J Fluoresc (2008) 18:701–706
DOI 10.1007/s10895-008-0342-1

ORIGINAL PAPER

Interaction between Pazufloxacin and DNA Mediated by Copper(II) Ions

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Received: 26 November 2007 / Accepted: 30 January 2008 / Published online: 22 February 2008

Abstract The influence of copper(II) ions on the binding of Pazufloxacin (PFLX), which is a forth generation quinolone antimicrobial agent, to calf thymus DNA (CTDNA) has been investigated by fluorescence, absorption spectroscopy and viscosity measurement. The results show that PFLX and Cu^{2+} can form binary complex with mole ratio 2:1, PFLX and CTDNA can form complex mediated by Cu^{2+}. A ternary system of PFLX–Cu^{2+}–DNA were proposed. The experimental results show that the PFLX binds to CTDNA by two kinds of binding modes, intercalative mode and groove binding mode.

Keywords Pazufloxacin · Calf thymus DNA · Fluorescence · Copper(II) ion

Introduction

Quinolones are a group of extremely potent synthetic antibacterial agents which are widely used for the treatment of many infections in clinical practice [1]. Compared with the first- (nalidixic acid, cinoxacin), second- (norfloxacin, ciprofloxacin) and third- (sparfloxacin, gatifloxacin) generation, the forth generation quinolones (pazufloxacin, balofloxacin) show a much broader spectrum of activity providing expanded gram-negative and gram-positive activity coverage as expanded activity against atypical pathogens [2]. The interaction of metal ions with diverse first, second and third-generation quinolones as ligands, and the influence of the metal ions on the binding between the quinolones and DNA have been studied [3–6]. Pazufloxacin (PFLX; Fig. 1) is a forth-generation quinolone antibacterial drug, which has broad-spectrum, potent antibacterial activity and lower toxicity than conventional quinolone antibacterial agents [2].

It is known that quinolones suppress cell growth by inhibiting one step of the multi-step activity of the enzyme DNA gyrase, which can introduce super-coils into closed-circular DNA using the free energy of ATP hydrolysis [7–8]. Although the exact mechanism of this action is still unclear, there is an evidence that quinolones interact directly with DNA in synergy with the gyrase enzyme [9–10]. Such interaction between quinolones and DNA undoubtedly contributes to the desired antibacterial activity, but it can also be responsible, at least in part, for the unwanted toxic effects [6]. Contributions to deeper insight into the mechanism of interaction of this class of antibiotics with DNA might be important for a better understanding of their therapeutic efficacy.

Shen et al. [11–12] proposed a cooperative quinolone–DNA binding model mediated by Cu^{2+} for the inhibition of DNA gyrase. Palu et al. [13] stressed the role of Mg^{2+} in the quinolone–DNA interaction, and proposed a model for the quinolone–DNA–Mg ternary complex, in which the Mg^{2+} acts as a bridge between the phosphate groups of

Electronic supplementary material The online version of this article (doi:10.1007/s10895-008-0342-1) contains supplementary material, which is available to authorized users.

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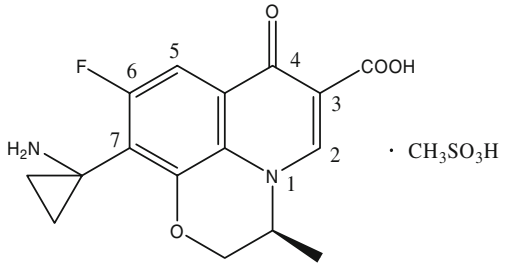

Fig. 1 Chemical structure of PFLX

nucleic acids and the carbonyl and carboxyl moieties of norfloxacin. Son et al. [9] found that norfloxacin bound to double-stranded calf thymus DNA (CTDNA) without requiring any mediation from ATP and Mg^{2+}. Ulrih and colleagues [14] investigated Mg^{2+} and Cu^{2+} mediated ciprofloxacin binding to DNA, and revealed different models of action of ciprofloxacin in the presence of Mg^{2+} and Cu^{2+}.Guo et al.[15] discussed the relationship between the binding mode of metal ions to DNA and the influence ability of metal ions on the binding between quinolones and DNA, and revealed the binding between sparfloxacin and the phosphate groups was the precondition for the combination between sparfloxacin and DNA, which is stabilized through stacking interactions between the condensed rings of the drug and DNA bases.

Although the influence of metal ions on the binding between quinolones and DNA has been studied for many years, the action of metal ions in the interaction between quinolones and DNA is still unclear. The interaction between metal ions and PFLX and the influence of metal ions on the binding between PFLX and DNA have not been reported yet. Fluorescence and UV–vis absorption spectroscopies are powerful tools for the reactivity of chemical and biological systems. We here used a combination of spectroscopic techniques (fluorescence and UV–vis absorption spectroscopies) and viscosity measurement to investigate the binding reaction between PFLX and CTDNA without and in the presence of copper(II) ions.

Experiments

Reagents

PFLX was provided by Hangzhou Greenda Chemistry Co. Ltd., China, CTDNA was purchased from Beijing Solarbio Science & Technology Co. Ltd., China. All reagents used were of analytical grade unless otherwise stated and doubly distilled water was used throughout the work. The samples were dissolved in Tris–HCl buffer solution (0.05 M Tris, 0.10 M NaCl, pH=7.4).

5.1 mg CTDNA was weighed up and dissolved to a 10-ml volumetric flask, then diluted to the scale with double-distilled water, and stored at about 0 °C. The stock solution was further diluted as working solution prior to use. Twenty-four hours or more were needed for dissolving DNA with occasional gentle shaking. The concentrations of stock solutions of nucleic acids were determined by the absorbance at 260.0 nm.

General methods

All fluorescence spectra were recorded on RF-5301PC spectrofluorimeter with a 1×105-cm cross-section quartz cell (Sahimadzu, Japan). A SPECORD 200 recording spectrophotometer (Jena, Germany) equipped with 1.00 cm quartz cell was used for scanning the UV–vis spectra.

In a dry 50 ml volumetric flask, 1 ml of 1.0×10^{-3} M PFLX solution and 4.0 ml of Tris–HCl solution were added. The mixture was diluted to 50 ml with doubly distilled water. Then the 1.0×10^{-3} M $CuCl_2$ solution was gradually added to the volumetric flask by means of a micro-injector, respectively. The concentration of PFLX was kept fixed at 2.0×10^{-5} M and the concentration of $CuCl_2$ solution was varied from 005 to 2.2×10^{-5} M, respectively.

The fluorescence spectra were measured with the following settings of spectrofluorimeter: excitation wavelength (λ_{ex}), 327 nm; emission wavelength (λ_{em}), 401 nm; both the excitation and emission slits were 1.5 nm in width at room temperature. Appropriate blanks corresponding to the buffer were subtracted to correct background fluorescence.

The UV–vis spectra were referenced versus a 1.0-cm quartz cell filled with Tris–HCl buffer solution. The absorbencies were read and spectral scanning curve was made.

Viscosity experiments used a Ubbelodhe viscometer, immersed in a thermostated water bath maintained at 30.0± 0.1 °C. Relative viscosities for CTDNA in the presence and absence of PFLX were calculated from the relation $\eta = (t - t_0)/t_0$, where t_0 is the buffer (Tris–HCl solution) flow time and t is the observed flow time.

Results and discussion

The influence of Cu^{2+} on fluorescence properties of PFLX

PFLX has very intense intrinsic fluorescence in aqueous solution at $\lambda_{ex}/\lambda_{em}$=327/401 nm. If the concentration of PFLX was constant, while the concentration of copper(II) ions was different and on the increasing, the fluorescence

spectra obtained was shown in Fig. 2. The results show the fluorescence intensity of 2.0×10^{-5} M PFLX solution at 401 nm dropped regularly with the increasing concentration of copper(II) ions. This may be due to a strong interaction between PFLX and copper(II) ions. Otherwise, the absorbance spectra of the binary system were studied. Electronic spectra of PFLX exhibited two bands with maximum wavelength at 250 and 330 nm, respectively. On addition of copper(II) ions, the maximal characteristic absorbance peak of PFLX located at 250 nm increased in the intensity. The above results indicate that there was a strong interaction between PFLX and copper(II) ions, and a non-fluorescent complex may be formed between PFLX and copper(II) ions.

Fluorescence quenching refers to any process in which the fluorescence intensity of a given fluorophore decreases upon adding a quencher. Assuming that the fluorescence intensity of a fluorophore–quencher complex is negligible compared to an unquenched fluorophore, the intensity in the presence (F) and absence (F_0) of the quencher is expressed by the Stern–Volmer equation [6]:

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

Where $[Q]$ is the concentration of the quencher, K_{SV} is the Stern–Volmer constant, which is the equilibrium constant of the complex formation in the static quenching process. If a system obeys the Stern–Volmer equation, a plot of F_0/F versus $[Q]$ will give straight line with a slope of K_{SV} and y-axis intercept.

Figure 3 is the Stern–Volmer plot of the PFLX–Cu^{2+} system. No changes in λ_{max} in the fluorescence emission spectra of PFLX at different concentration of Cu^{2+}, and fluorescence intensity of PFLX is decreased in the presence of Cu^{2+}.As show in Fig. 3, the term, $(F_0/F)-1$, linearly increases with increasing the concentration of the quencher Cu^{2+}, and the coefficients are 0.9959. The observed values of Stern–Volmer constant K_{SV} suggest that PFLX has a high binding affinity for Cu^{2+} ($K_{SV}=4.57\times10^4$ M^{-1}).

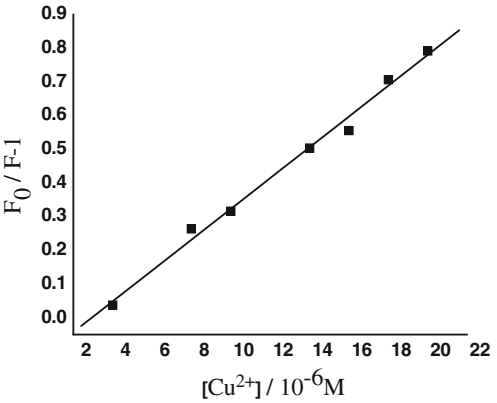

Fig. 3 Fluorescence quenching Stern–Volmer plots of PFLX with increasing concentration of Cu^{2+}, [(PFLX)]=2.0×10^{-5} M, [(Cu^{2+})]/10^{-5} M, (1→7)=0, 0.4, 0.8, 1.2, 1.6, 1.8, 2.2 (T=298 K)

The above results indicate that there is a strong interaction between PFLX and Cu^{2+}, and a non-fluorescent complex may be formed between PFLX and Cu^{2+}.

The composition of the binary complex

The composition of the binary complex can be deduced from the following formulae [6].

$$M + nL = ML_n \quad (2)$$

$$\log[(F_0/F) - 1] = \log K_a + n\log[M] \quad (3)$$

Where M is the quencher (metal ion), L is the drug molecule with a fluorophore, ML_n is binary complex whose resultant constant is K_a. A plot of $\log[(F_0/F) - 1]$versus $\log[M]$ will give straight line with a slope of n and y-axis intercept, $\log K_a$.

Figure 4 is obtained by keeping the PFLX concentration (2.0×10^{-5} M) constant and changing the concentration of Cu^{2+}. The data are well fitted to Eq. 3 and the slope is 1.8,

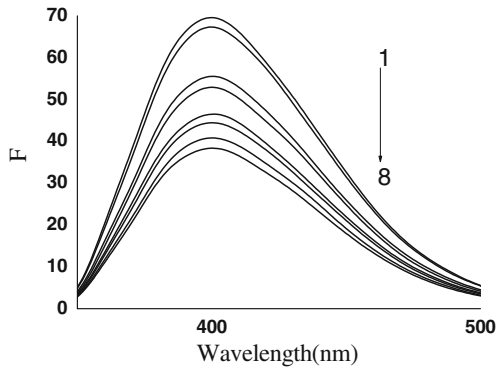

Fig. 2 Emission spectra of PFLX in the presence of various concentrations of Cu^{2+}, c(PFLX)=2.0×10^{-5} M, c(Cu^{2+})/10^{-5} M, (1→8)=0, 0.4, 0.8, 1.0, 1.4, 1.6, 1.8, 2.0 (T=298 K)

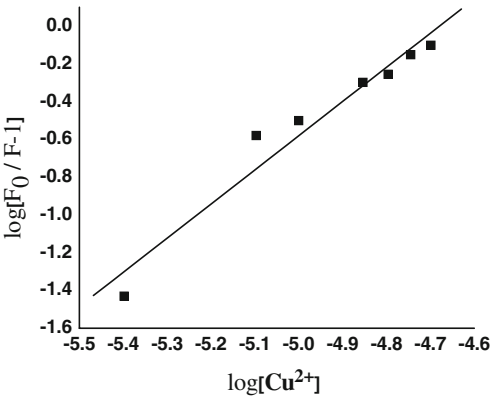

Fig. 4 Estimation of the composition of the PFLX–Cu^{2+} complex, λ_{ex}=327 nm: [PFLX]=2.0×10^{-5} M

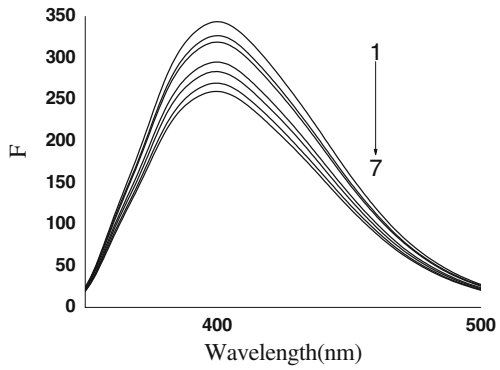

Fig. 5 Fluorescence emission spectra of PFLX at different concentration of CTDNA, λ_{ex}=327 nm: [PFLX]=1.0×10^{-5} mol l^{-1}, [DNA] (1→7) (10^{-5} M)=0, 0.8, 1.6, 2.4, 3.2, 4.0, 4.8

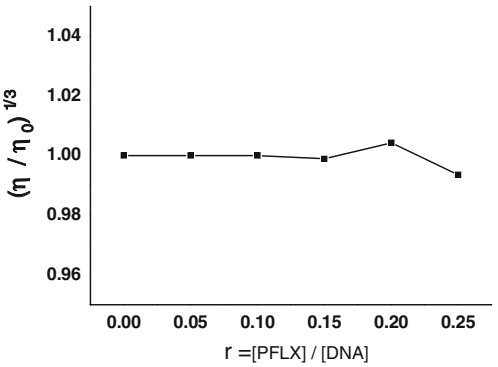

Fig. 7 The effect of PLFX on the viscosity of DNA solution

the correlation coefficient is 0.9747, respectively. The result indicates that PFLX can form a stable 2:1 complex with Cu^{2+}. Crystal structures of copper–quinolone complexes show that quinolones can form stable 2:1 complexes with Cu^{2+} and metal atoms are usually bonded to the oxygen atoms of carbonyl and carboxylic groups of the quinolone and exhibit octahedral, or square pyramidal geometry, with counter ions or additional water molecules in remaining coordination sites [3]. It is proposed that PFLX in the solution form the same bonds with copper ions.

The influence of CTDNA on fluorescence properties of PFLX

Figure 5 shows the fluorescence emission spectra of PFLX in the presence of different concentration of CTDNA. A significant decrease in the fluorescence intensity of PFLX in the presence of CTDNA has been observed with the shape and the maximal wavelength of the spectra remaining unaffected. And the addition of CTDNA made the maximal characteristic absorbance peak of PFLX located at 250 nm increase the intensity of absorbency in UV absorbance spectra. These phenomena mean that there must be some interaction happened in the binary system, and there might be a complex formed, too.

The composition of the complex PFLX–DNA was also determined by the above methods as PFLX–Cu^{2+} system. Figure 6 shows the result, a 1:1.16 mole ratio of PFLX to CTDNA was conformed by the experimental result and the correlation coefficient is 0.9982.

Viscosity measurements

The effect of PFLX on the viscosity of DNA solution is shown in Fig. 7. η_0, η are the relative viscosity of the DNA solution in the absence and presence of PFLX, r is [PFLX]/[DNA]. There is no remarkable change in the viscosity of DNA solution, and it seems that the binding mode between PFLX and CTDNA is groove binding, not intercalate binding [16].

The influence of CTDNA on fluorescence properties of PFLX in the presence of Cu^{2+}

The fluorescence spectra of PFLX and its decreased fluorescence intensity by CTDNA in the presence of

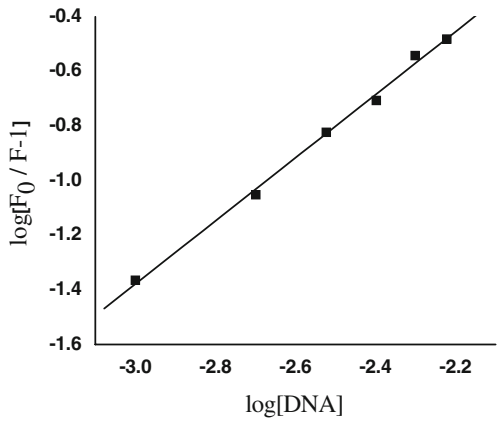

Fig. 6 Estimation of the composition of the PFLX–DNA complex

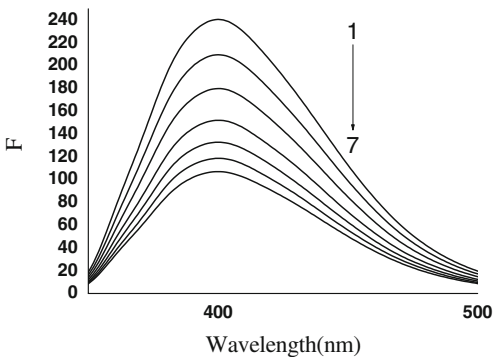

Fig. 8 Fluorescence spectra of PFLX–DNA–Cu^{2+}: λ_{ex}=327 nm, [PFLX]=1.0×10^{-5} M, [DNA]=4.0×10^{-5} M, [Cu^{2+}] (1→7) (10^{-5} M)=0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2

different concentration of Cu^{2+} are shown in Fig. 8. In the system, when CTDNA solution was added, the fluorescence intensity of PFLX was quenched obviously. In the presence of Cu^{2+}, the intensity of fluorescence of PFLX can be quenched more severely by CTDNA, and the quenching degree was aggravated with the increasing of the concentration of Cu^{2+} in aqueous solution. And special attention also has been paid to UV absorption properties of the ternary system. When Cu^{2+} solution was added gradually, there was no change happened in wavelength locality but the absorbency increased for the maximal characteristic absorption peak located 250 nm in the UV absorption spectra.

These results imply that there is the competition between the CTDNA and Cu^{2+} when metal ions coexisted with CTDNA in PFLX solution, and they also might suggest the formation of a ternary complex among CTDNA, PFLX and Cu^{2+}.

Conclusions

According to the experimental results, we think that there are two kinds of binding modes for the interaction between the PFLX and CTDNA.

Intercalative binding mode Deduced from the fluorescence experiment results, a ground-state binary complex is formed between PFLX and Cu^{2+}, and a ground-state ternary complex also can be formed among Cu^{2+}, PFLX and CTDNA. The intercalative binding of intercalated ligand into the base pairs of DNA has such characteristics as emission enhancements (or quenching) of the fluorescence of ligand [17]. Figure 5 showed the fluorescence emission spectra of the binary PFLX–DNA system has a strong fluorescence quenching, which is regarded as characteristics of intercalative binding. This conclusion is conformed by Long and Barton [18]. And this model is consistent with the denature DNA experimental result.

Groove binding mode The effect of drug on the relative viscosity of DNA is an effective method in all methods used in the research on the interaction between DNA and small drug molecules, because the viscosity of DNA is very sensitive to the length of DNA [16, 19]. If DNA and drug molecules combine through intercalate binding, the relative viscosity of DNA solution will increase; and there is no remarkable change in the viscosity of DNA solution when they combine through groove binding. According to viscosity experiment results, the interaction between PFLX and CTDNA should belong to groove binding mode.

To our knowledge, we cannot assert which mode is more suitable to explain the experimental results, but we think that there is the cooperative effect between the two modes. Earlier studies have pointed out that norfloxacin binds to DNA based via hydrogen bonding [19]. The hydrogen bonding donors thus become available for pairing with the acceptors on the drug molecules (carbonyl or fluoro groups). Both C_4 carbonyl group on the quinolone ring and C_3 carboxylic acid group are potential hydrogen-bond acceptors. The results of experiment indicated a possible interaction mechanism for PFLX binding to DNA. That is, firstly PFLX forms a binary complex with Cu^{2+}, then forms a ternary complex with DNA, and finally, releases Cu^{2+} to form hydrogen bonds between PFLX and DNA. Cu^{2+} plays an intermediary role in the interaction between PFLX and DNA.

Acknowledgements The authors gratefully acknowledge the financial support of the Nature Science Foundation of Zhejiang Province, China (No. Y407091) and the Medical Science Research Foundation of Zhejiang Province, China (No. 2005A071).

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