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Fluorescence quenching study of moxifloxacin interaction with calf thymus DNA

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Abstract: Moxifloxacin (MOX) is a fourth-generation synthetic fluoroquinolone antibacterial agent with many important therapeutic properties. Fluorescence quenching was used to study the interaction of MOX with calf thymus DNA (ct-DNA) in aqueous solution. The intercalative binding mode and a static quenching mechanism were confirmed by the Stern–Volmer quenching rate constant (K_q) of 3.48 × 10^{11} M^{-1} s^{-1} at 298 K. The thermodynamic parameters (ΔH = –118.4 KJ mol^{-1} and ΔS = –299.4 J mol^{-1} K^{-1}) were calculated at different temperatures, and they indicate that the main forces between MOX and ct-DNA are hydrogen bonding and Van der Waals force. We proved at the same time the presence of one single binding site on ct-DNA, and the binding constant is 1.28 × 10^5 M^{-1} at physiological pH. The results may provide a basis for further studies and clinical application of antibiotics drugs.

Key words: Moxifloxacin, ct-DNA, fluorescence quenching, binding

1. Introduction

As the most common genetic material of various biosomes in nature, deoxyribonucleic acid (DNA) plays a key role in different kinds of vital processes including gene expression, mutagenesis, cell death, etc. An increasing number of studies have suggested that there is an essential linkage between environmental factors (chemical drugs, nuclear radiations, etc.) and genetic damage.[1] Increasing attention has been paid to the study of molecular interactions between DNA and many drugs.[2–4] Studying the binding mechanism is of great importance in terms of life science, chemicals, pharmaceuticals, and clinical medicines.

The fluoroquinolones are one of the most useful types of synthetic antibacterial agents due to their broad spectrum of activity against gram-positive and gram-negative bacteria and mycoplasma pneumoniae.[5–7] Because of this, fluoroquinolones are used in the treatment of bacterial infections, including respiratory, soft tissue, urinary tract, and joint infections. Moxifloxacin (MOX), with fewer side effects and extended half-life, is a novel fourth-generation quinolone antimicrobial drug. Its chemical structure is shown in Figure 1. The molecular interactions between quinolones and bovine serum albumin (BSA) have been investigated successfully in our previous work.[8,9] The major binding mode of sinafloxacin with ct-DNA has been investigated.[10] As far as we know, the interaction between MOX and ct-DNA has not been investigated. MOX, however, is not limited to clinical applications. It is also widely used in the treatment and prevention of veterinary diseases in animals intended for human consumption and commercially farmed fish;[11] hence the interaction of MOX with ct-DNA is worthy of further study.

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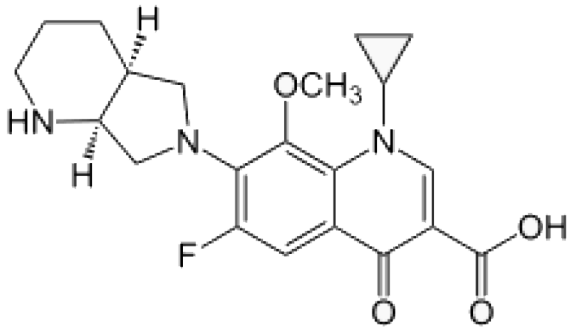

Figure 1. Chemical structure of MOX.

Consequently, this study is expected to provide important insight into the essence and the potential toxicity between drugs and ct-DNA in realistic situations and may also provide an available clinical reference for future combination and therapy guidelines for the development of new low toxicity and more efficient drugs. What is more, this study will contribute to our understanding of the interaction mechanism and the reason for the difference in the biological activity and clinical efficacy of MOX and its analogues.

2. Experimental

2.1. Materials and reagents

Commercially prepared calf thymus DNA (ct-DNA, purity $>$99.0%) purchased from Sigma Chem. Co. was dissolved in water. The concentration of ct-DNA solution was determined by UV absorbance at 260 nm using molar extinction coefficient $\varepsilon_{260\,nm} = 6600\ \mathrm{cm}^{-1}\ \mathrm{M}^{-1}$. Mox (CAS#, 151096-09-2) was obtained from Sigma (purity grade inferior 99.9%). Stock solutions of 2×10^{-3} M were prepared by dissolving the appropriate amount of the drug with double distilled water and then diluting to the mark of a 100-mL volumetric flask. Ethidium bromide (EB, CAS#, 1239-45-8) was purchased from Sigma (purity grade superior 99%). Exactly 11.6 mg of EB was dissolved with double distilled water and diluted to the mark of a 50-mL volumetric flask. The concentration of solution was 5.89×10^{-4} M. Tris (hydroxymethyl) aminomethane hydrochloride (Tris–HCl, pH 7.4) was used as standard buffer solution, the concentrations of HCl and NaCl were 0.6 M and 0.2 M, respectively, and double distilled water was used for all solution preparation. All the solutions were kept at 277 K. The excitation and emission wavelengths for MOX were 291 nm and 462 nm, respectively, and the excitation and emission slit widths were set at 10 nm.

2.2. Apparatus and instruments

All fluorescence spectra in our work were recorded with a Shimadzu RF-540 spectrofluorophotometer and a Hitachi F-4500 spectrofluorophotometer. All the reactions were kept at constant temperature with a water bath (CS501).

2.3. The effect of temperature on the fluorescence of MOX-ct-DNA

This part was conducted by fixing the concentration of MOX and pH 7.4 Tris–HCl while varying the ct-DNA concentration. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 25 min at 298 K, 308 K, and 313 K.

2.4. The effect of KI or NaCl on the fluorescence of MOX-ct-DNA

The salt effect experiments were conducted by adding various amounts of NaCl to MOX-ct-DNA mixture. Iodide quenching experiments were carried out by adding various concentrations of potassium iodide stock solution to MOX and MOX-ct-DNA mixture, respectively. The experiments were conducted at 298 K.

2.5. The competitive effect of EB on the fluorescence of MOX-ct-DNA

Fixed concentrations of EB and ct-DNA and varied concentrations of MOX were added to color comparison tubes. The samples were kept static for 25 min at 298 K.

3. Results and discussion

3.1. Fluorescence quenching studies

A variety of molecular interactions can lead to reduction of the fluorescence intensity of fluorescent substances, such as excited-state reactions, molecular rearrangements, energy transfer, and ground-state complex formation.[12,13] Such a decrease in intensity in these processes is called fluorescence quenching. K_{sv} is automatically used to evaluate fluorescence quenching efficiency. According to the classical Stern–Volmer equation (1):[14]

$$\frac{F_0}{F} = 1 + K_{sv}\,[Q] = 1 + K_q \tau_0\,[Q]\,, \tag{1}$$

where F_0 and F are the fluorescence intensities at 462 nm before and after the addition of the quencher, respectively. $[Q]$ represents the concentration of the quencher, K_{sv} is the quenching constant of the reaction, τ_0 is the fluorescence lifetime without quencher, the average life of fluorescence molecule is 10^{-8} s,[15] and K_q is the bimolecular quenching rate constant.

Figure 2 shows the characteristic changes in fluorescence emission spectra during the interaction of MOX with ct-DNA. The concentration of MOX (0.5 μM) was fixed and the concentration of ct-DNA was varied in assay solutions. The results show that the fluorescence intensity of MOX at 462 nm is decreased dramatically without a shift in the emission wavelength. This quenching spectrum is evidence of a direct interaction between MOX and ct-DNA.[10]

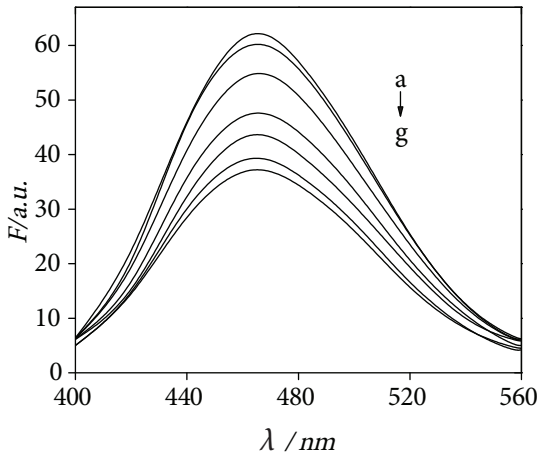

Figure 2. Fluorescence spectra of MOX-ct-DNA system (T = 298 K). $C_{MOX} = 0.5 \times 10^{-6}$ M, C_{ct-DNA} (a→g) = (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4) $\times$ 10^{-4} M.

As is well known, there are 2 quenching processes: static and dynamic quenching. In the former process, the fluorescence intensity of a given fluorophore is quenched by forming a nonfluorescent complex with a quencher molecule, and the excited energy transfer by collision in the latter process.[16,17] They can be distinguished by their different temperature dependence. Higher temperatures can lead to faster diffusion and extended collisional quenching, and so K_{sv} increases along with increasing temperature. For static quenching, higher temperatures will typically cause the dissociation of weak-bound complexes, and so K_{sv} decreases with increasing solvent temperature.[18] The nature of the quenching process was deduced by comparison of the different behavior of the Stern–Volmer plots at 298 K, 308 K, and 313 K. The Stern–Volmer graphs are shown in Figure 3.

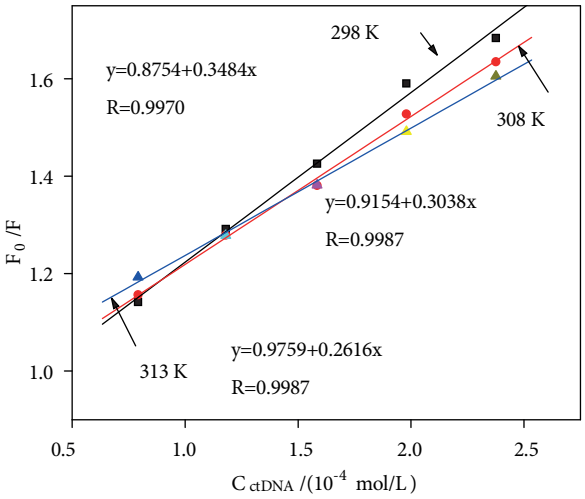

Figure 3. Stern–Volmer curves of MOX-ct-DNA system at different temperatures. $C_{MOX} = 0.5 \times 10^{-6}$ M, C_{ct-DNA} = (0.8, 1.2, 1.6, 2.0, 2.4) $\times 10^{-4}$ M.

The results show that the Stern–Volmer quenching rate constant K_q is inversely correlated with temperature; $K_{298\,K} = 3.48 \times 10^{11}$ M^{-1} s^{-1}, $K_{308\,K} = 3.04 \times 10^{11}$ M^{-1} s^{-1}, and $K_{313\,K} = 2.61 \times 10^{11}$ M^{-1} s^{-1}, all these statistics are greater than the diffusion rate constant (2.0×10^{10} M^{-1} s^{-1}) of all kinds of quenchers on the fluorescent molecule. What is more, K_q decreases with increasing solvent temperature. These results show that the probable quenching mechanism of MOX-ct-DNA is initiated by forming a nonfluorescent complex instead of dynamic collision according to the literature discriminant method.[19]

3.2. The composition of the binary complex

In terms of the mechanism, it is generally accepted that small molecules are bound to large biological molecules by 3 binding modes: intercalative binding, electrostatic binding, and groove binding.[20] The process of electrostatic binding involves cationic species reacting with the negatively charged ct-DNA phosphate backbone in the external ct-DNA double helix, and this kind of binding mode has low selectivity. The intercalation binding was first proposed by Lerman in 1961.[21] Typically intercalative small molecules with a planar aromatic system can insert into between 2 adjacent base pairs in a helix. In groove binding, direct hydrogen bonding or Van der Waals force is always formed between the t2 grooves of the ct-DNA double helix generally.[22]

If the static binding reaction exists, and if the binding capability of ct-DNA is equal at each binding site, then the composition of the binary complex can be deduced from Eqs. (2) and (3):[16,23]

$$M + nL = MLn \tag{2}$$

$$\lg\frac{F_0 - F}{F} = \lg K_a + n\lg(C_{DNA}) \tag{3}$$

M is the quencher, L is the pharmaceutical molecule with fluorescence, ML_n is the binary complex of which the binding constant is K_a, and F_0 and F are the fluorescence of the pharmaceutical molecules without and with ct-DNA, respectively. As shown in Figure 4, the plot of lg $[(F_0 - F)/F]$ vs. lg $[M]$ gives a straight line at different temperatures, and the binding stoichiometry (n) and lg K_a are calculated from the slope and y-axis intercept, respectively. The fluorescence titration data are well filled to Eq. (3) and can infer both the binding constant (K_a) and the binding stoichiometry (n) for the complex formation of MOX with ct-DNA. The values of K_a and n are found to be 1.28 $\times$ 10^5 M^{-1} and 0.99 at 298 K, respectively. The result indicates that MOX can form a stable 1:1 complex with ct-DNA. As we all know, EB is embedded in parallel into the ct-DNA double helix typically and the K_a of MOX-ct-DNA and that of EB-ct-DNA (4.94 $\times$ 10^5 M^{-1}) belong to the same order of magnitude. Therefore, the binding mode of MOX with ct-DNA should be intercalative.[24] Further, the K_a (2.71 $\times$ 10^4 M^{-1}) at 308 K and that (9.18 $\times$ 10^3 M^{-1}) at 313 K were less than that at 298 K, which proved further that the quenching mechanism of the MOX-ct-DNA binding reaction is static.

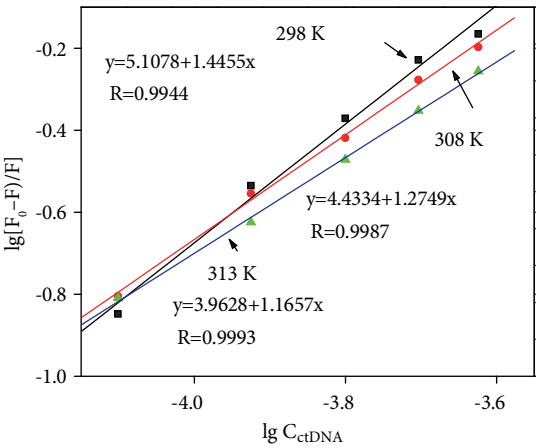

Figure 4. Curves of lg$[(F_0 - F)/F]$ vs. lg(C_{ct-DNA}) for MOX-ct-DNA system at different temperatures. C_{MOX} = 0.5 $\times$ 10^{-6} M, C_{ct-DNA} = (0.8, 1.2, 1.6, 2.0, 2.4) $\times$ 10^{-4} M.

3.3. Analysis of data and obtaining thermodynamic parameters

Given the temperature-dependent equilibrium constants (K_a) for the ct-DNA-quinolone complex formation, the entropy, enthalpy, and Gibb's free energy at 298 K can be easily obtained from the fundamental thermodynamic relationships coupled with the van't Hoff equation (4) and (5).[25]

$$R\ln K_a = \Delta S - \frac{\Delta H}{T} \tag{4}$$

$$\Delta G = -RT\ln K_a = \Delta H - T\Delta S \tag{5}$$

The thermodynamic parameters, namely the enthalpy change ΔH, the entropy change ΔS, and Gibb's free energy, can be calculated from the above equation; R denotes molar gas constant. ΔH and ΔS for the

association of MOX with ct-DNA can be obtained according to the basic thermodynamic relation. For the association of MOX with ct-DNA, the plot of $RlnK_a$ vs. 1/T gives a straight line and the ΔH and ΔS are calculated from the slope and y-axis intercept, respectively. Then we found that ΔS was –349.98 J mol^{-1} K^{-1} and ΔH was –133.55 KJ mol^{-1}. Previous research[26] showed that ionic and hydrophobic interactions are characterized by positive ΔS and ΔH; the nonbonded (Van der Waals) interactions and hydrogen-bond formation in low dielectric media and protonation accompanying association are characterized by predominantly negative ΔS and ΔH, and electrostatic interactions are characterized by positive ΔS, ΔH $\approx$ 0. Therefore, we can conclude that the main force between MOX and ct-DNA is hydrogen bonding or Van der Waals force.

3.4. Effect of ionic strength on the fluorescence properties

Studying the ionic strength effect is also an efficient method to determine the binding mode of molecules-ct-DNA. Increasing the cation concentration will add to the combination probability between cations and ct-DNA phosphate backbone, and weaken the surface-binding mode of interaction between ct-DNA and MOX due to competition for phosphate anion.[21,27] It is obvious that both intercalative binding and groove binding react with the groove in the ct-DNA double helix, but the electrostatic binding can take place out of the groove. In our experiments, the fluorescence of the MOX-ct-DNA ($C_{MOX-ct-DNA}$ = 0.5 μM) system with gradually increasing NaCl concentration (0 $\sim$ 0.06 M) was studied. The results showed that the fluorescence intensity ratio underwent no significant change when we increased the concentration of NaCl. Such data indicated that the interaction between MOX and ct-DNA was not surface-binding mode.

3.5. Iodide quenching studies

Compared with the intercalation, groove binding exposes much more the bound MOX to the solvent surrounding the helix, and so the iodide quenching experiment was chosen to further decide the ct-DNA binding affinity of MOX. Iodide ions are effective collision fluorescence quenchers for small fluorescent molecules like MOX. The binding mode of the small molecule with ct-DNA can be deduced from the different behavior of the fluorescence with and without the presence of ct-DNA. If MOX is intercalated into the helix stack, it should be protected from the iodide by DNA, owing to the base pairs located above and below the intercalator and plus the electrostatic repelling between ct-DNA phosphate backbone and the iodide anions.[28] The magnitude of K_{sv} of the free MOX should be much higher than that of the bound MOX.[29,30] However, groove binding should provide much less protection for the chromophore, and iodide anions can still quench its fluorescence. The quenching behaviors of KI in the MOX-ct-DNA system are shown in Figure 5. In aqueous solutions, iodide quenched the fluorescence of MOX efficiently. The K_{sv} values (13.9185 M^{-1}) of MOX-iodide anions with the presence of ct-DNA decreased slightly, which indicated that MOX could be partly protected. Therefore, the binding mode of MOX with ct-DNA should be intercalative.

3.6. EB competitive experiment

EB, a very convenient fluorescence spectroscopic probe of ct-DNA conformation, is a planar aromatic dye that readily intercalates between the base pairs of ct-DNA,[31] and because of this feature EB is widely used to study the binding properties of other species to ct-DNA through competition studies.[32] EB itself has weak fluorescence just like ct-DNA, and a compound with strong fluorescence would be formed once EB was parallel embedded into the ct-DNA double helix.[33] Energy that excites EB to emit fluorescence is derived from 2 sources. One is that nucleic acid absorbs UV light at 260 nm and then transfers the energy to EB. The other one is that bound

EB absorbs UV light at 300 nm and 360 nm. All of the above energy will excite EB to emit red fluorescence, which lies in the red–orange region of the visible spectrum, emitting fluorescence at 587 nm. The fluorescence of bound EB is stronger than that of free EB.

If the binding mode of MOX with ct-DNA is intercalative, then the fluorescence intensity of EB-DNA will decrease with the mixing of MOX with EB-DNA. This phenomenon can be explained as competition between MOX and EB for absorption sites in DNA. As shown in Figure 6, the fluorescence intensity of EB-ct-DNA dropped when adding increasing amounts of MOX to the solution. Since MOX was shown not to quench the fluorescence of EB, we can come to the conclusion that the binding mode of MOX with ct-DNA is intercalative.

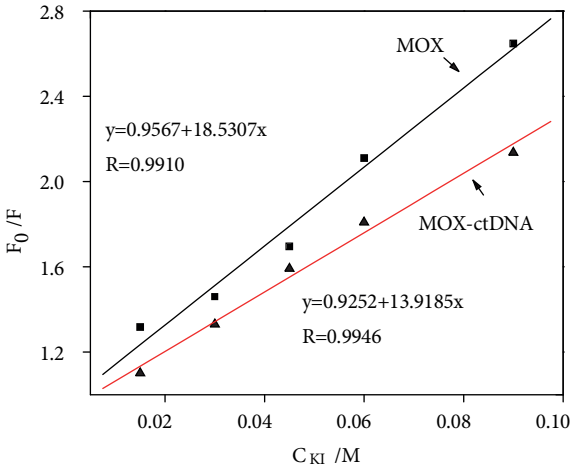

Figure 5. Effect of KI on the fluorescence intensity of MOX and MOX-ct-DNA. C_{MOX} = 0.5 × 10^{-6} M, C_{ct-DNA} = 4.0 × 10^{-5} M, C_{KI} = (1.5, 3.0, 4.5, 6.0, 9.0) × 10^{-2} M.

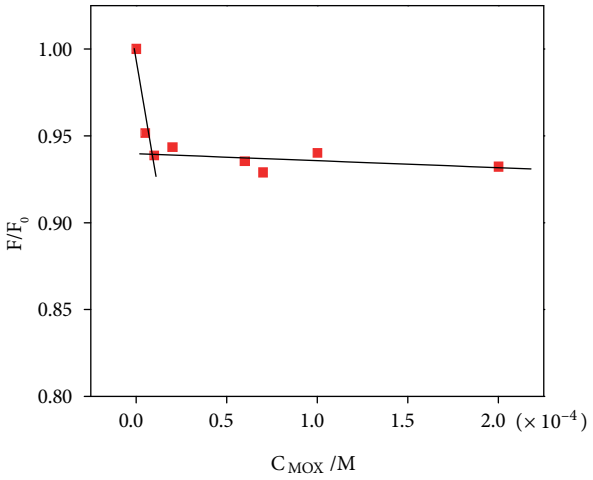

Figure 6. Effect of EB on the fluorescence intensity of MOX-ct-DNA (λ_{ex} = 524 nm, λ_{em} = 587 nm). C_{EB} = 2.9 × 10^{-6} M, C_{ct-DNA} = 1.0 × 10^{-5} M, C_{MOX} = (0.00, 0.05, 0.10, 0.20, 0.60, 0.70, 1.00, 2.00) × 10^{-4} M.

4. Conclusions

We have investigated the interaction mechanism of MOX and ct-DNA by fluorescence spectroscopy. Moxifloxacin binding with ct-DNA formed a new nonfluorescent complex, which caused static quenching. The binding constant of this binary system was 1.28 × 10^5 M^{-1} (298 K). Hydrogen bonding or Van der Waals force played an important role in the conjugation reaction between ct-DNA and MOX. In addition, 3 experiments (effect of ionic strength, quenching experiment of I^-, and the competition of EB) indicated that the conjugation mechanism could be considered as MOX embedded into 2 adjacent base-pairs of ct-DNA.

Acknowledgments

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