

## The Investigation of the Interaction between Lomefloxacin and Human Serum Albumin by Spectroscopic Methods

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**Abstract:** Mechanism of the binding of lomefloxacin (LMF) with human serum albumin has been studied at physiological pH (7.4) using fluorescence spectroscopic technique. LMF is a third-generation fluoroquinolone antibiotic that exhibits striking potency against a broad spectrum of Gram-negative and Gram-positive bacteria through inhibition of DNA gyrase. Lomefloxacin is a drug that is excreted in urine and has very variable systemic absorption. Human serum albumin (HSA) is the most important and abundant constituent of blood plasma and serves as a protein storage component. Recently, the three-dimensional structure of HSA was determined through X-ray crystallographic measurement. Fluorescence studies showed that (LMF) has an ability to quench the intrinsic fluorescence of HSA through a static quenching procedure according to the Stern-Volmer equation. LMF showed two types of binding sites, the first having a very high affinity ( $1/72 \times 10^7 M^{-1}$ ) and a secondary binding site with an affinity two orders lower than the primary site. The number of binding sites for complex: HSA-LMF at 280 nm was calculated 1 and 0.5. The microenvironment of tryptophan and tyrosin residues and more hydrophobic of fluorophores microenvironment were changed and disturbed by the blue shift in maximum wavelength and decreased in fluorescence intensity in the presence of lomefloxacin revealed decreased polarity of the fluorophores. The binding site for LMF is in a hydrophobic pocket in the sub-domain II A of HSA.

**Keywords:** Human serum albumin; Lomefloxacin; Fluorescence spectroscopy; Fluorophore; Fluoroquinolone

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### INTRODUCTION

HSA is the most abundant plasma protein, which accounts for approximately 60% of the total protein corresponding to a concentration of 40 mg ml<sup>-1</sup> (0.6 mM) in the blood. The protein contains 585 amino acids with known sequences (Quan, 2007) and a molecular weight of 66,500 Da. HSA is served as a transport carrier for a variety of small species, such as fatty acids, cations and many diverse drugs (Tang 2008, Xie 2006) present in the systematic circulation, due to its very unique single-polypeptide globular multi domain structure (Xie 2006). Furthermore, the protein renders the feasibility to bind and carries many drugs through the bloodstream, which are poorly soluble in water (Xie, 2006).

It has been shown that the distribution, free concentration, and the metabolism of various molecules can be significantly altered as a result of their binding to HSA (Yue, 2008). LMF is a third-generation fluoroquinolone antibiotic that exhibits striking potency against a broad spectrum of Gram-negative and Gram-positive bacteria through inhibition of DNA gyrase (Dufour, 2005). Lomefloxacin is a bactericidal agent with in vitro activity against a wide range of gram-negative and gram-positive organisms. The bactericidal action of lomefloxacin results from interference with the activity of the bacterial enzyme DNA gyrase, which is needed for the transcription and replication of bacterial DNA.

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The minimum bactericidal concentration (MBC) generally does not exceed the minimum inhibitory concentration (MIC) by more than a factor of 2, except for staphylococci, which usually have MBCs 2 to 4 times the MIC. Lomefloxacin has been shown to be active against most strains of the following organisms both in vitro and in clinical infections. Measurement of the quenching of the natural fluorescence of albumin is an important method for the study of its interaction with several substances (Dufour 2005). This technique can reveal the accessibility of quenchers to fluorophore groups in the protein, provide an understanding of binding mechanisms to drugs, and yield clues as to the chemistry of the binding phenomenon. The fluorescence quenching technique has been widely applied in the investigation of drug-protein binding interactions because of the presence of aromatic amino acid residues such as tryptophan.

Therefore, a study of the interaction of LMF with HSA is of major biochemical importance, and can be used as a model for an elucidation of LMF-protein complication. HSA binding can extend the metabolic half-life of a compound, providing a convenient way in which to extend the duration of the activity.

#### **MATERIALS**

HSA, LMF and potassium phosphate were all purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purifications. All reagents were used as supplied without further purification. The protein was dissolved in 50 mM phosphate buffer solutions at pH 7.4 and the stock solution was kept in the dark at 4 °C. All other reagents were of analytical grade and doubly distilled water used through out all the experiments. LMF (0.005mM) stock solution was prepared by dissolving in the same buffer and storing in a

refrigerator at 4 °C in the dark. A digital pH-meter (Metrom, Berlin, Germany) was used for pH adjustment.

#### **INSTRUMENTATION**

Fluorescence spectra were recorded on a F-2500 spectrofluorometer (Hitachi, Tokyo, Japan) linked to a personal computer and equipped with a 150-W xenon arc lamp, gating excitation and emission monochromators, and a Hitachi recorder. Slit widths for both monochromators were set at 10 nm. A 2.0-mL solution containing an appropriate concentration of plasma proteins (separately or mixed) was titrated. The excitation wavelength for Trp and Tyr was 280nm and the emission wavelength was 300–600 nm.

#### **RESULTS AND DISCUSSIONS**

The fluorescence of HSA results from the Trp, Tyr and Phe. Even so, the intrinsic fluorescence of HSA is almost totally achieved by Trp alone. The fluorescence of Tyr is almost totally quenched if it is ionized, or near a carboxyl group, an amino group or a Trp. A single tryptophan residue, Trp 214, located in the depth of subdomain IIA of HSA is largely responsible for the intrinsic fluorescence of HSA. Fluorescence spectroscopy was carried out to investigate whether LMF interacts with HSA. Fig. 1 displays fluorescence emission spectra for HSA in the presence of increasing of LMF. The fluorescence intensity of HSA decreased regularly with the increasing of LMF concentration. The observations reflect that LMF causes a decrease in the tryptophan fluorescence quantum yield of HSA. A blue shift in the fluorescence maximum also suggests a reduction in the polarity of the microenvironment. Fluorescence quenching can proceed via different mechanisms, usually classified as static quenching, dynamic quenching, and nonradioactive energy transfer. In general, dynamic and static quenching can be distinguished by the quenching constant (KSV).

According to the Hill equation, the values of  $n_1$  and  $n_2$  for LMF in the HSA complex were obtained as 0.5 and 1, respectively.

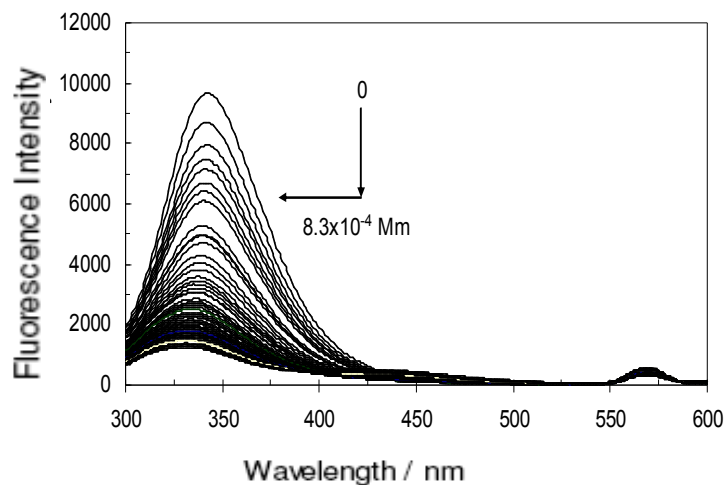


Fig. 1. Emission spectra of HSA in the presence of various concentrations of LMF at pH=7.4 and  $\lambda_{ex} = 280$  nm.

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