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Interaction of Cefdinir with Iron in Aqueous Solution

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Cefdinir (CFDN) is an orally active, semisynthetic cephalosporin antibiotic and its structure is characterized by an oxyimino side chain which is able to form complexes with various metal ions. We observed that bioavailability of CFDN in dogs was reduced when it was co-administered with iron(II) salts. To assess possible effects of extraneous iron such as iron supplements on the bioavailability of CFDN, stability constants of the complexes formed between CFDN and iron(II) and iron(III) have been determined in aqueous solution by potentiometric and spectrophotometric titration methods. The stability constants were as follows: log $\beta_{110}=7.53$, log $\beta_{120}=14.44$, log $\beta_{130}=18.33$ for the iron(II) complexes and log $\beta_{110}=10.43$, log $\beta_{120}=20.40$ and log $\beta_{130}=27.54$ for the iron(III) complexes. Theoretical species distribution diagrams as a function of pH for solutions of metal (M) (0.1 mM) and ligand (L) (0.3 mM) showed that $M_1L_1H_0$ and $M_1L_2H_0$ existed as major species for the iron(II) system and $M_1L_2H_0$ and $M_1L_3H_0$ were main species for the iron(III) system under neutral conditions. From these results it is believed that complex formation in the digestive tract is involved in the reduction of the bioavailability of CFDN in dogs. In addition, spectrophotometric studies indicated that iron(II) coordinated to CFDN *via* the thiazole-ring and deprotonated oxime nitrogen atoms and iron(III) coordinated *via* the amide and deprotonated oxime oxygen atoms.

Key words Cefdinir; stability constant; metal complex; iron complex; coordination mode

Cefdinir (CFDN) (Fig. 1) is an orally active, semisynthetic cephalosporin antibacterial agent and shows excellent activities against both gram-positive and gram-negative bacteria.[1] The structure of CFDN is characterized by an oxyimino side chain which is able to form complexes with various metal ions. When an iron supplement, iron sulfate, was added to an aqueous solution of CFDN under neutral conditions, the solution turned pure red indicating that iron complexes of CFDN had formed.

This finding suggests that iron may form complexes with CFDN in the digestive tract when multivitamins, powdered milk or other foods containing iron supplements are co-administered with the drug. In fact, red coloration was observed in the stools of some patients taking CFDN, suggesting formation of iron complexes in the digestive tract.[2] It was reported that bioavailabilities (BA) of some pyridone carboxylic acids[3] and tetracyclines[4] were decreased when iron supplements were orally co-administered with the drugs, and these phenomena were considered due to high affinities of the drugs to iron. We have observed in studies on dogs that the BA of CFDN was reduced when it was co-administered with iron(II) salts.[5] This is presumably due to a complex formation of CFDN and iron. It is important to study the interaction between CFDN and iron, especially to evaluate affinities of CFDN to iron(II) and iron(III), considering that irons are contained in various foods and drugs as mentioned above and possibly are co-administered with orally active CFDN.

Stability constants are useful in investigating the interactions between CFDN and irons, since they can indicate in a quantitative manner the extent of complexations under various conditions. We have determined the stability constants of the complexes formed between CFDN and iron(II) and iron(III). In addition, coordination sites of CFDN have been proposed by spectral studies.

Experimental

Materials Two orally active antibiotics, (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-hydroxyiminoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (CFDN) and (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid (cefixime (CFIX)), and their related compound (*Z*)-2-(2-amino-4-thiazolyl)-*N*-(2-hydroxyethyl)-2-hydroxyiminoacetamide (**1**) were produced at the Technological Research Laboratories of Fujisawa Pharmaceutical Company, Ltd. (Osaka, Japan). Iron(II) chloride *n*-hydrate and iron(III) chloride hexahydrate were purchased from Wako Pure Chemical Industries (Osaka, Japan). 2,2′-Bipyridil (bipy), acetylacetone (acac) and 2,2,6,6-tetramethyl-3,5-heptanedione (tmhd) were from Nakarai Tesque, Ltd. (Tokyo, Japan). All chemicals used were of reagent grade, and water was purified with a Milli-Q system (Millipore Co., Ltd., U.S.A.) and decarbonated with nitrogen gas.

pH Titrations Solutions of iron(II) chloride (0.01 M) and iron(III) chloride (0.01 M) were standardized by chelatometry.[6] Ligand (CFDN or **1**) of 0.1 mmol and 3 ml of the metal solution were dissolved in water

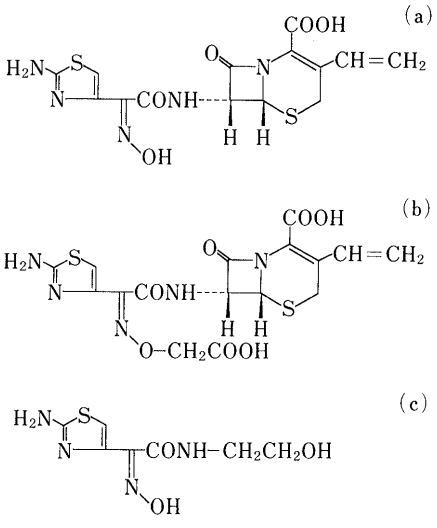

Fig. 1. Structures of CFDN (a), CFIX (b) and Compound **1** (c)

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so that the total volume was 100 ml. Solutions containing the ligand (0.1 mmol) and the metal solution (5 or 10 ml) were also prepared. The concentrations of the ligand and the metal of these solutions were as follows: ligand: 1.0 mM and metal: 0.3, 0.5 or 1.0 mM. Ionic strength (I) of the solutions was adjusted to 0.1 M with KNO_3. The solution was placed in a glass cell equipped with a water-jacket for temperature control at 25±0.2 °C, and titrated with carbonate-free 0.1 M NaOH in an atmosphere of nitrogen. pH values were recorded on a TOA autotitrator AUT-301 (TOA Electronics, Ltd., Japan) equipped with a TOA HGS-4005 glass electrode and a TOA HS-205S reference electrode. Calibration of the equipment was made with standard buffer solutions (pH 4.01 and 6.86 at 25 °C, Nakarai Tesque, Ltd.). Conversion of pH meter reading (pH_M) to $-\log[H]$, where [H] refers to hydrogen ion concentration, was made by correction of the difference (0.07) between pH_M and $-\log[H]$ obtained by titrating 0.01 M HCl with 0.1 M NaOH at the ionic strength of 0.1 M (KNO_3). The hydroxide ion concentration [OH] was calculated from the apparent ion product of water $pK_{w'}=13.93$ ($=pH_M-\log[H]$) determined by titrating 0.1 M KNO_3 with 0.1 M NaOH.

Calculation of Equilibrium Constant The stability constants are expressed as overall constants, β_{pqr}, for species containing metal ion M, ligand L and proton H in the molar ratio of p, q and r, respectively (charges are omitted for simplicity):

$$pM+qL+rH \overset{\beta_{pqr}}{\rightleftharpoons} M_pL_qH_r \quad (1)$$

$$\beta_{pqr}=\frac{[M_pL_qH_r]}{[M]^p[L]^q[H]^r} \quad (2)$$

where a negative value of r refers to deprotonation from the complex. The β_{pqr} values were calculated by the method of nonlinear least squares with a computer program MINIQUAD[7] by a FACOM M1800 computer.

Spectrophotometric Titrations Spectrophotometric measurements were made on a Hitachi 320 UV spectrophotometer (Hitachi, Ltd., Japan). The spectra were recorded over the wavelength range of 250—450 nm. Four μl increments of the iron(III) solution (0.01 M) were added to a cuvette containing 4.0 ml of CFDN solution (0.1 mM), and the absorption spectrum was recorded after each addition. Ten additions were made in total so that the total volume added did not exceed 1% of the volume of the original solution. Non-coordinating buffer solutions, acetate buffer and Tris buffer (0.1 M), were used to maintain constant pH. Refinement of the stability constants was carried out by a non-linear least squares method with a computer program SQUAD[8] implementing the Beers law.

Calculation of Species Distributions The species distributions of complexes as a function of pH were calculated from the stability constants for iron(II) (0.1 mM)–CFDN (0.3 mM) and iron(III) (0.1 mM)–CFDN (0.3 mM) systems with a computer program COMIC.[9]

Absorption Spectral Studies Absorption spectra were recorded for metal (0.1 mM)–ligand (0.3 mM) solutions on a Shimadzu UV 2200 spectrophotometer (Shimadzu, Ltd., Japan). pH of these solutions was maintained at pH 7 using 0.1 M phosphate buffer.

Coloration Appearances of metal (0.1 mM)–ligand (0.3 mM) solutions were observed at pH 7 for iron(II)–CFDN, iron(II)–CFIX, iron(III)–CFDN and iron(III)–CFIX systems.

Results and Discussion

Stability Constants and Species Distributions The stability constants could be determined by pH titration method except for the iron(III)–CFDN systems since the systems gave a small amount of precipitate between pH 3 and 6 during the potentiometric titration. However, it was possible to determine the stability constants for iron(III) complexes of CFDN spectrophotometrically since no precipitation was observed. The stability constants obtained are reported in Table I. Stability constants for complexes of CFDN are quite high, indicating that CFDN has high affinities to iron(II) and iron(III) in water.

Species distributions calculated from the stability constants as a function of pH are depicted for solutions of metal ions (0.1 mM) and CFDN (0.3 mM) (Fig. 2). These diagrams show that CFDN forms stable complexes with iron(II) and iron(III) under neutral conditions. In the iron(II)–CFDN system, $M_1L_1H_0$ and $M_1L_2H_0$ exist as major species under neutral conditions. In the iron(III)–CFDN system, $M_1L_2H_0$ and $M_1L_3H_0$ are main species under neutral conditions.

It is interesting to note that the stability constants for complexes of CFDN are as large as or larger than those of corresponding complexes of tetracycline (Table I),[10] whose BA was reported to be reduced by complexation of tetracycline with iron.[4] Based on those results it is supposed that the reduction of BA of CFDN observed in

TABLE I. Stability Constants for Iron Complexes of CFDN, **1** and Tetracycline at 25 °C and I=0.1 M (KNO_3)

Metal	Ligand	Stability constants			Reference
		$\log\beta_{110}$	$\log\beta_{120}$	$\log\beta_{130}$	
Fe(II)	CFDN	7.53	14.44	18.33	This work
Fe(II)	Tetracycline	5.3	9.3	—	10
Fe(II)	**1**	6.48	13.34	15.86	This work
Fe(III)	CFDN	10.43	20.40	27.54	This work
Fe(III)	Tetracycline	9.9	18.5	25.3	10
Fe(III)	**1**	10.61	21.37	30.02	This work

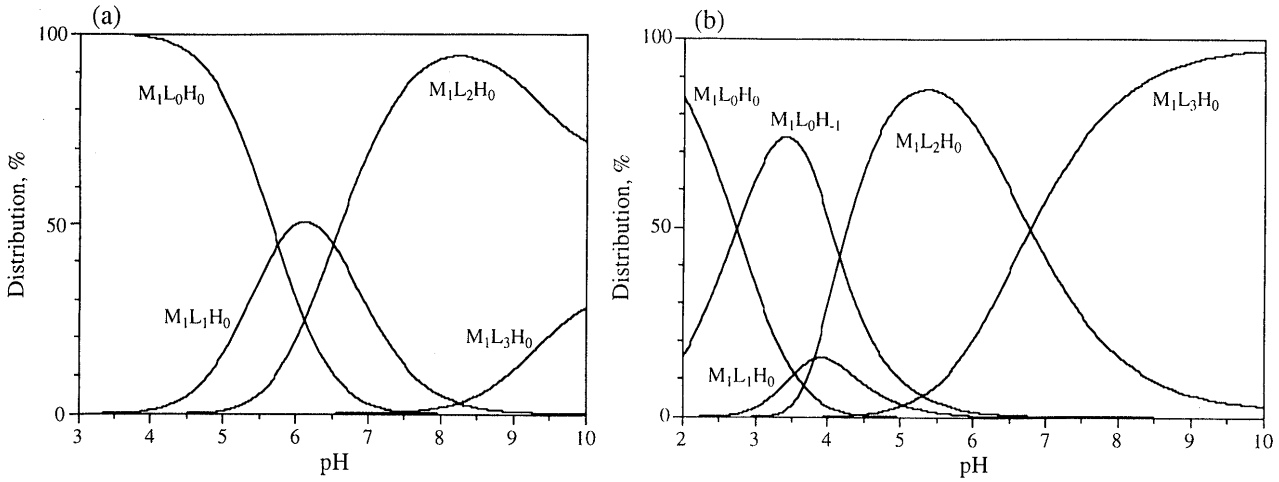

Fig. 2. Species Distributions in 1:3 Iron(II)–CFDN (a) and Iron(III)–CFDN (b)

Calculated from the stability constants listed in Table I. Concentration: metal, 0.1 mM; ligand, 0.3 mM.

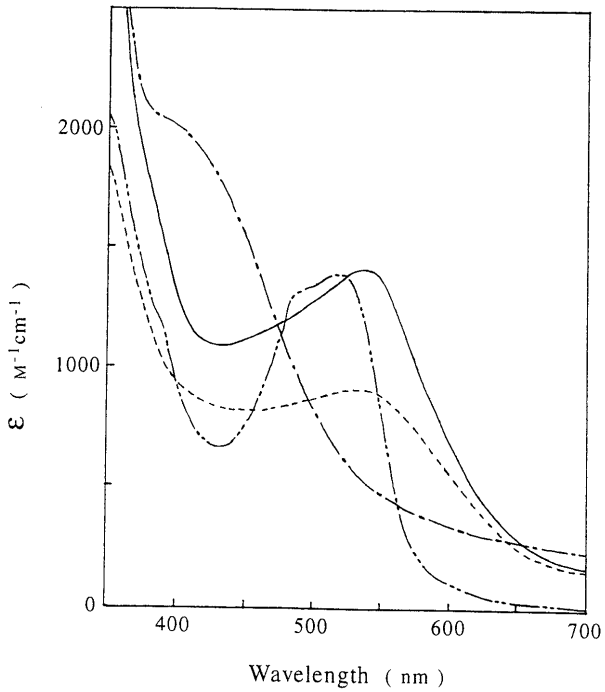

Fig. 3. Absorption Spectra of Iron(II) Complexes of Iron(II)–CFDN (——), Iron(II)-**1** (------), Iron(II)–Tmhd (— - —) and Iron(II)–Bipy (— - - —) Systems

Concentration: metal, 0.1 mM; ligand, 0.3 mM. pH 7.0.

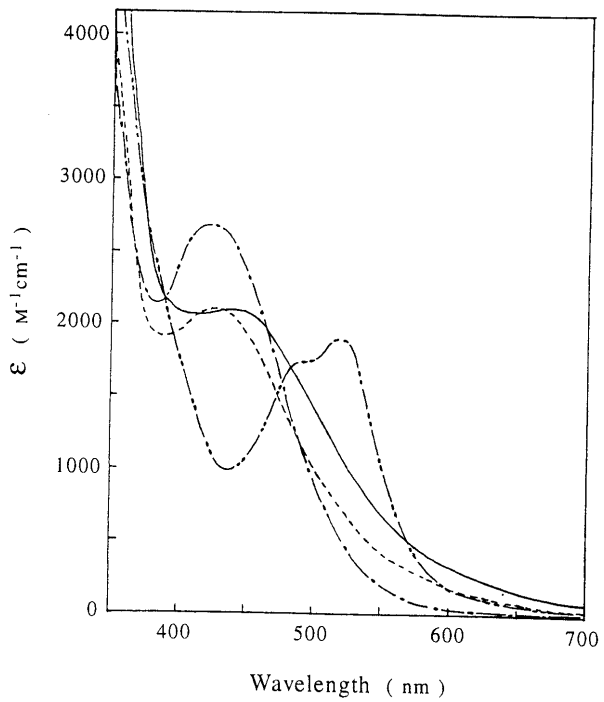

Fig. 4. Absorption Spectra of Iron(III) Complexes of Iron(III)–CFDN (——), Iron(III)-**1** (------), Iron(III)–Tmhd (— - —) and Iron(III)–Bipy (— - - —) Systems

Concentration: metal, 0.1 mM; ligand, 0.3 mM. pH 7.0.

dogs is due to complexation with iron in the digestive tract.[5]

Coordination Modes The compound **1** is the side chain moiety of CFDN at 7 position of the cephalosporin ring. Stability constants for iron–**1** complexes, which are almost the same as those of corresponding complex of CFDN, indicate that coordination sites of CFDN to both iron(II) and iron(III) are in the side chain. The absorption spectra of iron–CFDN systems showed similar charge transfer (CT) bands to those of iron–**1** systems (Figs. 3, 4). Not only the stability constants but also these spectral data suggest that coordination sites of CFDN to iron(II) and iron(III) are in the (*Z*)-2-(2-amino-4-thiazolyl)-2-hydroxyiminoacetamido group.

There could be three possible coordination modes of CFDN in which the oxime moiety is involved, given the chelate effect: The first is a coordination mode through two nitrogen atoms of the thiazole ring and the oxime (2N coordination). The second is through two oxygen atoms of the deprotonated oxime and the amide (2O coordination). The last is a mode through the oxygen atom of the deprotonated oxime and the nitrogen atom of the deprotonated amide. However, the last one does not actually exist, since no complexes with the amide being deprotonated ($M_1L_1H_{-1}$, $M_1L_2H_{-1}$, $M_1L_3H_{-1}$, *etc.*) were detected in the titration studies.

Absorption spectral studies using acac, tmhd and bipy were carried out to be able to suggest whether coordination mode 2N or 2O is formed in iron(II) and iron(III) complexes of CFDN. Acac and tmhd were employed as model ligands for 2O coordination, and bipy was employed as a ligand of 2N coordination. Tmhd, which does not form multinuclear complexes with iron(II) due to the bulky tertiary butyl group, was employed as a model ligand of 2O coordination for the iron(II) system, since iron(II) forms precipitate of multinuclear complexes with acac.[11] The spectra are shown in Figs. 3 and 4.

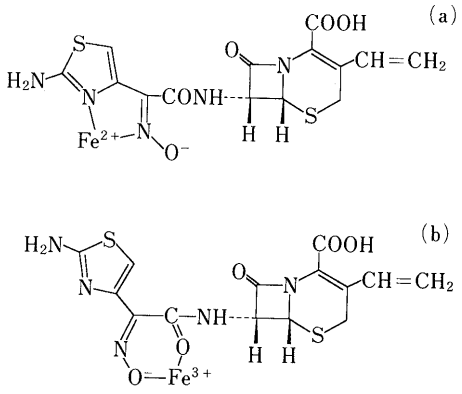

Fig. 5. A Possible Coordination Modes of CFDN to Iron(II) (a) and Iron(III) (b)

The λ_{max} value of the iron(II)–CFDN system (540 nm) is very similar to that of the iron(II)–bipy system (523 nm). Thus, iron(II) must be coordinated to CFDN through the two nitrogen atoms of the thiazole ring and the oxime. In addition, the oxime moiety of CFDN in iron(II) complexes should be deprotonated under neutral conditions, since pK_a values of the oxime moiety (pK_a 9.63) apparently went down to about 5.5 in the system of iron(II) (0.5 mM)–CFDN (1.0 mM), and no complexes with the oxime moiety being protonated were observed in the titration study (Fig. 2a). The possible coordination mode of CFDN to iron(II) is shown in Fig. 5a. The 2N coordination in the iron(II) complexes may be the cause

of the red coloration of the stools.

The λ_{max} value of the iron(III)–CFDN system (432 nm) is very similar to that of the iron(III)–acac system (421 nm). Thus, iron(III) should be coordinated to CFDN through the two oxygen atoms of the oxime and the amide. The possible coordination mode of CFDN to iron(III) is shown in Fig. 5b.

Coloration The iron(II)–CFDN and iron(III)–CFDN systems were colored pure red and orange, respectively. However, neither the iron(II)–CFIX nor the iron(III)–CFIX system showed coloration of complexation. These phenomena indicate that CFIX having carboxymethoxyimino group in place of oxime group of CFDN does not form complexes with either iron(II) or iron(III). In view of these results and the fact that the oxime group of CFDN is deprotonated in both of the iron(II) and iron(III) complexes, the coordination property of CFDN is probably mainly based on the deprotonation property (pK_a 9.63) at the oxime moiety. In general, although a ligand with higher pK_a values of the coordination sites gives larger stability constants, deprotonation at the coordination site occurs in many cases when a coordinate bond is formed. In the case of CFDN, the deprotonation at the oxime is believed to increase electron donating properties of the coordination atoms (nitrogen and oxygen atoms) of the oxime group and to stabilize the complexes.

Conclusion

Both iron(II) and iron(III) form stable complexes with CFDN. CFDN is believed to coordinate to iron(II) *via* the oxime and thiazole-ring nitrogen atoms, and to iron(III) *via* the amide and deprotonated oxime oxygen atoms. The oxime group participates in the formation of both the iron(II) and iron(III) complexes. The deprotonation property of the oxime group may be deeply involved in the complexation of CFDN. These data indicate the possibility of complex formation of CFDN in the digestive tract with extraneous iron(II) and iron(III), for example, with multivitamins, powdered milk or other foods containing iron supplements. It is considered that the iron complex formation depresses the BA.

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