified that CPL was bound to subdomain IIA better than other sites. The results of further analysis proposed that CPL interacts with BSA of the hydrophobic cavity of subdomain IIA of BSA (Fig. 11 A). CPL was surrounded by the hydrophobic and negatively charged residues such as Arg194, Arg198, Arg256, Ala290, His241, His287, and Leu237 through hydrogen bond between CPL and Arg198 (3.03A^0, 2.92A^0) and Arg256 (2.86A^0) (Fig. 11 D). The existence of the hydrogen bond makes a reduction in the hydrophilicity and a rise in the hydrophobicity which becomes stable in the CPL-BSA complex.[38] It shows that CPL-BSA binding is mainly hydrophobic in nature. Distance between Trp213 residue and CPL was 15.9 Å and free energy for the CPL-BSA complex found from the docking simulation was -6.93 kcal mol^{-1} (-28.99 kj mol^{-1}) (Table 5), which is close to experimental ΔG of binding (-21.349 kj mol^{-1}) (Table 2).

Conclusion

In conclusion, this investigative research on the interaction of CPL and BSA was studied by spectroscopic

Table 3. The binding constants (L mol^{-1}) between CPL and BSA at 293 K in the presence of common ions

Ions	Association constant(K)
0	3650.32
Fe^{3+}	3850.72
K^+	4007.52
Na^+	4237.43
Zn^{2+}	4251.78

Table 4. ΔG^0 of binding CPL-BSA (kcal mol^{-1}) for docking positions

Complex	Subdomain					
	IA	IIA	IIIA	IB	IIB	IIIB
CPL-BSA	-5.2	-8.9	-7.5	-7.6	-5.2	-6.0

Table 5. Amino acid residues involved in CPL-BSA intraction with ΔG0 and Hydrogen bond distance

Grid size	Subdomain	Inhibition Constant, Ki (μM)	$\Delta G^{0\,a}$ kcal mol^{-1}	$\Delta E_1{}^{b}$ kcal mol^{-1}	$\Delta E_2{}^{c}$ kcal mol^{-1}
60×60×60	IIA	8.33	-6.93	-8.66	+0.54

$\Delta G^{0\,a}$ is estimated Free Energy of Binding in the binding process. $\Delta E_1{}^{b}$ is Vander Waals energy. $\Delta E_2{}^{c}$ is electrostatic energy.

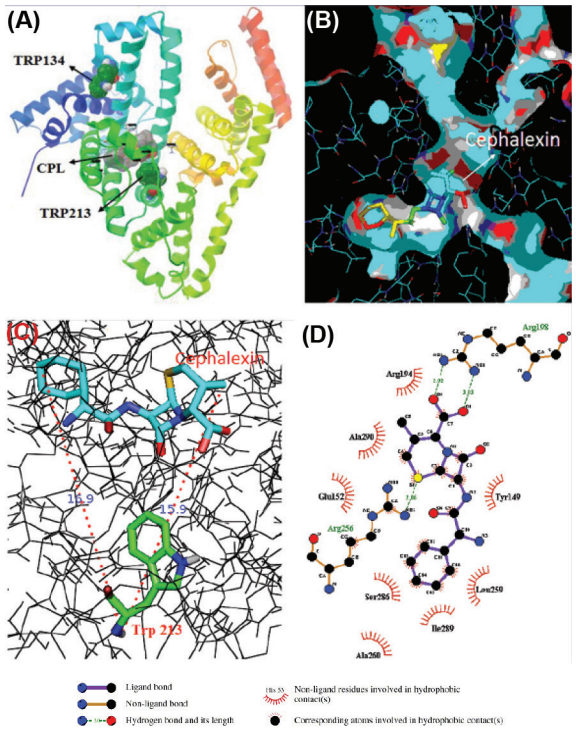


Fig. 11. (A) Minimum energy docking conformation obtained from docking in subdomain (IIA) of BSA, Trp-213, Trp 134 and CPL have been identified. (B) the surface representation of BSA showing the binding pocket of CPL by PyMOL. (C) The distance between Trp-213 and CPL from docking simulation in minimum energy is shown by dotted line.(D) Representation of CPL interaction with its binding pocket in 2- dimensional view, generated by LIGPLOT.

techniques with fluorescence and UV–Vis absorption spectroscopy. The experimental outcomes confirmed that the mechanism of interaction between BSA and CPL is static.

Based on the synchronous fluorescence technique, the secondary construction of BSA was reformed in the presence of CPL. It was also demonstrated that Trp residue participates more than Tyr in the interaction between CPL and BSA. The results of the current research revealed the presence of a single binding site and hydrophobic interaction in the CPL - BSA complex. Gentamicin changed the binding constants of the CPL–BSA complex. Therefore, the variation of the kinetic and dynamic properties of CPL by gentamicin through alteration of the binding capacity of CPL to BSA cannot be negligible. Furthermore, the displacement investigation demonstrated that the location of CPL is in site I of BSA. In addition, the molecular docking studies confirmed the results of experimental studies.

Research Highlights

What is current knowledge?

√ Participation of both Tyr and particularly Trp residues in the interaction between CPL and BSA.

What is new here?

√ Docking simulation for CPL-BSA and comparing to the results of experimental methods.

Ethical approval

There is no ethical issue to be considered.

Competing interests

We wish to confirm that there are no known conflicts of interests associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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