Interaction of Quinolones with Metal Cations in Aqueous Solution

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Quinolones are a group of potent antibacterial agents. Many reports show that oral absorption of quinolones is reduced by coadministration of metallic preparations. For better understanding of this phenomenon, we investigated the complex formations of quinolones (DU-6859a, DV-7751a, DR-3862, levofloxacin) with various metal cations by pH titrations, NMR and spectrophotometry. Several metal complexes including hydroxo species were confirmed by pH titrations. The order of stability constants among trivalent metal cations was $Fe^{3+} > Al^{3+}$, and those among divalent metal cations was $Cu^{2+} > Fe^{2+} > Zn^{2+} > Mg^{2+} > Ca^{2+}$. The differences in stability constants among quinolones were not as large. Complex structures and metal complex formation equilibria in aqueous solution were proposed. Quinolones were believed to bind metal cations through carboxyl group and carbonyl group of the quinolone ring, and these equilibria show that there are two types of deprotonation reactions from metal complex: the several Fe^{3+} complexes and Al^{3+} complexes liberate protons from coordinated water molecules under acidic to neutral conditions, while the other complexes liberate protons from amine groups under neutral to alkaline conditions. Moreover, quantitative behavior of metal complex in aqueous solution has been demonstrated by simulation for the first time. The simulation especially showed that quinolones interact with Al^{3+} in the stomach, but with Mg^{2+} in the intestines when coadministered with antacid containing Al^{3+} and Mg^{2+}. These results suggest that the complex formation of quinolones with metal cations is an important factor affecting absorption of the former in the gastrointestinal tract.

Key words quinolone; stability constant; metal complex; coordination mode

Some drugs are known to have complex forming abilities with metal cations which substantially affect their pharmacological effects. Oral absorption of tetracyclines was reportedly reduced by coadministration of metallic preparations.[1] This reduction seems to be caused by complex formation of the drugs with metal cations; thus interaction of drugs with such cations needs to be understood. It has recently been reported that oral absorption of quinolones also was reduced by coadministration of metallic antacid containing Al^{3+}, Mg^{2+} and Ca^{2+}, and mineral supplements such as Fe^{2+} and Zn^{2+}.[2] Quinolones, which have common basic structural features as shown in Fig. 1, are a group of potent antibacterial agents active against gram-positive and negative microorganisms. Quinolones have a complex forming ability with metal cations through carboxyl group at 3-position and carbonyl group at 4-position of the quinolone ring. For example, Köppenhofer *et al.* demonstrated using the crystallographic data that the β-keto acid group of nalidixic acid, a classic quinolone, acts as a chelating ligand towards zinc.[3] In relation to the reduction of quinolone absorption, the stability constants of metal

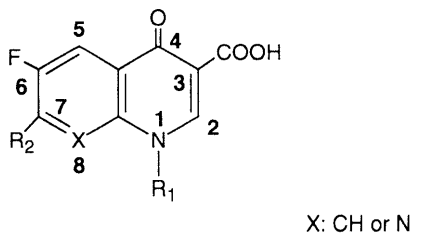
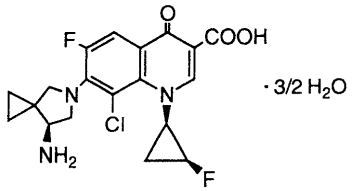
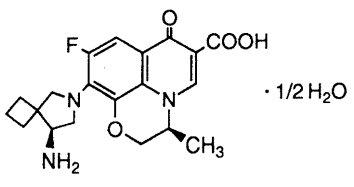


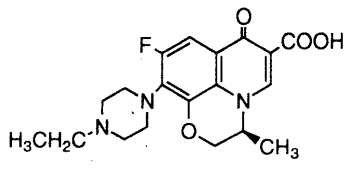
Fig. 1. Basic Required Structure of Quinolone Antibiotics



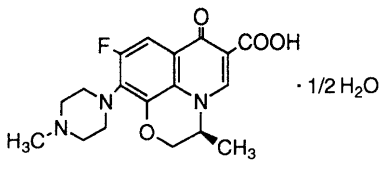
DU-6859a



DV-7751a



DR-3862



levofloxacin

Fig. 2. Chemical Structure of Quinolones

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complexes of quinolones had been measured, and the relationship between the stability constants of metal cations and their inhibitory abilities in oral absorption of quinolones had been discussed by many researchers.[4] The details of quantitative behavior and the structures of metal complexes in the solution are not completely clear, however. In this report, the complex formation of several quinolones (Fig. 2) with various kinds of metal cations was confirmed by NMR and spectrophotometry, and was investigated quantitatively by pH titrations.

Experimental

Materials 7-[7(*S*)-7-Amino-5-azaspiro[2,4]heptan-5-yl]-8-chloro-6-fluoro-1-[(1*R*,2*S*)-2-fluoro-1-cyclopropyl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid sesquihydrate (DU-6859a), (*S*)-10-[(*S*)-(8-amino-6-azaspiro[3,4]octan-6-yl)]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid hemihydrate (DV-7751a), (*S*)-(−)-9-fluoro-2,3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid (DR-3862) and (*S*)-(−)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid hemihydrate (levofloxacin) were synthesized at the Production Technology Research Laboratories of Daiichi Pharmaceutical Co., Ltd. (Tokyo). Iron(III) nitrate nonahydrate, aluminum(III) nitrate nonahydrate, zinc(II) nitrate hexahydrate and calcium(II) nitrate tetrahydrate were purchased from Kanto Chemical Co. Inc. (Tokyo). Copper(II) nitrate trihydrate and magnesium(II) nitrate hexahydrate were purchased from Nakarai Tesque, Ltd. (Kyoto). Iron(II) chloride tetrahydrate was purchased from E. Merck (Darmstadt, Germany). All chemicals used were of reagent grade and water was purified with a Milli-Q system (Millipore Co., Ltd., Massachusetts, U.S.A.) and degassed with nitrogen gas.

pH Titrations The metal salt (8.0 mmol) was dissolved in 200 ml of water. The metal solution (1.0 or 4.0 ml) and the quinolone (0.04, 0.08 or 0.12 mmol) were dissolved in water so that the total volume was 80 ml. Potentiometric titrations of the solutions containing metal cations and quinolones with carbonate-free 0.1 M KOH were carried out under nitrogen atmosphere at 25 °C and ionic strength(I) of 0.1 M (KNO_3). Concentration ratios of metal cation to quinolone were 1 : 2 and 1 : 3 for Fe^{3+}, 1 : 1, 1 : 2 and 1 : 3 for Al^{3+}, 1 : 2 for Cu^{2+} and Zn^{2+}, 1 : 3 for Fe^{2+}, 2 : 1 for Mg^{2+} and 4 : 1 for Ca^{2+}. Concentrations of metal cations were 0.5 mM for Fe^{3+}, Al^{3+}, Cu^{2+}, Fe^{2+} and Zn^{2+}, and 2.0 mM for Mg^{2+} and Ca^{2+}. Reproducibility of the results was checked by repeated titrations. The solution of 0.1 M KOH was standardized against standard potassium hydrogen phthalate (Kanto Chemical Co. Inc.). Measurements of pH were performed with an automatic titrator GT-05 (Mitsubishi Kasei Co.) equipped with a GTPH10 glass electrode and a GTPH50 reference electrode. Calibration of the equipment was made with standard buffer solutions (pH 4.01, pH 6.86 and pH 9.18 at 25 °C, Horiba, Ltd.). Conversion of pH meter reading (pH M) to $-\log[H]$, where [H] refers to hydrogen ion concentration, was made by correcting the difference (0.05) between pH M and $-\log[H]$ obtained by titrating 1.25 mM HNO_3 with 0.1 M KOH at I = 0.1 M (KNO_3). The hydroxide ion concentration [OH] was calculated from the apparent ion product of water, $pK_{w'} = 13.88$ (= pH M − log[OH]), determined by titrating 0.1 M KNO_3 with 0.1 M KOH.

Calculation of Equilibrium Constants The overall stability constants, β_{pqr}, are calculated for species containing metal cation M, quinolone L and proton H in the molar ratio of p, q and r, respectively.

$$pM + qL + rH \xleftrightarrow{\beta_{pqr}} M_pL_qH_r$$

$$\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$$

(charges are omitted for simplicity)

where a negative value of r refers to deprotonation from the complex. The $\log \beta_{pqr}$ values were calculated with the computer programs PKAS[5] and BEST[5] by a Gateway 2000 computer system. Since, in the case of DR-3862 and DV-7751a, potentiometric titrations of the solutions containing Fe^{3+}, Fe^{2+} and Al^{3+} (Al^{3+} to quinolone ratios = 1 : 1 and 1 : 2) were not carried out, the $\log \beta_{pqr}$ values for these systems were not calculated. The hydrolysis constants of metal cations were taken from the literature[5-8]; those used were: $\log \beta_{10-1} = -2.76$, $\log \beta_{10-2} = -5.88$, $\log \beta_{20-2} = -3.08$ and $\log \beta_{10-4} = -20.76$ for Fe^{3+},[5] $\log \beta_{10-1} = -5.69$, $\log \beta_{10-2} = -11.2$, $\log \beta_{30-4} = -14.0$, $\log \beta_{10-3} = -17.3$, and $\log \beta_{10-4} = -24.5$ for Al^{3+},[5] $\log \beta_{10-1} = -7.22$ and $\log \beta_{30-4} = -21.05$ for Cu^{2+},[6] $\log \beta_{10-1} = -8.3$ for Fe^{2+},[7] $\log \beta_{10-1} = -8.7$ for Zn^{2+},[8] $\log \beta_{10-1} = -11.7$ for Mg^{2+},[5] and $\log \beta_{10-1} = -12.2$ for Ca^{2+}.[5]

Spectroscopic Measurements ^{13}C-NMR spectra of the solution containing only DR-3862 as solute and solutions containing Al^{3+} and DR-3862 in deuterium oxide were measured at 25 °C with a JEOL JNM-GSX500 NMR spectrometer. Concentration of DR-3862 was 52 mM and its ratio of Al^{3+} : DR-3862 was 1 : 1. Sodium 3-trimetylsilylpropionate-2,2,3,3-d_4 was used as external standard. The pH values of the solution of DR-3862 and solution containing Al^{3+} and DR-3862 were adjusted to pD 3 with DCl or NaOD. The pH values were checked with a Horiba M-8 pH meter. Attenuated total reflection spectra (ATR spectra) of the solution of DR-3862 and solution containing Al^{3+} and DR-3862 in water were measured with a JEOL JIR-5300 FT-IR spectrometer equipped with a micro-circle cell (Spectra-Tech Inc., Connecticut, U.S.A.). Concentration of DR-3862 was 52 mM and its ratio of Al^{3+} : DR-3862 was 1 : 1. The pH values of these solutions were adjusted to pH 3 with HCl or NaOH.

Calculation of Species Distributions The species distributions of complexes as a function of pH were calculated from the stability constants obtained with computer program SPE[4] by a Gateway 2000 computer system. The solubility products of metal cations and stability constants for Fe^{2+} complexes of citric acid were taken from the literature.[5,7,9]

Results and Discussion

Stability Constants The complex formation constants between metal cations (Fe^{3+}, Al^{3+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}) and quinolones were investigated by pH titrations in 0.1 M KNO_3 solution at 25 °C. For example, pH titration curve of DU-6859a is shown as curve a in Fig. 3. pH titration of DU-6859a in the diprotonated form shows that the two protons dissociate in individual steps, m = 1—2 and m = 2—3, corresponding to the dissociation

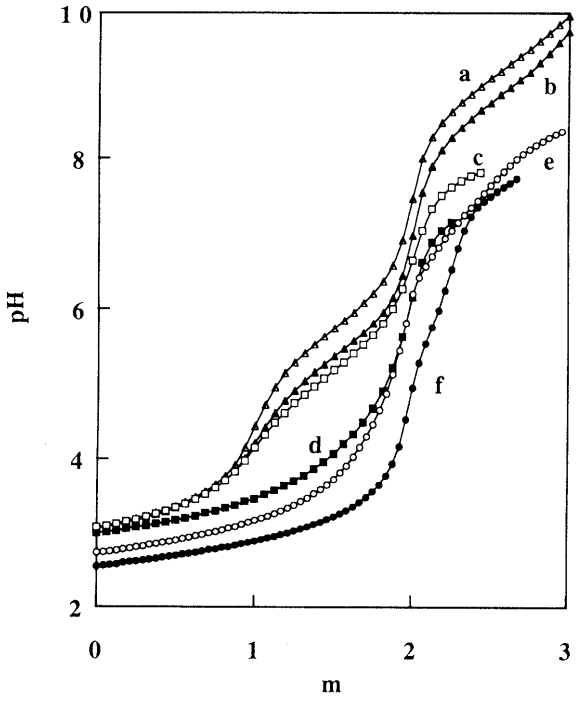

Fig. 3. Titration Curves for DU-6859a with KOH at 25 °C and I = 0.1 M (KNO_3)

m: Moles of KOH added per mole of DU-6859a. a, free ligand; b, Mg^{2+} + ligand (2 : 1); c, Zn^{2+} + ligand (1 : 2); d, Cu^{2+} + ligand (1 : 2); e, Al^{3+} + ligand (1 : 3); f, Fe^{3+} + ligand (1 : 3). A curve similar to b was obtained with Ca^{2+}. A curve similar to c was obtained with Fe^{2+}. Concentration: Fe^{3+}, Al^{3+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, 0.5 mM; Mg^{2+}, Ca^{2+}, 2.0 mM.

Table 1. Overall Stability Constants, $\log\beta_{pqr}$, for Proton–Quinolone and Metal–Quinolone Complexes at 25 °C and I=0.1 M (KNO_3)

		$\log\beta_{pqr}$[a]			
	$p\,q\,r$	DU-6859a	Levofloxacin	DR-3862	DV-7751a
	0 1 1	9.1 (0)	8.2 (0)	8.5 (0)	8.8 (0)
	0 1 2	14.8 (0)	14.3 (0)	14.6 (0)	15.3 (0)
Fe^{3+}	1 1 1	16.6 (1)	16.1 (1)		
	1 2 2	32.9 (0)	31.5 (0)		
	1 3 3	48.8 (1)	46.7 (1)		
	1 3 2	42.0 (3)	40.6 (2)		
	1 3 1	34.5 (4)	33.2 (2)		
	1 3 0		25.6 (3)		
	1 1 0	13.6 (1)	13.0 (0)		
	1 2 1	28.4 (1)	27.0 (1)		
	1 2 0	21.0 (1)	19.7 (3)		
	1 2 −1	13.4 (1)	12.2 (1)		
Al^{3+}	1 1 1	16.5 (1)	15.3 (1)	15.8 (0)	16.1 (3)
	1 2 2	31.5 (1)	29.6 (2)	30.4 (1)	31.5 (2)
	1 3 3	45.0 (0)	42.6 (2)	43.8 (1)	45.9 (2)
	1 3 2	38.3 (0)	35.4 (2)	36.4 (1)	38.2 (2)
	1 3 1	30.4 (1)	27.6 (2)	28.5 (1)	30.2 (3)
	1 3 0	22.3 (1)	19.7 (3)	20.3 (1)	
	1 1 0	11.7 (1)	10.0 (1)		
	1 1 −1	5.9 (1)	4.3 (0)		
	1 1 −2	−1.8 (1)	−3.3 (0)		
	1 2 1	26.1 (0)	23.5 (1)		
	1 2 0	18.6 (1)	16.0 (1)		
	1 2 −1	10.6 (1)	8.2 (1)		
Cu^{2+}	1 1 1	15.0 (0)	14.4 (0)	14.9 (1)	15.3 (0)
	1 2 2	28.8 (0)	27.3 (1)	28.1 (1)	29.5 (0)
	1 2 1	21.7 (0)	19.6 (1)	20.1 (2)	
	1 2 0		11.7 (1)	12.0 (2)	
Fe^{2+}	1 1 1	13.4 (0)	12.6 (1)		
	1 2 2	25.9 (0)	23.9 (1)		
	1 2 1	18.5 (0)	16.4 (2)		
	1 2 0		8.8 (2)		
Zn^{2+}	1 1 1	13.1 (0)	12.1 (0)	12.5 (0)	13.4 (1)
	1 2 2	25.4 (0)	23.3 (0)	24.0 (1)	25.8 (0)
	1 2 1	18.4 (0)	16.3 (0)	16.5 (1)	
	1 2 0		8.1 (0)	8.2 (2)	
Mg^{2+}	1 1 1	12.0 (0)	11.1 (0)	11.5 (0)	12.1 (0)
	1 1 0	3.4 (0)	3.2 (0)	3.3 (0)	3.7 (0)
Ca^{2+}	1 1 1	11.2 (0)	10.3 (0)	10.6 (0)	11.1 (0)
	1 1 0	2.7 (1)	2.4 (0)	2.5 (0)	2.7 (0)

a) Values in parentheses denote estimated standard deviations.

Table 2. Stepwise Stability Constants, $\log K_1$, for Metal–Quinolone Complexes at 25 °C and I=0.1 M (KNO_3)

	$\log K_1$			
	DU-6859a	Levofloxacin	DR-3862	DV-7751a
Fe^{3+}	7.5	7.9		
Al^{3+}	7.4	7.1	7.3	7.3
Cu^{2+}	5.9	6.2	6.4	6.5
Fe^{2+}	4.3	4.4		
Zn^{2+}	4.0	3.9	4.0	4.6
Mg^{2+}	2.9	2.9	3.0	3.3
Ca^{2+}	2.1	2.1	2.1	2.3

of carboxyl group and amine group, respectively, where m represents the number of moles of potassium hydroxide added per mole of DU-6859a. The portion of the curve corresponding to m values from 0 to 1 indicates neutralization of 1 mol of HNO_3. Titration curves obtained for the systems containing DU-6859a and metal cations are illustrated as curves b—f in Fig. 3. In the presence of metal cations, the titration curves of DU-6859a are depressed due to the formation of metal complexes. These curves showed that complex formation with Fe^{3+}, Al^{3+} and Cu^{2+} occurred from a low pH region, whereas complex formation with Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+} occurred from the deprotonation region of carboxyl group of DU-6859a. The pH titration curve patterns of other quinolones were similar to that of DU-6859a. However, precipitations were observed between pH 7 and 8 during the pH titrations in the systems involving Fe^{3+}, Cu^{2+}, Fe^{2+} and Zn^{2+} for DU-6859a and Al^{3+}, Cu^{2+} and Zn^{2+} for DV-7751a. The precipitations were considered to be metal complex of DU-6859a or DV-7751a, because they began to occur from formation of neutral complex, for example FeL_3 or CuL_2. The solubility of DU-6859a and DV-7751a is low compared with that of levofloxacin and DR-3862. Therefore, the solubility of metal complex of quinolone was believed to depend on that of quinolone. The stability constants were calculated from potentiometric data by using the nonlinear least-squares refinement program BEST. Overall stability constants, $\beta_{pqr}=[M_pL_qH_r]/[M]^p[L]^q[H]^r$, are shown in Table 1, and stepwise stability constants, $K_1=[M(LH)]/[M]\cdot[LH]$, in Table 2. Several metal complexes including ML_2H, ML_2, ML_3, MLH_{-2} were obtained for the first time in this study. Since the precipitations were observed during the pH titrations in the systems involving Fe^{3+}, Cu^{2+}, Fe^{2+} and Zn^{2+} for DU-6859a and Al^{3+}, Cu^{2+} and Zn^{2+} for DV-7751a, the stability constants for ML_3, ML_2H, ML_2 were not obtained. The order of stability constants among trivalent metal cations was $Fe^{3+}>Al^{3+}$, and then that among divalent metal cations was $Cu^{2+}>Fe^{2+}>Zn^{2+}>Mg^{2+}>Ca^{2+}$. The stability constants for metal complex of quinolone were large and quinolones were demonstrated to have high affinities to metal cation. It was thought that quinolones formed stable complexes with metal cations because of the chelate effect in the carbonyl group and carboxyl group of the quinolone ring. The differences in stability constants among quinolones were not large. However, the stability constants for the metal complex of DV-7751a, which has the highest pK_a value of carboxylic group in four quinolones, tended to be slightly larger than those for the metal complex of other quinolones.

Coordination Mode of Quinolone Coordination modes to metal cation of quinolones were confirmed by comparing the ^{13}C-NMR spectra of DR-3862 with that of a 1:1 mixture of Al^{3+} and DR-3862. Figure 4 shows the differences between ^{13}C-NMR chemical shift of DR-3862 with and without Al^{3+}. The chemical shift changes of the carbons near the carboxyl group of DR-3862 were very large, while those of the carbons near the protonated amine group of DR-3862 did not change with the addition of Al^{3+}. Moreover, the absorption band at *ca.* 1600—1700 cm^{-1} of the carbonyl and carboxyl groups of DR-3862 in the ATR spectrum also shifted to a lower wavenumber by addition of Al^{3+} (Fig. 5). These results indicate that coordination sites of DR-3862 are in the carbonyl group and carboxyl group, and that the amine group of Al^{3+} complex, MLH, is protonated (Fig. 6). The

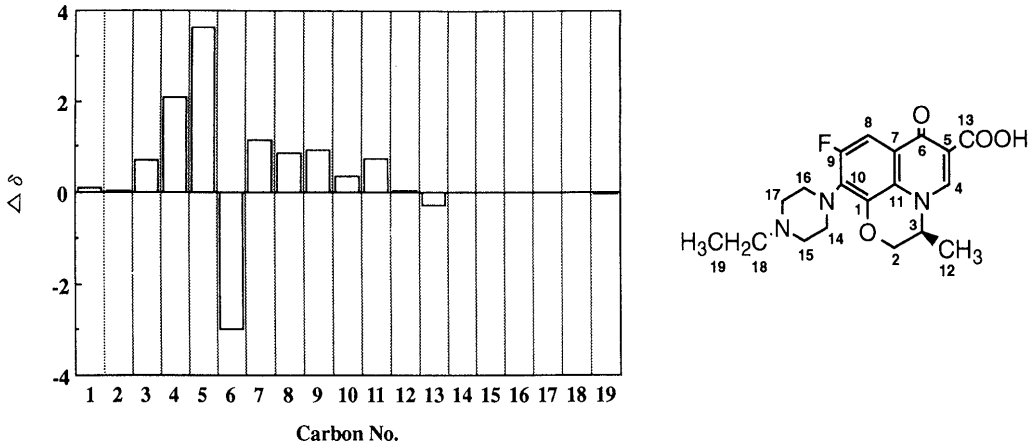

Fig. 4. Difference of ^{13}C-NMR Chemical Shift between DR-3862 (pD 2.8) and Al^{3+}–DR-3862 System (pD 3.0)
Concentration: Al^{3+}, 52 mM; DR-3862, 52 mM.

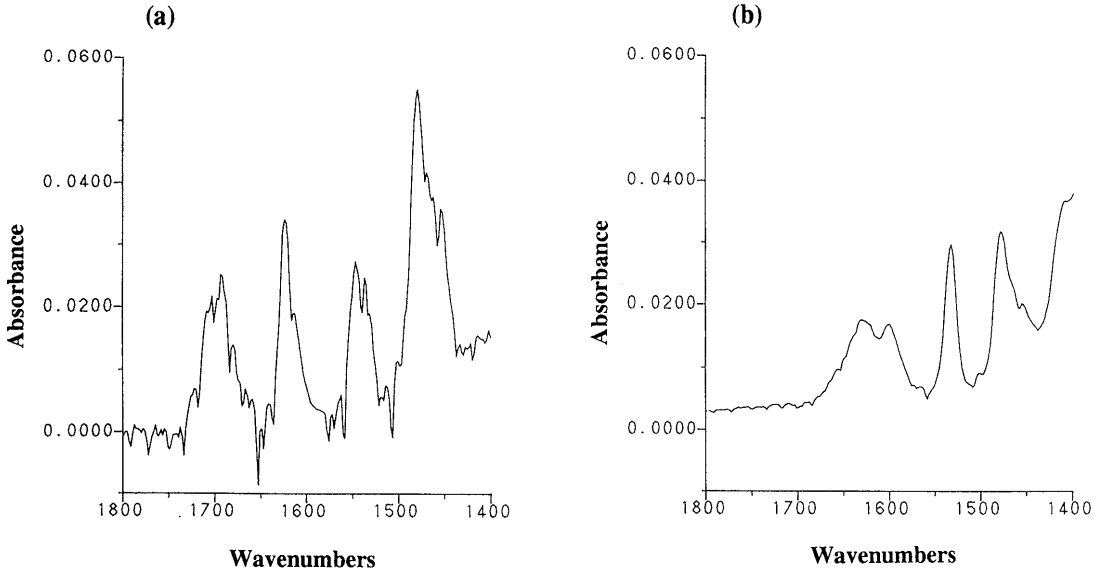

Fig. 5. ATR Spectra of DR-3862 (a) and 1 : 1 Al^{3+}–DR-3862 (b) in Water
Concentration: Al^{3+}, 52 mM; DR-3862, 52 mM. a, pH 2.8; b, pH 3.0.

coordination sites of nalidixic acid were demonstrated to be in the β-keto acid group by the crystallographic data.[3] The stability constants for metal complexes of nalidixic acid were similar to those of quinolones obtained in this experiment.[10] Consequently, the coordination site of other quinolones are believed to be the same as that of DR-3862 or nalidixic acid. This speculation was supported by the pattern of ATR spectrum in water, which was similar to that of IR spectrum of solid product isolated after reaction of ofloxacin, racemate of levofloxacin, and metal cation.[11]

Deprotonation Reaction from Metal Complex The important complex formation equilibria in the aqueous solution were elucidated based on the titration data obtained. For example, the equilibria for Al^{3+} and Mg^{2+} (Fig. 7) show that there are two types of deprotonation reactions from metal complex, because metal complexes have coordinated water molecules and a protonated amine group as possible deprotonation sites.

In the deprotonation from MLH and ML_2H_2 for Fe^{3+} complex, and MLH, ML and ML_2H_2 for Al^{3+} complex, the deprotonation constants given by $\log\beta_{111}-\log\beta_{110}$ and $\log\beta_{122}-\log\beta_{121}$ for Fe^{3+} complex and by $\log\beta_{111}-\log\beta_{110}$, $\log\beta_{110}-\log\beta_{11-1}$ and $\log\beta_{122}-\log\beta_{121}$ for Al^{3+} complex were closer to the hydrolysis constant of each metal cation than to the dissociation constants of protonated amine groups. These data suggested that the deprotonation of these complexes occurs from the coordinated water molecule under acidic to neutral conditions.

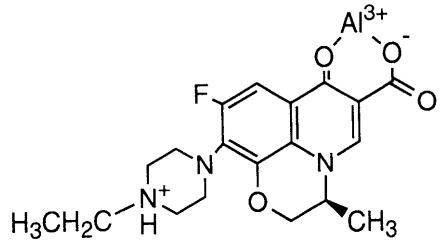

Fig. 6. Proposed Structure of Al^{3+}–DR-3862 Complex for MLH

As for the deprotonation from ML_3H_3, ML_3H_2 and ML_3H for Fe^{3+} complex and Al^{3+} complex, these complexes have only protonated amine group as a possible deprotonation site. Consequently, their deprotonation

$M=Al^{3+}$	M	+	LH_2	$\rightleftharpoons$	M(LH)	+	H
	M(LH)			$\rightleftharpoons$	M(OH)(LH)	+	H
	M(OH)(LH)			$\rightleftharpoons$	$M(OH)_2(LH)$	+	H
	$M(OH)_2(LH)$			$\rightleftharpoons$	$M(OH)_2(L)$	+	H
	M(LH)	+	LH_2	$\rightleftharpoons$	$M(LH)_2$	+	H
	$M(LH)_2$			$\rightleftharpoons$	M(OH)$(LH)_2$	+	H
	M(OH)$(LH)_2$			$\rightleftharpoons$	M(OH)(L)(LH)	+	H
	M(OH)(L)(LH)			$\rightleftharpoons$	$M(OH)(L)_2$	+	H
	$M(LH)_2$	+	LH_2	$\rightleftharpoons$	$M(LH)_3$	+	H
	$M(LH)_3$			$\rightleftharpoons$	M(L)$(LH)_2$	+	H
	M(L)$(LH)_2$			$\rightleftharpoons$	$M(L)_2(LH)$	+	H
	$M(L)_2(LH)$			$\rightleftharpoons$	$M(L)_3$	+	H
$M=Mg^{2+}$	M	+	LH_2	$\rightleftharpoons$	M(LH)	+	H
	M(LH)			$\rightleftharpoons$	M(L)	+	H

Fig. 7. Proposed Metal Complex Formation Equilibrium

was thought to occur from the protonated amine group.

For ML_2H and ML_2 for Fe^{3+} complex, MLH_{-1}, ML_2H and ML_2 for Al^{3+} complex, and MLH and ML_2H_2 for Cu^{2+} complex, Fe^{2+} complex and Zn^{2+} complex, it was difficult to determine deprotonation sites clearly. However, the deprotonation constants for these complexes were close to the dissociation constants of the protonated amine group for ML_3H_3, ML_3H_2 and ML_3H. Therefore, the deprotonation of these complexes appeared to occur from the protonated amine group.

As for the deprotonation site from MLH for Mg^{2+} complex and Ca^{2+} complex, deprotonation constants given by $\log\beta_{111}-\log\beta_{110}$ were closer to the dissociation constants of protonated amine group than to the hydrolysis constant of each metal cation. This finding suggested that the deprotonation occurs from the protonated amine group.

Species Distributions To determine the metal complex formation in the solution, the percentage of each species in a hypothetical solution of metal cations and quinolones was calculated from the stability constants by the simulation program SPE. A metal complex formation of quinolones was first analyzed in the binary system [(metal–quinolone 1:3), [M]=0.5 mM]. For example, the results of metal cation-DU-6859a system are shown in Figs. 8 and 9. The species distributions are expressed as relative concentrations to the total concentration of M. Figure 8 shows that Al^{3+} complexes were formed over the pH range 2.0—8.0; the total relative concentration of metal complexes formed was more than 80% of Al^{3+} over this pH range. Then, AlLH, AlL_2H_2, AlL_3H_3 and AlL_3H_2 were predominantly formed over the pH range 2.0—8.0, while Mg^{2+} complexes were formed over the pH range 4.0—8.0 (Fig. 9). The total concentration of the Mg^{2+} complexes formed was smaller over the pH range 2.0—8.0 than that of Al^{3+} complexes. These results indicated that complex formation of quinolones with metal cations was influenced by pH value of the aqueous solution.

Next, we calculated the percentages of Al^{3+} complex and Mg^{2+} complex in a hypothetical solution (100 ml) of

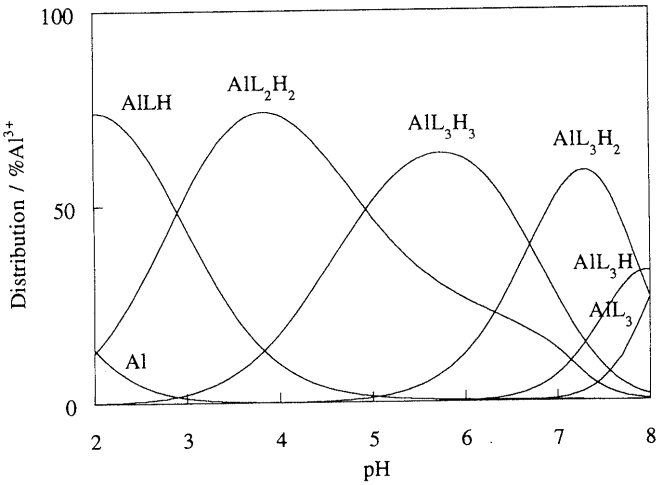

Fig. 8. Species Distributions in Al^{3+}–DU-6859a System as a Function of pH (25 °C; I=0.1 M (KNO_3))

Concentration: Al^{3+}, 0.5 mM; DU-6859a, 1.5 mM.

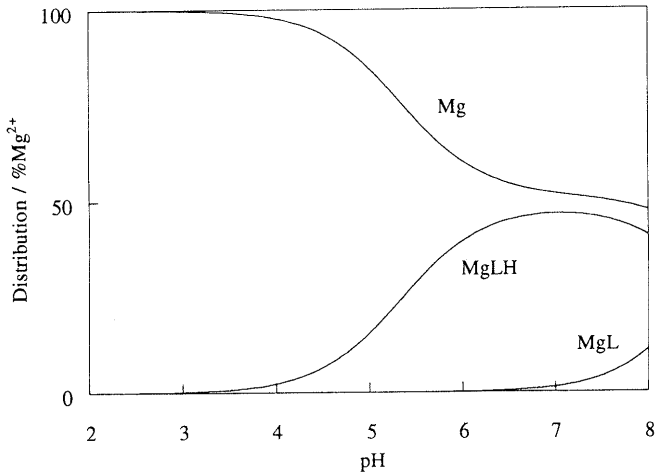

Fig. 9. Species Distributions in Mg^{2+}–DU-6859a System as a Function of pH (25 °C; I=0.1 M (KNO_3))

Concentration: Mg^{2+}, 0.5 mM; DU-6859a, 1.5 mM.

DU-6859a (100 mg), a new quinolone, and Maalox tablet, metallic antacid, containing $Al(OH)_3$ (200 mg/tablet) and $Mg(OH)_2$ (400 mg/tablet), as a model of the contents of the digestive organs. In the stomach, pH range is *ca.* 2—5 on account of administration of antacid, and intestinal pH range is *ca.* 6—8.[12] So, we simulated the complex formation over the pH range 2—8 in this calculation. Since the metal cation has the potential to form insoluble hydroxide, we took into account the solid phase formed by precipitation of the hydroxides, for example, $Al(OH)_3$, by including the K_{sp}'s of metal cations in this calculation, where K_{sp} represents solubility product (Fig. 10). The species distributions are expressed as relative concentrations to the total concentration of L. The total relative concentration of metal complexes formed is more than 95% of the administered DU-6859a over the pH range 2.0—8.0. Under acidic conditions (pH 2.0—5.0), which represented the stomach contents, Al^{3+} complexes were predominantly formed, and under weakly acidic to neutral conditions (pH 5.0—8.0), which represented the intestinal contents, Mg^{2+} complexes were predominantly formed. Al^{3+} does not form any stable complexes due to its precipitation as $Al(OH)_3$ over the pH range 6.0—8.0.

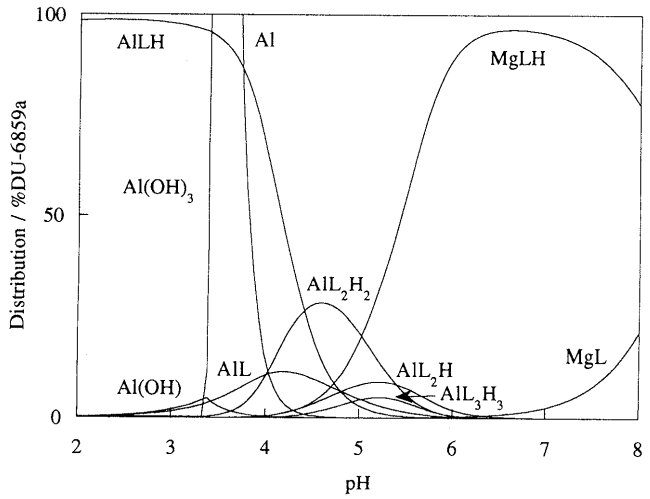

Fig. 10. Species Distributions in Al^{3+}–Mg^{2+}–DU-6859a (11.1 : 30.0 : 1) System as a Function of pH (25 °C; I=0.1 M (KNO_3))

Concentration: Al^{3+}, 25.6 mM; Mg^{2+}, 69.0 mM; DU-6859a, 2.3 mM.

Moreover, metal complexes confirmed under this condition are cationic complexes, and then these complexes are believed soluble in aqueous solution. These metal complex formations in the digestive tract reduce the absorption of quinolones in this organ.

We also simulated the complex formation in a hypothetical solution (100 ml) of DU-6859a (100 mg) and a ferrous tablet, containing ferrous citrate (Fe 50 mg/tablet), as a model for the coadministration of preparations containing ligands, which have higher affinities to metal cations than the quinolones. In this model, citrate is such a component working as a strong chelating reagent. The results show that citrate considerably inhibited complex formation of DU-6859a in the pH range 6.0—8.0, and strongly suggest that complex formation of quinolones with metal cations could be influenced by the coexistence of other components having strong chelating ability.

In this paper, quantitative behavior of metal complex in aqueous solution has been described here for the first time. Especially, it is believed that quinolones interact with Al^{3+} in the stomach, but with Mg^{2+} in the intestines when coadministered with antacid containing Al^{3+} and Mg^{2+}. These findings suggest that the complex formation of quinolones with metal cations is an important factor affecting the quinolone absorption in the gastrointestinal tract.

In the use of drugs having a high affinity for metal cations, it is necessary to prevent their complex formation in order to obtain the desired effect. Thus, simultaneous use of these drugs and metallic preparations or foods containing metal cations should be done with care given to the relationship among absorption part in the digestive tract and strength of the interaction with metal cations and coexisting compounds. The results of our study could provide a useful key to solving clinical problems correctly in quinolone medication.

In this paper and in most previous reports dealing with homogeneous and equilibrated systems, however, the contents in the digestive tract are not homogeneous, and the phenomena that happen there have dynamic reactions. Although the complex formation is fast, dissolution of quinolones and metallic preparations is relatively slow. Thus, the dissolution rates of quinolone and metallic preparation such as insoluble metal hydroxides in the digestive tract are thought to be important factors affecting the complex formation of quinolones.[13] As the next step, these processes should also be studied and taken into consideration for full understanding of the inhibition of quinolone absorption by metal cations.

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