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Short communication

Study of the interaction between fluoroquinolones and bovine serum albumin

Bhalchandra P. Kamat *

KLS's Gogte Institute of Technology, Department of Chemistry, Udyambag, Belgaum 590 008, India

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Abstract

The mechanism of interaction between norfloxacin (NRF) and ciprofloxacin (CPF) with bovine serum albumin has been investigated using circular dichroism, fluorescence and absorption spectroscopy. The quenching mechanism of fluorescence of bovine serum albumin by fluoroquinolones was discussed. The binding sites number *n* and apparent binding constant *K* were measured by fluorescence quenching method. The thermodynamic parameters obtained from data at different temperatures were calculated. The distance *r* between donor (bovine serum albumin) and acceptor (fluoroquinolones) was obtained according to Forster theory of non-radiation energy transfer. The effect of common ions on binding constant was also investigated. The results of synchronous fluorescence spectra, UV–vis absorption spectra and circular dichroism of BSA in presence of fluoroquinolones show that the conformation of bovine serum albumin changed.

Keywords: Fluoroquinolones; Bovine serum albumin; Fluorescence quenching; UV–vis spectroscopy; Thermodynamic parameters; Energy transfer

1. Introduction

Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms. Being the major macromolecule contributing to the osmotic blood pressure they can play a dominant role in drug disposition and efficacy. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells in vivo and in vitro. Consequently, it is important to study the interaction of drugs with this protein. The effectiveness of drugs depends on their binding ability [1–4]. Ciprofloxacin (CPF) and norfloxacin (NRF) belong to fluoroquinolones (FQ), which are bacteriostatic at low concentration and bactericidal at high concentrations. They are highly active against most Gram-negative pathogens including *Pseudomonas aeruginosa* and the *Enterobacteriaceae*. Fluoroquinolones are used to treat upper and lower respiratory infections, gonorrhea, bacterial gastroenteritis, skin and soft tissue infections and both uncomplicated and complicated urinary tract infections, especially those caused by Gram-negative than Gram-positive infections.

Fluorescence and UV–vis absorption spectroscopies are powerful tools for the study of the reactivity of chemical and biological system. The aim of this work was to determine the affinity of fluoroquinolones to bovine serum albumin (BSA), and to investigate the thermodynamics of their interaction. To resolve this problem the UV, circular dichroism and fluorescent properties of FQ as well as BSA were investigated [5].

2. Experimental

2.1. Materials

BSA, Fraction V 99% protease-free essentially γ-globulin free prepared from pasteurized serum. Purified by heat treatment and organic solvent precipitation was obtained from Sigma Chemical Company, St. Louis, USA. CPF and NRF

* Fax: +91 831 2441909.
E-mail address: bp_kamat@yahoo.co.in.

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drugs were obtained as gift samples from CIPLA Ltd., India. The solutions of FQ and BSA were prepared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl. All other materials were of analytical reagent grade and doubly distilled water was used throughout.

2.2. Apparatus

Fluorescence measurements were performed on a Hitachi spectrofluorometer Model F-2000 equipped with a 150 W Xenon lamp and slit width of 10 nm, and using a 1.00 cm quartz cell was used. Peltier Accesory (temperature control) attached Varian CARY 50 BIO UV–vis spectrophotometer was used for scanning UV–vis spectra. CD measurements were made on a JASCO-810 spectropolarometer using a 1.00 cm cell at 0.2 nm intervals, with three scans averaged for each CD spectrum in the range of 195–260 nm.

2.3. Spectroscopic measurements

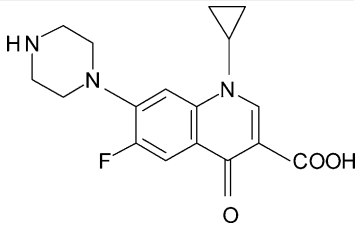
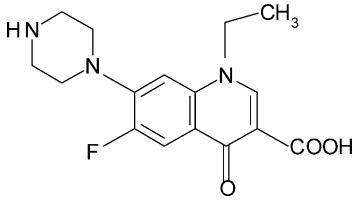
2.3.1. Drug–BSA interaction

Some preliminary studies were carried out to select optimum protein and FQ concentrations for FQ–BSA interaction. On the basis of preliminary experiments, BSA concentration was kept fixed at 10 μM and FQ concentration was varied from 10 to 140 μM. Fluorescence spectra were recorded at room temperature 29 °C in the range 300–500 nm after excitation at 296 nm in each case. The fluorescence measurements were performed at different temperatures (298, 302, 306 and 310 K) in the range 300–500 nm. BSA concentration was kept fixed at 10 μM and FQ concentration was varied from 10 to 140 μM. Excitation wavelength was 296 nm. Appropriate blanks corresponding to the buffer were subtracted to correct background fluorescence. The absorption spectra of BSA, FQ and their mixture were performed at room temperature. A stock solution of 0.1 μM BSA was prepared in 0.01 M phosphate buffer containing 0.15 M NaCl. The BSA to FQ concentration was varied (1:1 and 1:3) and the CD spectrum was recorded.

3. Results and discussion

The structures of FQ used in the present study are shown in Table 1.

Table 1
Structures of FQ in the present investigations

Drug	Structure
Ciprofloxacin (CPF)	
Norfloxacin (NRF)	

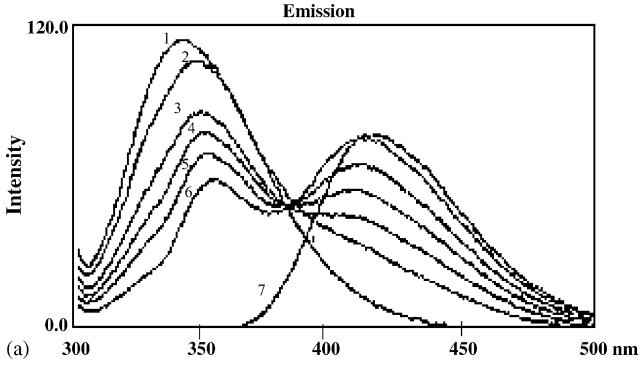
3.1. Fluorescence studies

Fluorescence spectra of BSA in the presence of increasing amounts of various FQ are shown (Fig. 1a and b). It could be interpreted that the complex formed between CPF/NRF and BSA quenched the fluorescence of tryptophan moiety of BSA with red shift. The fraction of drug bound, θ, was determined [1,6] using the equation,

$$\theta = \frac{(F_0 - F)}{F_0}, \quad (1)$$

where F and F_0 denote the fluorescence intensities of protein in a solution with a given concentration of drug and without drug, respectively. The θ represents the fraction of site on the protein occupied by drug molecule. Fluorescence data was analyzed using the method described by Ward [7]. It has been shown that for equivalent and independent binding sites

$$\frac{1}{(1-\theta)K} = \frac{[D_t]}{\theta} - n[P_t] \quad (2)$$



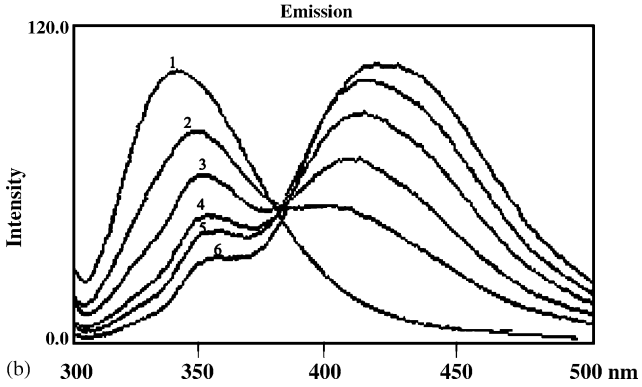


Fig. 1. (a) Fluorescence spectra of BSA (10 μM) in the presence of (a) CPF (1–0, 2–20, 3–40, 4–80, 5–100, 6–140 μM and 7-only CPF) and (b) NRF (1–0, 2–20, 3–40, 4–80, 5–100, 6–140 μM).

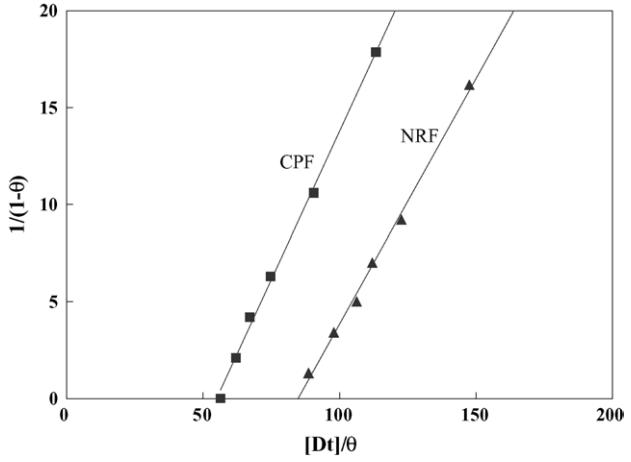


Fig. 2. The $1/(1-\theta)$ vs. $[D_t]/\theta$ plot for the binding of CPF and NRF to BSA.

where K is the association constant for drug–protein interaction, n the number of binding sites, $[D_t]$ the total drug concentration and $[P_t]$ total protein concentration. A plot of $1/(1-\theta)$ versus $[D_t]/\theta$ shown in Fig. 2. The values of K and n, obtained from the slope and intercept of such plots were found to be 2.38×10^4 and 3.09×10^4 L mol^{-1} and, 2.5 and 4.5 for CPF and NRF, respectively. Since the data fits Eq. (2) in all cases, it may be concluded that under the conditions of the experiment, all the binding sites are equivalent and independent.

3.2. Stern–Volmer analysis

The fluorescence quenching data are usually analysed by the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \tag{3}$$

where F_0 and F are the steady-state fluorescence intensities in the absence and presence of quencher (FQ), respectively. K_{SV} is the Stern–Volmer quenching constant and $[Q]$ the concentration of quencher. The Stern–Volmer quenching constant K_{SV} of BSA and tryptophan residues fluorescence by FQ at different temperatures are shown in Table 2. These results indicate that the probable quenching mechanism of fluorescence of BSA by FQ is a static quenching procedure because the K_{SV} decreased with rising temperature.

Table 2
Stern–Volmer quenching constant K_{SV} at pH 7.4

T (K)	10^{-4} K_{SV} (L mol^{-1})		R[a]	S.D.[b]
	NRF	CPF		
298	3.231	4.124	0.9985	0.0274
302	2.787	3.226	0.9976	0.0312
306	2.523	3.185	0.9979	0.0277
310	2.212	2.296	0.9982	0.0339

[a] R is the linear quotient.
[b] S.D. is the standard deviation.

3.3. The determination of the force acting between FQ and BSA

The interaction forces between drug and biomolecule may include hydrophobic force, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc. The slope of a plot of the bimolecular quenching constant versus $1/T$ (T, absolute temperature) allows one to calculate the energy change for the quenching process since,

$$\text{Log}\, K = \frac{-\Delta H^\circ}{2.303RT} + \frac{\Delta S^\circ}{2.303R} \tag{4}$$

Log K versus $1/T$ plot enabled determination of standard enthalpy change, ΔH° and standard entropy change, ΔS° for the binding process. The ΔH°, ΔS° and ΔG° values were found to be +13.94 kJ mol^{-1}, +131.7 J K^{-1} mol^{-1} and −39.78 kJ for NRF and +46.48 kJ mol^{-1}, +67.97 J K^{-1} mol^{-1} and −20.57 kJ for CPF, respectively. The positive enthalpy (ΔH°) and entropy (ΔS°) values of the interaction of FQ and BSA indicate that the bonding is mainly entropy-driven and the enthalpy is unfavorable for it, the hydrophobic forces playing a major role in the reaction [8]. These results together with spectral changes in the fluorescence emission spectra of BSA induced by CPF/NRF suggest that the interaction may take place in subdomain IA and IIA since these have been proposed to bind drugs and other hydrophobic materials.

3.4. Energy transfer between FQ and BSA

The overlap of the UV absorption spectra of FQ with the fluorescence emission spectra of BSA is shown in Fig. 3. According to Forster's non-radiative energy transfer theory, the energy transfer will happen under conditions: (a) the donor can produce fluorescence light; (b) fluorescence emission spectrum of the donor and UV absorbance spectrum of the acceptor have more overlap; (c) the distance between the donor and the acceptor is approach and lower than 7 nm. The energy transfer effect is related not only to the distance between the acceptor and the donor, but also to the critical

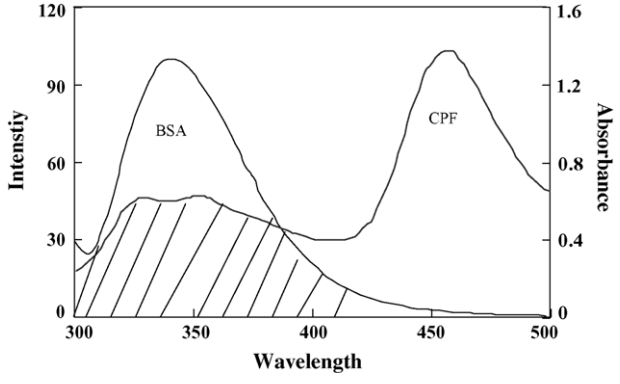


Fig. 3. Spectral overlap of FQ absorption with BSA fluorescence c(BSA) = c(CPF) = 1.0×10^{-5} mol L^{-1}.

energy transfer distance, that is

$$E = \frac{R_0^6}{(R_0^6 + r^6)} \quad (5)$$

where r is the distance between the acceptor (A) and the donor (D) and R_0 is the critical distance when the transfer efficiency is 50%, which can be calculated by

$$R_0^6 = 8.8 \times 10^{-25} k^2 \phi n^{-4} J \quad (6)$$

where k^2 is the spatial orientation factor of the dipole, n the refractive index of the medium, ϕ the fluorescence quantum yield of the donor, J the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. Therefore,

$$J = \frac{\Sigma F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\Sigma F(\lambda)\Delta\lambda} \quad (7)$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at wavelength λ and $\varepsilon(\lambda)$ is the molar absorptivity of the acceptor at wavelength λ. The energy transfer efficiency is given by

$$E = \frac{(F_0 - F)}{F_0} \quad (8)$$

J can be evaluated by integrating the spectra Fig. 3. It has been reported for BSA that, $k^2 = 2/3$, $\phi = 0.10$, $N = 1.46$. Based on these data, we found $R_0 = 2.23$ and $r = 2.48$ nm for BSA. So the distance between FQ and tryptophan residue in BSA is 2.48 nm. Obviously, they are lower than 7 nm after interaction between FQ and BSA. These accord with conditions of Forster's non-radiative energy transfer theory, indicating again the static quenching interaction between FQ and BSA.

3.5. The effect of other ions on the binding constant

The effect of common ions on the binding constants was investigated at 29 °C. The results are summarized in Table 3. It is shown that the binding constant between the protein and FQ increased in the presence of common ions, implying stronger binding between FQ and BSA. The higher binding constant obtained in the presence of metal ions might be resulted from the interaction of metal ion with FQ to form a complex, than the complex interacted with BSA. Thus, prolonging the storage time of the FQ in blood plasma and enhancing the maximum effectiveness of the drug. Therefore, in the presence of common ions FQ can be stored and removed better by BSA.

Table 3
The binding constants (L mol^{-1}) between NRF, CPF and BSA at 29 °C in the presence of common ions

Ions	Association constant	
	NRF	CPF
Na^+	3.25×10^4	3.21×10^4
K+	3.69×10^4	3.42×10^4
Ca^+	4.06×10^5	3.65×10^4
Ba^{2+}	3.95×10^4	4.25×10^4
Mg^{+2}	4.23×10^4	4.65×10^5
Al^{3+}	5.21×10^4	4.21×10^5
Cl^-	4.23×10^4	3.19×10^4
Br^-	4.25×10^4	3.69×10^4
F^-	4.96×10^5	4.23×10^4
CO_3^{2-}	5.21×10^4	4.91×10^4
SO_4^{2-}	5.06×10^4	4.16×10^5
PO_4^{3-}	4.22×10^4	4.36×10^4

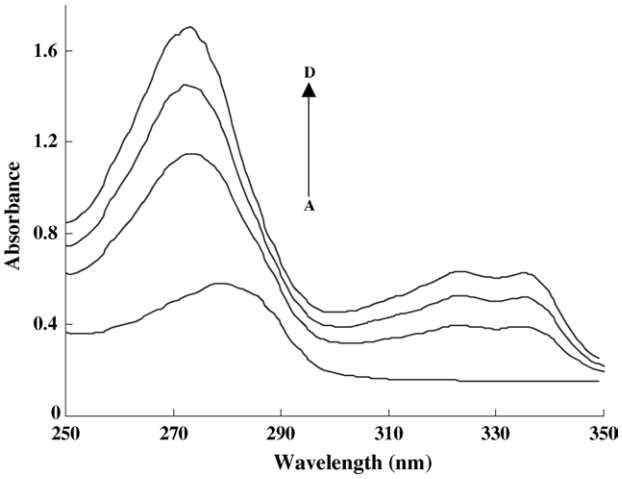


Fig. 4. UV–vis spectra of BSA in the presence of various concentrations of FQ A to D, c(BSA) = 1.0×10^{-5} mol L^{-1}; c (FQ) (10^{-5} mol L^{-1}): 0; 2.6; 3.5; 6.

3.6. Conformation investigation

To explore the structural change of BSA by addition of FQ, we measured UV–vis spectra (Fig. 4) and circular dichroic of BSA (Fig. 5) with various amounts of FQ. The UV–vis spectra of BSA at different contents of FQ show that in the visible region, the absorption peaks of these solutions showed moderate shifts towards shorter wavelength indicating that with the addition of FQ, the peptide strands of BSA molecules

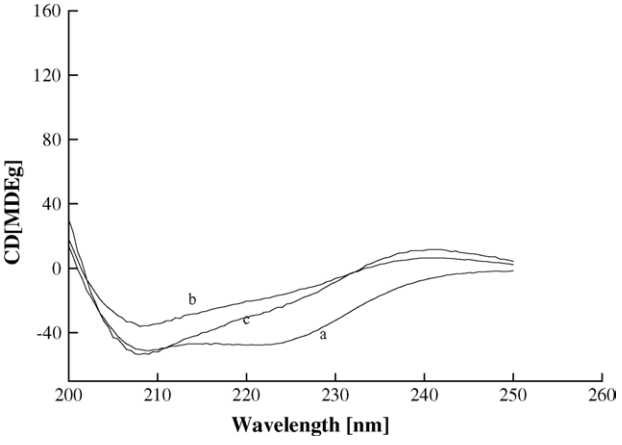


Fig. 5. Circular dichroic spectra in the 200–250 nm range; (a) BSA, 0.1 μM; (b) BSA:NRF = 1:1; (c) 1:3.

extended more and the hydrophobicity was decreased. The binding of FQ was also confirmed by circular dichroism (CD) spectra. As expected the α-helices of protein show a strong double minimum at 220 and 209 nm [9]. The intensities of this double minimum reflect the amount of helicity of BSA and further these indicate that BSA contains more than 50% of α-helical structure. Upon the addition of the NRF to BSA (1:1 and 3:1) the extent of α-helicity of the protein decreased and hence, the intensity of double minimum was reduced. This is indicative of change in helicity when the CPF is completely bound to BSA.

4. Conclusion

In this paper, the interaction of FQ with BSA was studied by spectroscopic methods including fluorescence spectroscopy, UV–vis absorption spectroscopy and circular dichroic spectroscopy. The results of synchronous fluorescence spectroscopy, circular dichroic and UV–vis spectra indicate that the secondary structure of BSA molecule is changed dramatically in the presence of FQ. The experimental results also indicate that the probable quenching mechanism of fluorescence of BSA by FQ is a static quenching procedure, the binding reaction is mainly entropy-driven, and hydrophobic interactions played a major role in the reaction.

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