Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 1046–1050
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

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

Received 3 March 2005; received in revised form 26 April 2005; accepted 11 May 2005
Available online 27 June 2005

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.

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.05.013

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

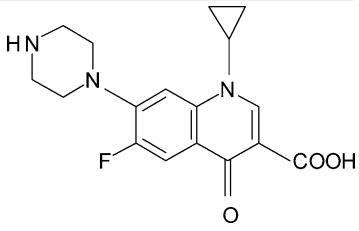
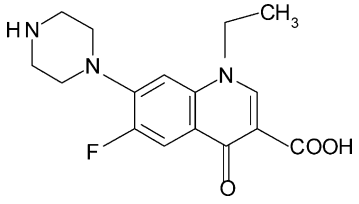
2.3.1. Drug–BSA interaction

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)	

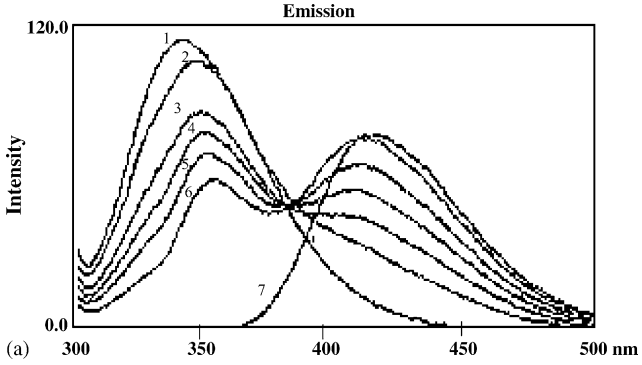
3.1. Fluorescence studies

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

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



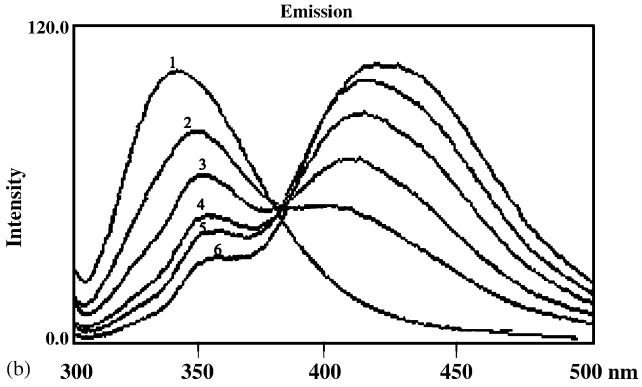


Fig. 1. (a) Fluorescence spectra of BSA (10 μM) in the presence of (a) CPF (1–0, 2–20, 3–40, 4–80, 5–100, 6–140 μM and 7-only CPF) and (b) NRF (1–0, 2–20, 3–40, 4–80, 5–100, 6–140 μM).