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Comparison of the complexation of fluoroquinolone antimicrobials with metal ions by nuclear magnetic resonance spectroscopy

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Abstract

The complexation of fluoroquinolone antimicrobials with various metal ions have been studied in aqueous solution (pD 2.5, 37°C) by ^{1}H and ^{13}C-NMR spectroscopy. The compounds examined are levofloxacin, ciprofloxacin and lomefloxacin. In each drug, new signals have appeared by the addition of Al^{3+}, suggesting that the complexes are formed between the drug and Al^{3+} and that the ligand exchange is slow on the NMR time scale. Solution structure of the major species in the presence of 2.0 mol equiv of Al^{3+} has been proposed based on the large downfield shifts of some specific protons. Signals of both the coordinated and free drugs have shown slight broadening at 90°C due to the enhanced rate in ligand dissociation process, though the coalescence phenomena are not observed even at this temperature. Thus, the complexes are supposed to be stable at the physiological condition. Titration experiments have revealed that the binding ability of levofloxacin toward Al^{3+} is much stronger than that of ciprofloxacin and lomefloxacin at pD 2.5. In contrast to the complexation with Al^{3+}, the binding of these drugs with other metal ions such as Ca^{2+} and Mg^{2+} is much weaker; NMR signals have shown no appreciable downfield shift by the addition of Ca^{2+} and Mg^{2+}. Based on these results, it is concluded that the fluoroquinolone antimicrobials examined in the present study at pD 2.5 exist as stable complexes in the presence of Al^{3+} and the absorptivity of the drugs on oral administration could be affected by Al^{3+}.

Keywords: Quinolone antimicrobials; Metal ions; Complexation; ^{1}H-NMR; ^{13}C-NMR

1. Introduction

The advantages of fluoroquinolone antimicrobials are their excellent activities against various bacteria, a low frequency of adverse effects, and a good absorptivity on oral administration. It has been reported, however, that the ciprofloxacin (CPFX) level in plasma decreases after the oral administration of a metallic antacid containing Al^{3+}, Mg^{2+} and Ca^{2+}, Fe^{2+} and Zn^{2+} [1–8]. Similar observations have been reported for other fluoroquinolone antimicrobials such as ofloxacin and rufloxacin [1,9]. The decrease in absorption of

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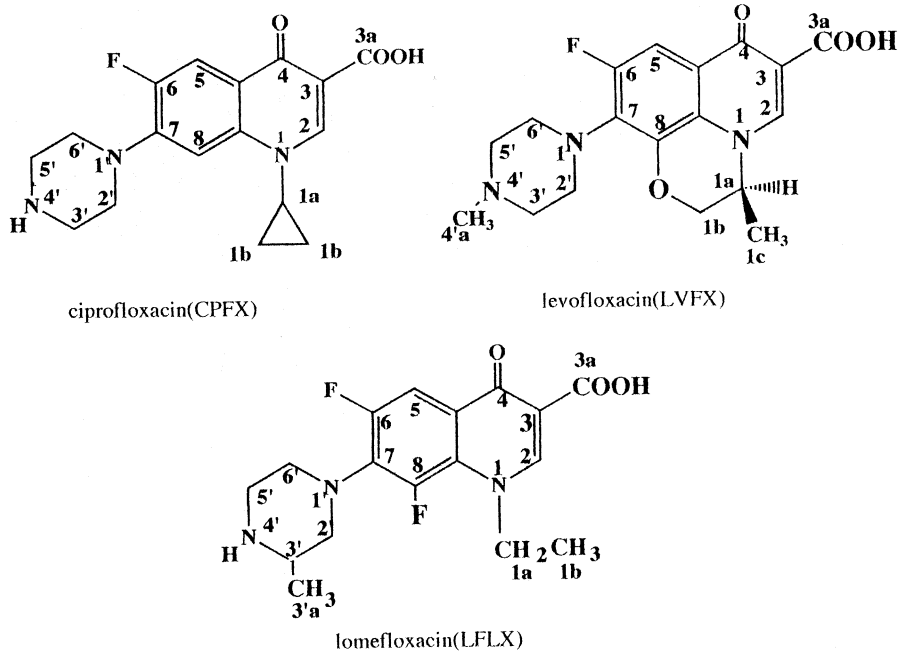

Fig. 1. Structures of levofloxacin(LVFX), ciprofloxacin(CPFX), and lomefloxacin (LFLX).

these drugs might be ascribed to the chelate formation with the metal ions, although little has been reported on the interaction of fluoroquinolone antimicrobials with metal ions. Nuclear magnetic resonance spectroscopy (NMR) must be a suitable method to elucidate the metal-drug interaction in solution because it can provide not only the stoichiometry of the chelate formation but also some kinetic and thermodynamic aspects of the complexes [10]. Riley et al. have reported based on the ^{1}H- and ^{13}C-NMR study that the complexes derived from lomefloxacin (LFLX) and Al^{3+} have 2:1 and 3:1 drug to metal stoichiometries [11]. Lecomte et al. have reported based on the ^{19}F-NMR study that Mg^{2+} is situated between the ketone and the carboxylate groups in fluoroquinolones–Mg^{2+} complexes and that the complexes have 1:1 drug to metal stoichiometry [12]. In view of the therapeutic importance of these drugs, we have studied the complexation ability of three kinds of commonly used fluoroquinolone antimicrobials, ciprofloxacin(CPFX), lomefloxacin (LFLX), and levofloxacin (LVFX) as shown in Fig. 1, with various metal ions such as Al^{3+}, Mg^{2+} and Ca^{2+} by using ^{1}H- and ^{13}C-NMR method. In this paper, we would like to report that these drugs have different affinity toward Al^{3+} and that LVFX has larger binding ability than CPFX and LFLX.

2. Experimental

2.1. Chemicals

The fluoroquinolones were kindly provided by their manufacturers; ciprofloxacin (CPFX), levofloxacin (LVFX) and lomefloxacin (LFLX) are gifts from Bayer, Daiichi Seiyaku and Shionogi, respectively.

2.2. NMR Measurement

^{1}H- and ^{13}C-NMR spectra of fluoroqinolones and their metal complexes were recorded on a JEOL LA-300 spectrometer operating at 300.40 MHz for proton. In every measurement, D_2O was used as a solvent and the pD was regulated at 2.5

Table 1
Chemical shifts (ppm) for levofloxacin, levofloxacin:metal (1:1) at 37°C and pH 2.5 in D_2O

Carbon atom	^1H (ppm)			
	–	Ca^{2+}	Mg^{2+}	Al^{3+}
2	8.70	8.70	8.69	9.06
5	7.35(d,J = 12 Hz)	7.24(br)	7.22(d,J = 12 Hz)	7.90(d,J = 12 Hz)
2′,3′,5′,6′	3.65(m)	3.66(m)	3.66(m)	3.64(m)
lb	3.37(m)	3.38(br)	3.36(br)	3.35(br)
4′a	3.04	3.04	3.06	3.00
lc	1.60(d,J = 6.4 Hz)	1.59(d,J = 6.4 Hz)	1.59(d,J = 6.3 Hz)	1.62(d,J = 6.5 Hz)

by the additions of HCl or NaOH solutions. NMR probe was maintained at 37°C throughout the measurement. Each of the metal complexes was prepared in an NMR sample tube by the addition of a D_2O solution of metal salt such as $AlCl_3 \cdot 6H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 6H_2O$ or $FeCl_3 \cdot 6H_2O$ into a D_2O solution of a drug. Initial concentration of a drug was ca. 0.2 M. The reaction was monitored by ^1H-NMR in each time after the addition of the salts. Typical conditions for ^1H-NMR measurement were as follows: spectral width: 5 kHz; pulse delay time: 1.0 s; scans; 100 times. Chemical shifts (δ ppm) were obtained from DSS (sodium 2,2-dimetyl-2-silapentane-5-sulfonate) as an internal standard ($\delta = 0.000$). ^{13}C-NMR spectra were similarly taken on a JEOL LA 300 spectrometer using DSS as internal standard.

3. Results

3.1. Assignment of ^1H-NMR signals of the drugs

The ^1H-NMR spectral data for LVFX, CPFX and LFLX are given in Tables 1–3, respectively. The aryl protons in LVFX and LFLX, 2-H and 5-H, were easily distinguished since 5-H showed splitting due to the coupling with the ^{19}F nucleus at the adjacent position. Thus, the two doublets at 7.35 and 7.64 ppm were assigned to the 5-H of LVFX and LFLX, respectively. The aryl protons in CPFX, 2-H, 5-H and 8-H, were also assigned based on the magnitude of the coupling constants of these protons with the ^{19}F nucleus at position 6. Thus, the signals at 7.46 and 7.52 ppm, both appeared as doublet due to the $^1H-^{19}F$ coupling, were assigned to 5-H and 8-H, respectively, since the coupling constant of the former is 13.1 Hz while that of the latter is 7.3 Hz; the coupling constant of the protons *ortho* to the fluorine is much larger than that *meta* to it [13].

3.2. ^1H-NMR spectra of the complexes

1H NMR spectra of LVFX, CPFX, and LFLX were measured in the presence of various metal ions such as Al^{3+}, Mg^{2+}, Ca^{2+} and Fe^{3+}. In the presence of Fe^{3+}, every peak of the drug broadened considerably due to the paramagnetic effect of Fe^{3+}. Thus, the complexation reaction with Fe^{3+} was not studied. The ^1H-NMR spectral data in the presence of excess amount of metal ions are also listed in Tables 1–3. As is clear from the data, new signals were observed only when Al^{3+} was added; addition of Mg^{2+} and Ca^{2+} induced no appreciable change in the NMR spectra of the drugs except for some signal broadening in the case of Ca^{2+}. As a typical example, the ^1H-NMR titration results of CPFX by Al^{3+} are given in Fig. 2. It is noticeable that the spectrum became very simple after the addition of 1.0 mol equiv of Al^{3+}; three kinds of aromatic proton signals appeared at 7.77(d), 8.20(d), and 9.11(s) ppm. CPFX and LFLX showed a similar spectral change by the addition of Al^{3+}.

The drugs examined showed different coordination ability toward Al^{3+}. By the addition of 0.2 mol equiv of Al^{3+}, more than 56% of LVFX were involved in the coordination to Al^{3+} to form

Table 2
Chemical shifts (ppm) for ciprofloxacin, ciproflaxacin:metal (1:1) at 37°C and pH 2.5 in D_2O

Carbon atom	^1H (ppm)			
	–	Ca^{2+}	Mg^{2+}	Al^{3+}[a]
2	8.63	8.63	8.60	9.11
5	7.46(d,J = 13 Hz)	7.50(br)	7.42(d,J = 13 Hz)	8.20(d,J = 13 Hz)
8	7.52(d,J = 7.3 Hz)	7.50(m)	7.49(d,J = 6.9 Hz)	7.77(d,J = 7.1 Hz)
2′,6′ or 3′,5′	3.66(d,J = 2.0 Hz)	3.66(m)	3.66(m)	3.64(m)
2′,6′ or 3′,5′	3.56(d,J = 3.7 Hz)	3.56(m)	3.56(m)	3.56(m)
1b	1 47(d,J = 6.5 Hz)	1.45(d,J = 6.1 Hz)	1.46(d,J = 6.6 Hz)	1.51 (d,J = 6.2 Hz)
	1.22	1.20	1.20	1.31

[a] The ratio for Al^{3+} is 1:2.

stable complexes. In contrast, only 28% of CPFX and 10% of LFLX were involved in the complex formation. In Fig. 3(a–c) are given the ^1H-NMR spectra of the three drugs, LVFX, CPFX, and LFLX, respectively, in the presence of 0.2 mol equiv of Al^{3+} at pD 2.5. In Fig. 4 is given how the ratios of the coordinated species increase by the addition of Al^{3+}.

3.3. Effect of pD on the complex formation

In general, complex formation is affected by the difference in pD. Thus, 0.2 mol equiv of Al^{3+} was added to the LFLX solution at pD 2.5 and 5.3. Integration of the peaks ascribed to the 2-H of the free and the coordinated species clearly indicated that the concentration of the coordinated species at pH 5.3 is twice as much as that at 2.5.

3.4. ^{13}C-NMR of LVFX

^{13}C-NMR spectrum of LVFX showed carbonyl and carboxylate signals at 179.1 and 171.5 ppm, respectively. While the aliphatic carbon signals did not show appreciable shift by the addition of 0.2 mol equiv of Al^{3+}, some of the aromatic carbon signal showed a 1–2 ppm shift. Especially clear is the shift of the carbonyl carbon signals; at least two new signals due to the coordinated species were observed at 174.1 and 176.3 ppm. Further addition of Al^{3+} made spectrum much more complicated by the formation of several coordinated species.

3.5. ^1H-NMR spectra at higher temperatures

As mentioned, ^1H-NMR spectra of the drugs were quite different in the presence of Al^{3+} due to the formation of stable complexes. In order to find out the kinetic and thermodynamic stability of the complexes, the ^1H-NMR spectra were measured at higher temperature. In Fig. 5(a) are given the temperature dependent ^1H-NMR spectra of LVFX taken between 37 and 90°C in the presence of 0.2 mol equiv of Al^{3+}, where the concentration of the free and the coordinated drugs is nearly 1:1 at 37°C. As the temperature was raised to 90°C, the 2-H signals at δ 9.0–9.3 ppm broadened. However, coalescence phenomena of the H2 signals of the free and coordinated drugs was not observed even at 90°C. In Fig. 5(b) are given the temperature dependent ^1H-NMR spectra of LFLX in the presence of 1.2 mol equiv. of Al^{3+}, where the concentration of the free and the coordinated drugs is nearly 1:1 at 37°C. In this case also, no coalescence phenomenon was observed even at 90°C. It is noticeable that the integral intensity of the 2-H signal of the free drug, which was ca. 50% at 37°C, decreased gradually as the temperature was raised and reached as small as 28% at 90°C. It should also be noted that the new signals appeared at 8.0 and 9.1 ppm as a broad doublet and a broad singlet, respectively, and increased their intensities as the temperature was raised. Similar results were obtained in the ^1H-NMR spectra of CPFX in the presence of 0.6 mol equiv of Al^{3+}.

Table 3
Chemical shifts (ppm) for lomefloxacin, lomefloxcacin:metal(1:1) at 37°C and pH 2.5 in D_2O

Carbon atom	^1H (ppm)			
	–	Ca^{2+}	Mg^{2+}	Al^{3+}[a]
2	8.70	8.66	8.66	9.06
5	7.64(d,J = 12 Hz)	7.62(d,J = 12 Hz)	7.63(d,J = 12 Hz)	8.18(d,J = 12 Hz)
5′,6′	3.70(d,J = 15 Hz)	3.70(m)	3.70(m)	3.68(m)
2′	3.57(d,J = 14 Hz)	3 70(m)	3.70(m)	3 68(m)
3′	3.44(d,J = 11 Hz)	3.70(m)	3.70(m)	3.68(m)
1b	1.54(d,J = 6.5 Hz)	1.53(t,J = 6.5 Hz)	1.53(t,J = 6.4 Hz)	1.61 (m)
3′a	1.43(d,J = 6.4 Hz)	1.43(d,J = 6.2 Hz)	1,43(d,J = 6 4 Hz)	1,43(d,J = 6.4 Hz)

[a] The ratio for Al^{3+} is 1:4.3.

4. Discussion

4.1. Complexaton with metal ions

Addition of Mg^{2+} into the LVFX solution at pD 2.5 did not show any new signals ascribed to the complex formation. Addition of Ca^{2+} caused a small effect on the ^1H-NMR spectra; although the chemical shift of each signal showed no appreciable change, the 2-H and 5-H signals exhibited some broadening to give a single line instead of a doublet in the free drug. These results suggest either that the ligand exchange between free and chelated species is fast on the NMR timescale or that the difference in chemical shifts is too close each other. In contrast to the cases of Mg^{2+} and Ca^{2+}, addition of Al^{3+} into the drug solution induced a great change on the spectrum. The signals due to the free drug decreased in intensities and some new signals appeared as the amount of Al^{3+} increased. When 0.5 mol equiv of Al^{3+} was added, the signals of the free LVFX completely disappeared. The result clearly indicates that the new species, formed by the complexation with Al^{3+}, has kinetically stable ligand at 37°C; ligand exchange between free and coordinated LVFX is slow on the NMR timescale. Observation of the clearly separated signals in the Al^{3+} complex in turn indicate that the ligand exchange is quite fast in the Mg^{2+} and Ca^{2+} complexes. Furthermore, stability constants of LVFX with Mg^{2+} and Ca^{2+} must be very small, since the chemical shift of each signal showed no appreciable change; most of the LVFX molecules exist as free ligand even in the presence of 2.0 mol equiv of Mg^{2+} and Ca^{2+} at pD 2.5. Similar results were obtained in the experiments using other drugs such as CPFX and LFLX; formation of the complexes were observed only by the addition of Al^{3+} complex. These results suggest that, while Al^{3+} forms stable complexes with the drugs, Mg^{2+} and Ca^{2+} form only unstable complexes in aqueous solution. Broadening of some signals in the presence of Ca^{2+} might be the indication that binding with Ca^{2+} is much stronger than that with Mg^{2+}. Thus, the binding affinity of the metal ions toward the drugs is $Al^{3+} \gg Ca^{2+} > Mg^{2+}$. For this reason, the complexation of the three drugs with Al^{3+} will be mentioned in the following discussion. It should be noted that the order of the binding affinity described above is obtained in the acidic solution of pD 2.5. According to Okabayashi and co-workers, the binding affinity is $Al^{3+} \gg Mg^{2+} > Ca^{2+}$ in more basic solution [14].

4.2. Solution structure of the complexes

When Al^{3+} was added to the D_2O solutions of three kinds of drugs, 2-H, 5-H and 8-H (in the case of CPFX) signals in the quinolone ring showed large downfield shifts. Other signals showed no appreciable shift even by the addition of 2.0 mol equiv of Al^{3+} as the data in Tables 1–3 indicate. The average downfield shifts of 2-H and 5-H signals of the three drugs were 0.42 and

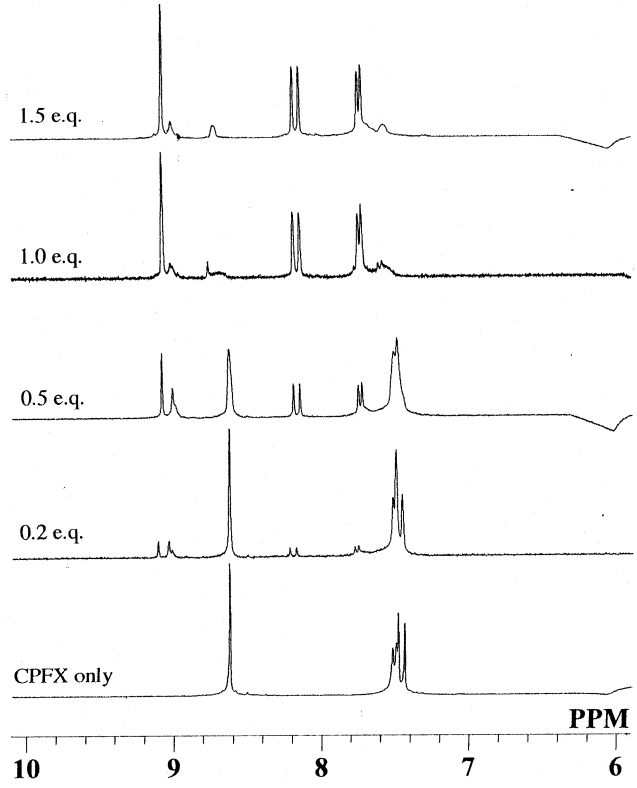

Fig. 2. Aromatic region of the ^{1}H-NMR spectra of ciprofloxacin obtained after the addition of various amount of Al^{3+} at pD 2.5.

0.62 ppm, respectively, in the presence of 2.0 mol equiv of Al^{3+}. The downfield shift of the 8-H signal in CPFX was much smaller, 0.28 ppm. As Fig. 2 shows, the spectral change is very complicated in the presence of 0.2–1.0 mol equiv of Al^{3+}. After the addition of 2.0 mol equiv of Al^{3+}, however, the spectrum is greatly simplified. It gives only three kinds of aromatic proton signals, indicating the presence of a single species in solution. It is reasonable to assume that the species is a 1:1 complex as shown in Fig. 6 where the Al^{3+} ion is located between 4-keto oxygen and the ionized 3-carboxylate. This is consistent with the structure proposed by Nakano et al. [15] based on the UV data. By the coordination of highly charged Al^{3+}, the 4-keto group would have some enolic character, which results in the induction of positive charges on the C-5 and C-7 positions as shown in Fig. 6. This must be one of the reasons for the larger downfield shift of the 5-H signal as compared with 8-H signal. In the presence of less amount of Al^{3+}, complexes with 3:1 and 2:1 drug to metal ratios are present, making the spectrum much more complicated.

4.3. Coordination ability of the drugs toward Al^{3+}

In order to find out the coordination ability of these drugs toward Al^{3+}, titration experiments were carried out at pD 2.5. ^{1}H-NMR spectra of the three drugs were quite different as shown in Fig. 3 when 0.2 mol equiv of Al^{3+} was added. In the case of LFLX, nearly 90% of the drug remained unreacted, exhibiting an intense singlet at 8.70 ppm ascribed to the 2-H of the free drug. On the contrary, only 44% of the unreacted drug was observed in the case of LVLX. Fig. 4 clearly shows that the ratio of the coordinated species in LVFX increases to a greater extent as compared

with that in LFLX by the addition of Al^{3+}; free LVFX disappeared completely after the addition of 0.6 mol equiv of Al^{3+}. This indicates that two or three molecules of LVFX pick up an Al^{3+} ion to form 3:1 and/or 2:1 complexes. It is noteworthy that nearly 80% of LFLX still remained unreacted when most of the free LVFX disappeared. The results suggest that the coordination ability

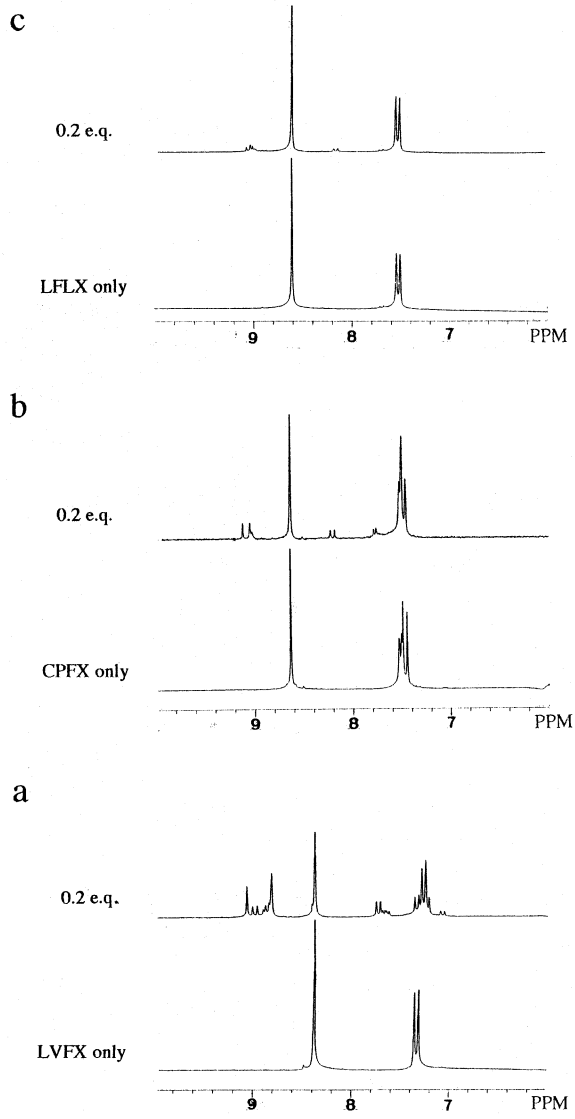

Fig. 3. ^{1}H-NMR spectral change of (a) LVFX; (b) CPFX and (c) LFLX caused by the addition of 0.2 mol equiv of Al^{3+} at pD 2.5.

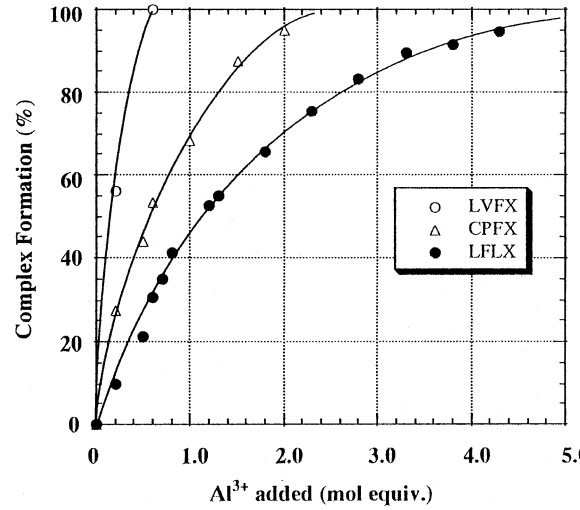

Fig. 4. Change in the ratios of the chelated species by the addition of Al^{3+} at pD 2.5.

of LVFX toward Al^{3+} is much larger than that of CPFX and LFLX at pD 2.5. The ratio of the coordinated species increased as the solution became much more basic. At pD 5.3, ca. 35% of LFLX existed as the coordinated species. This is reasonable because the p*K*a values of the carboxyl groups in CPFX and LFLX were reported to be 6.09 and 5.82, respectively [16]; the carboxyl groups of these drugs exist as protonated form at pD 2.5. Although the p*K*a value of LVFX is not reported, the value is expected to be the same as that of ofloxacin, its enantiomer, p*K*a = 6.05 [16]. On raising the pD value of the solution, the ratio of the carboxylate increases relative to the protonated form resulting in the increase in the coordinated species.

The large coordination ability of LVFX toward Al^{3+} can be explained in terms of electronic effect of the substituents of the quinolone ring. While LVFX has ether oxygen at the C-8 position, LFLX carries fluoro substituent and CPFX has no substituent at the same position. The Hammet sp values for methoxyl and fluoro groups are -0.27 and $+0.06$, respectively. Thus, the ether oxygen at the C-8 position can stabilize the structure given in Fig. 6 by its electron donating ability as compared with the fluoro group. As a result, coordination ability of the three drugs becomes LVFX > CPFX > LFLX at pD 2.5.

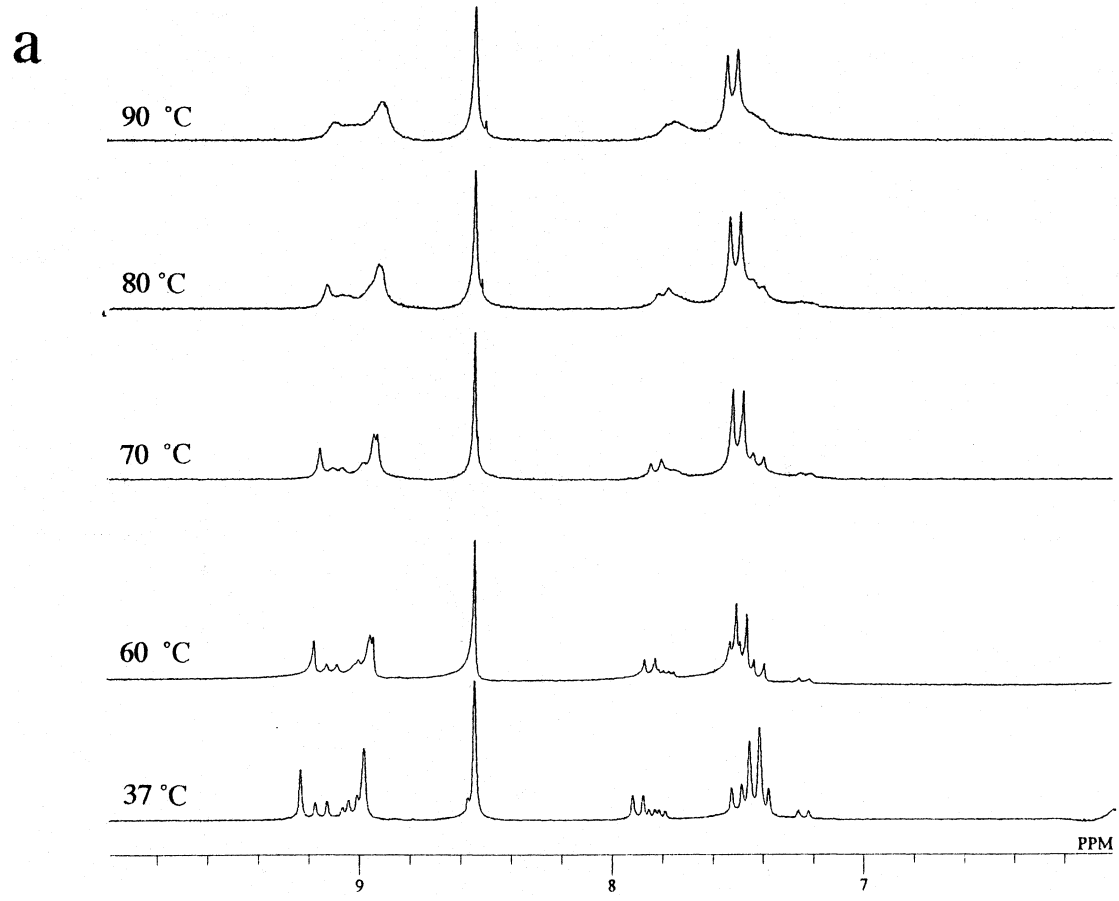

Fig. 5. Temperature dependent ^{1}H-NMR spectra of (a) LVFX in the presence of 0.2 mol equiv of Al^{3+} at pD 2.5 and (b) LFLX in the presence of 1.2 mol equiv of Al^{3+} at pD 2.5.

4.4. Stability of the complexes

Complex formation of the fluoroquinolone antimicrobials with Al^{3+} could influence the absorptivity of these drugs on oral administration if the complexes are stable even in the acidic condition. In order to find out the stability of these complexes, we have measured the high temperature NMR spectra of these drugs in the presence of Al^{3+}. Although each signal of LVFX broadened to some extent at 90°C as shown in Fig. 5(a), no coalescence phenomena of the signals was observed between the free and coordinated species. If we assume that the coalescence temperature is 100°C, then the activation free energies for dissociation of the drugs are estimated to be ca. 19 kcal/mol. Since this is the minimum value for the activation free energy, it might be reasonable to assume that the drug is tightly fixed in the aluminum complex at 37°C.

Temperature dependent ^{1}H-NMR spectra of LFLX in the presence of 1.2 mol equiv of Al^{3+} is given in Fig. 5(b). Although no coalescence was observed even at 90°C, new signals appeared at 8.0 and 9.1 ppm and increased their intensities at higher temperature. Correspondingly, the intensities of 2-H and 5-H of the free drug decreased from 50% (37°C) to 28% (90°C). The results might be the indication that there are three types of metal-drug complexes, $Al^{3+}(L^-)$, $Al^{3+}(L^-)_2$, and $Al^{3+}(L^-)_3$, as presented by Eqs. (1)–(3), where LH and L^- stand for free drug and its carboxylate form.

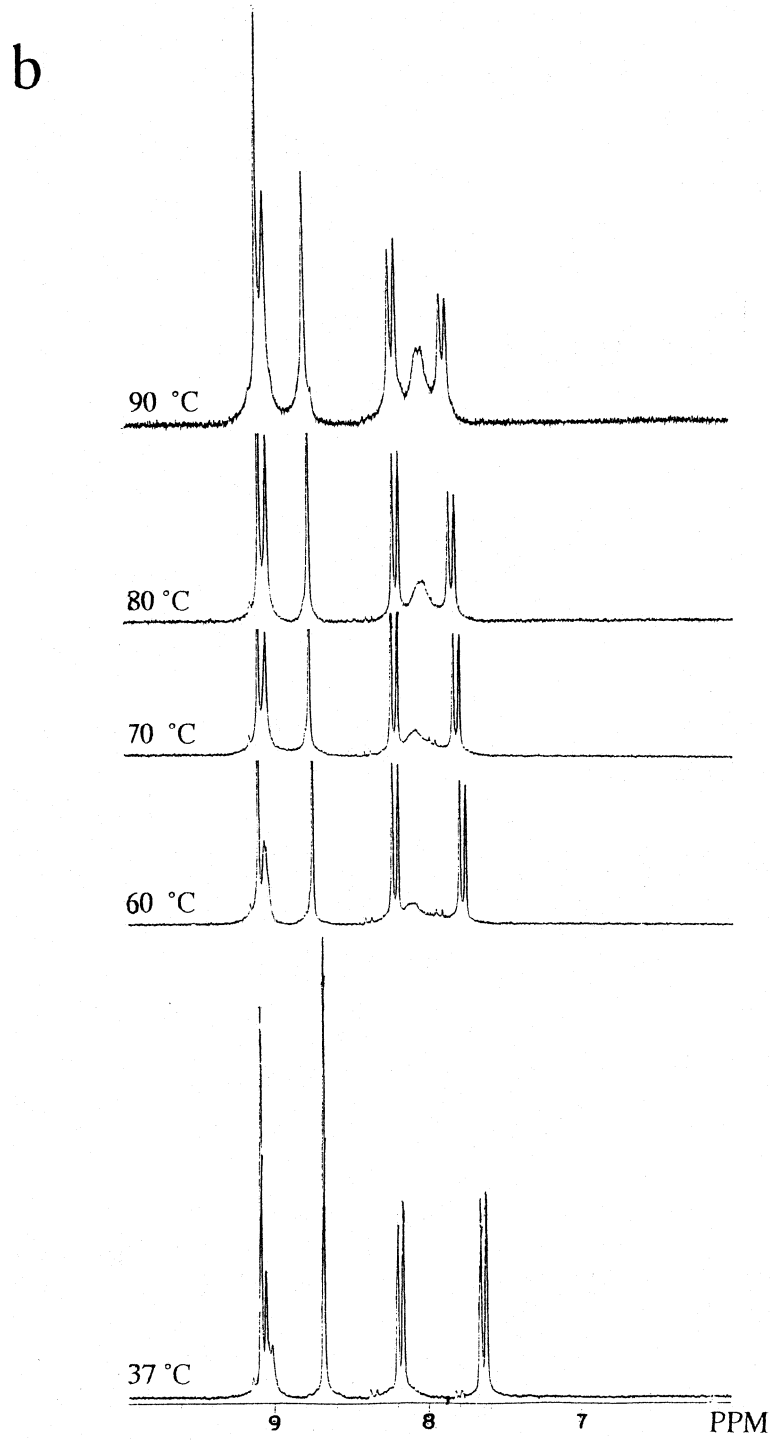

Fig. 5. (*Continued*)

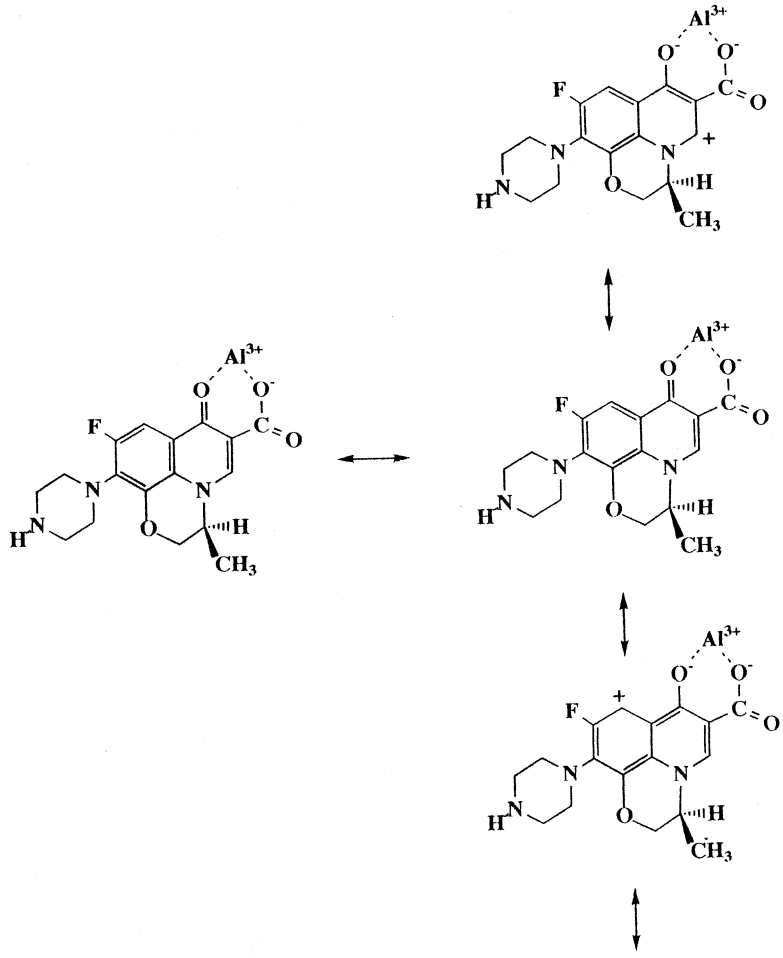

Fig. 6. Possible solution structure of LVFX in the presence of large excess of Al^{3+}.

$$Al^{3+} + LH \overset{k_1}{\rightleftharpoons} Al^{3+}(L^-) + H^+ \quad (1)$$

$$Al^{3+}(L^-) + LH \overset{k_2}{\rightleftharpoons} Al^{3+}(L^-)_2 + H^+ \quad (2)$$

$$Al^{3+}(L^-)_2 + LH \overset{k_3}{\rightleftharpoons} Al^{3+}(L^-)_3 + H^+ \quad (3)$$

In the presence of 1.2 mol equiv of Al^{3+}, the 1:1 complex, $Al^{3+}(L^-)$, is the major species at 37°C as is clearly seen in Fig. 5(b). At higher temperature, the concentrations of $Al^{3+}(drug)_2$ and/or $Al^{3+}(drug)_3$ are supposed to increase. As a result, new signals ascribable to $Al^{3+}(L^-)_2$ and/or $Al^{3+}(L^-)_3$ appeared at 8.0 and 9.1 ppm at the expense of the free drug. The temperature dependence described above is possible if ΔH_2^0 and/or ΔH_3^0 for Eq. (2) and/or Eq. (3) are positive. It should be noted here that the rate of ligand dissociation in $Al^{3+}(L^-)_2$ and/or $Al^{3+}(L^-)_3$ is still slow on the ^{1}H-NMR time scale even at 90°C, since they gave separate signals for 2-H and 5-H.

5. Conclusion

We examined the complex formation of the three fluoroquinolone antimicrobials with metal ions such as Al^{3+}, Ca^{2+} and Mg^{2+} at pD 2.5. Extensive ^{1}H- and ^{13}C-NMR studies revealed that these drugs coordinate to Al^{3+} to form stable complexes. Variable temperature NMR studies indicated that the activation free energies for ligand dissociation is larger than 19 kcal mol^{-1}. The

NMR studies also revealed that the drugs have different coordination affinities toward Al^{3+}. Titration experiment showed that the order of the affinity is LVFX > CPFX > LFLX at pD 2.5. These results suggest that the drugs exist as aluminum complexes in a physiological condition in the presence of comparable amount of Al^{3+} and that the absorptivity of the drugs on oral administration could be affected.

Acknowledgements

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