Studies on the energy transfer system of terbium-norfloxacin chelate and its interaction with serum albumins

XU, Yan SHEN, Han-Xi*

Department of Chemistry, Nankai University, Tianjin 300071, China

HUANG, Han-Guo

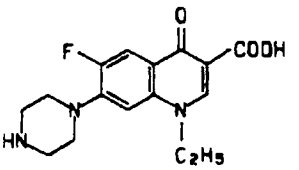
Department of Chemistry Engineering, Hebei Institute of Technology, Tangshan, Hebei 063009, China

Abstract The energy absorbed by norfloxacin could be transferred to terbium(III) through chelation of norfloxacin with terbium(III), then the characteristic fluorescence emission could be observed. The interaction of serum albumins with norfloxacin have been investigated in this paper. The results showed that HSA could inhibit the energy transfer between norfloxacin and terbium(III). But, BSA could not. It was shown that the binding properties of norfloxacin to HSA and BSA were totally different.

Keywords Norfloxacin, terbium(III), energy transfer, HSA, BSA

Introduction

Norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl) quinoline-3-carboxylic acid] (NF) is a synthetic broad-spectrum fluoroquinolone antibacterial agent for oral administration, which has activities against Gram-negative and Gram-positive bacteria.



NF

By the excitation of ultraviolet light norfloxacin emits characteristic fluorescence which has been applied to determine the drug contents in pharmaceutical preparation and biological samples.[1,2] Some lanthanides such as terbium and europium ions have their characteristic fluorescence emission because of possession of 4f electron shell. The fluorescence emission intensity may be enhanced especially when terbium or europium ions are chelated with appropriate organic ligands. The main advantages of lanthanides

[0]Received May 30, 1996; revised October 7, 1996.

[0]Project supported by the National Natural Science Foundation of China.

chelates in fluorescence spectra include large Stokes shifts, narrow emission bands and long fluorescence lifetimes.[3] These have led to numerous applications in various fields,[4] such as fluorescent labels[5] in clinical chemistry, molecular biology[6] as well as high sensitive time-resolution fluorimetric immunoassays.[7,8] In this paper the energy transfer between norfloxacin and terbium(III) in aqueous solution and interaction of serum albumins with norfloxacin-terbium chelate were studied. According to the effect of BSA and HSA on the energy transfer process different binding properties of the albumins with norfloxacin were discussed.

Experimental

Apparatus

A Hitachi-850 spectrofluorimeter was used for collection of fluorescence spectra and intensity measurements. The excitation and emission slits were set at 10 nm band pass for all measurements. A 350 nm cut-off filter was placed in front of the emission mono-chromator in order to minimize second order scatter. Fused silicon cuvettes with a 1 cm path-length were used.

A Hitachi-220A spectrophotometer was used for the absorption studies. A pHS-2 meter (Shanghai 3rd Analytical Instrumental Factory) was used for the measurements of pH values.

Reagents

Stock solution of norfloxacin (1×10^{-3} mol/L): Norfloxacin (Taiyuan Pharmaceutical Factory) (0.0319 g) was dissolved in a small amount of 0.05 mol/L HCl solution and diluted to 100 mL with distilled water. In use, it was further diluted to 5×10^{-5} mol/L.

Terbium chloride solution (6.3×10^{-4} mol/L): Terbium oxide (Yuelong Chemical Factory) (0.0294 g) was dissolved in a small amount of 1.0 mol/L HCl solution, then diluted to 100 mL with distilled water.

HSA and BSA (electrophoretic pure, MW 66000) (Blood Institute of Chinese Medical Academy, Tianjin) were used as 5×10^{-5} mol/L aqueous solution.

Ammonium acetate buffer solution (0.5 mol/L, pH 7.0) was used.

Procedure

Norfloxacin, terbium chloride and 5.0 mL of NH_4Ac buffer solutions were taken into a 25 mL volumetric flask, mixed well, then $\leq$4 mL of HSA or BSA solutions were added. Final volume was made up with distilled water. The fluorescence emission intensities of analyte solutions was recorded at the appropriate excitation and emission wavelengths of terbium-norfloxacin chelate. Rigorous clearing of the sample cuvettes was required before all measurements to avoid contamination by the analyte solutions and memory effects with terbium ions. For clearing the cuvettes were rinsed thrice with distilled

water, then three small portions of the analyte solutions were measured.

Results and discussion

Excitation and emission spectra

The terbium-norfloxacin chelate exhibits three excitation bands at 278, 320 and 334 nm, which correspond well to these in the absorption spectrum (Fig. 1). It was shown that the excitation of terbium-norfloxacin chelate originates from the energy absorption of norfloxacin itself other than the chelate with terbium ions.

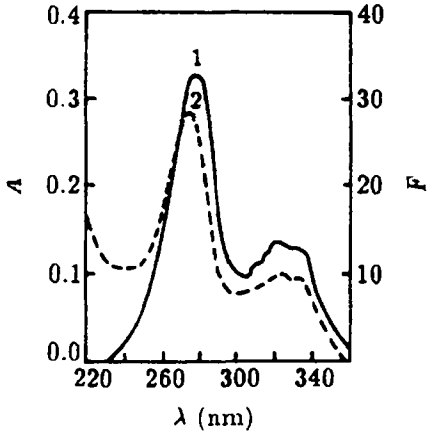


Fig. 1 Correspondence between absorption and fluorescence excitation spectra of Tb(III)-norfloxacin chelate system. 1, Excitation; 2, Absorption. [NF]=2.0×10^{-6} mol/L.

The emission spectrum of terbium-norfloxacin system is shown in Fig. 2. Besides the 415 nm emission of norfloxacin itself, the narrow emission bands of terbium ions were also observed at 489, 545 and 585 nm. Their intensities were enhanced by the formation of the terbium-norfloxacin chelate. From these results it could be concluded that the energy transfer occurs between norfloxacin and terbium ions.

The singlet norfloxacin, excited by its absorption, emits fluorescence through the transition from the lowest singlet state (S_1) to ground state, and convert to the first triplet state (T_1) through intersystem crossing (ISC) which transfers the energy to the terbium(III) $^5d_4$ state. Therefore, it exhibited the characteristic fluorescence spectrum of Tb(III).

According to the literature,[9] in the 2-naphthylacetic acid-terbium(III)-bis(2-ethylhexyl) sulfosuccinate energy transfer system, the fluorescence intensity of 2-naphthylacetic acid (energy donor) did not change in the presence of terbium(III). From Fig. 2 the fluorescence intensity of norfloxacin was decreased as the rise of characteristic emission bands of terbium ions. This difference could be considered as that the former

was intermolecular energy transfer system and the latter intramolecular.

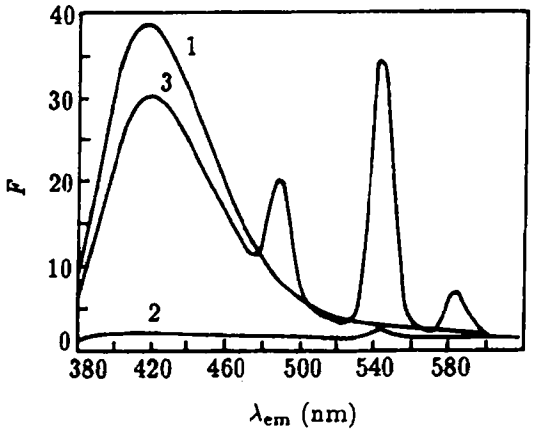


Fig. 2 Fluorescence emission spectra of Tb(III)-norfloxacin chelation system. 1, Norfloxacin; 2, terbium chloride; 3, Tb(III)-norfloxacin. [NF]=2.0×10^{-6} mol/L; [Tb(III)]=2.5×10^{-5} mol/L; λ_{ex}=320 nm.

Optimal experimental conditions

The pH effect of medium on the fluorescence intensity of Tb(III)-norfloxacin chelate showed that the maximum value of fluorescence intensity was observed at pH 7.0.

The effect of irradiation time was also tested. The solution containing norfloxacin and terbium ions was irradiated with 278 nm excitation light. Continuous irradiation for 15 min resulted in no change in the fluorescence signal. If the sample solution was kept at room temperature the signal magnitude retained at least for 24 h.

The interaction of serum albumins with terbium-norfloxacin chelate

The bovine serum albumin (BSA) and human serum albumin (HSA) have maximum absorption at 280 nm, overlapping with the 272 nm absorption of norfloxacin (Fig. 3). At 278 nm excitation wavelength of terbium-norfloxacin, the serum albumins have competitive absorption with norfloxacin and quenched the fluorescence of terbium-norfloxacin by the "inner filtering effect".[10,11] In order to investigate the chemical interaction of the two albumins with terbium-norfloxacin, 320 nm of excitation wavelength was taken. The emission spectra of terbium-norfloxacin in the presence of BSA and HSA were scanned and shown in Fig. 4.

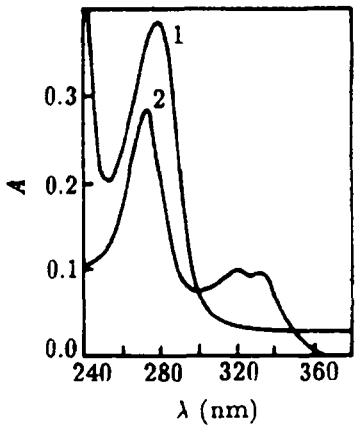


Fig. 3 Absorption spectra of BSA (1) and norfloxacin (2). [BSA]=4.0×10^{-6} mol/L; [NF]=4.0×10^{-6} mol/L.

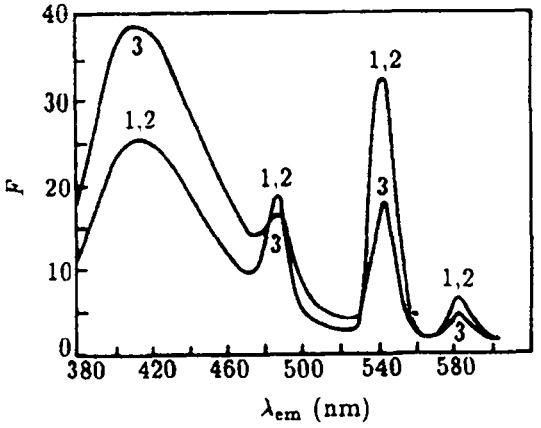


Fig. 4 The effect of serum albumins on the emission spectrum of Tb-norfloxacin chelate. 1, [NF]=2.0×10^{-6} mol/L; [Tb(III)]=2.5×10^{-5} mol/L. 2, idem(1); [BSA]=8.0×10^{-6} mol/L. 3, idem(2); [HSA]=8.0×10^{-6} mol/L.

It could be seen from Fig. 4 that in the presence of HSA, the characteristic emission intensity (545 nm) of terbium ions decreased, but the fluorescence intensity of norfloxacin (415 nm) increased appropriately. It was proved that HSA inhibited the energy transfer process. In the presence of BSA no fluorescence change occurred for both terbium ions (489, 545 and 585 nm) and norfloxacin (415 nm). So it was suggested that HSA and BSA possessed different binding sites with norfloxacin.

On HSA, there were specific binding sites called site I (warfarin site) and site II (diazepam site).[12,13] The two binding regions on HSA were revealed by crystallography that they existed in subdormin II A and subdormin III A, respectively.[14] Since there was approximately 80% homology of the amino acids sequences between HSA and BSA,[15,16] BSA was thought to have binding sites similar to those of HSA. But in the studies on

the binding of carprofen to HSA and BSA,[17] it was found that HSA had two major sites, Site I and Site II, whereas BSA had a single primary site, Site II. It was thought that the difference of binding characteristic was caused by the variation in hydrophilic and hydrophobic amino acids residues at binding sites between HSA and BSA.

The energy transfer took place through the chelation of norfloxacin with terbium(III) ions via carboxylate moiety, 4-keto functional group. At binding sites of HSA the carboxyl of norfloxacin might be caught by the basic amino acid residues, which prevented the chelation of norfloxacin with terbium ions and inhibited the energy transfer process. On BSA, the amino acid residues at binding of drug might be through the interaction of other functional group or just hydrophobic combination.

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