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Equilibrium and Kinetic Studies of the Iron(III) Complex with *N*-Pyridoxylidene-L-tryptophan: Electronic Absorption and Circular Dichroism Spectral Characteristics, Stability, and Formation and Ligand-Substitution Reaction Mechanisms

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Studies have been made of the electronic absorption and circular dichroism (CD) spectral characteristics, thermodynamic stability, and formation and ligand-substitution reaction kinetics of an iron(III) complex with *N*-pyridoxylidene-L-tryptophan (Hpdx-L-Htrp). The 1:2 (metal:ligand) complex forms in an acidic aqueous solution with the stability constant (β_2) of $\log \beta_2 = 18.38 \pm 0.10\ \mathrm{mol^{-2}\,dm^6}$ ($0.50\ \mathrm{mol\,dm^{-3}}$ (NaCl), 25 °C). The complex shows a characteristic CD spectrum in the charge-transfer region. The kinetics were studied by a stopped-flow CD method in a $0.50\ \mathrm{mol\,dm^{-3}}$ aqueous NaCl solution at 25 °C. The formation of the complex proceeds through six parallel pathways of $\mathrm{Fe^{3+}}$, $\mathrm{FeOH^{2+}}$, and $\mathrm{FeCl^{2+}}$ with $\mathrm{H_2pdx\text{-}L\text{-}Htrp^+}$ and $\mathrm{H_2pdx\text{-}L\text{-}trp}$, with the coordination of the carboxylato group to iron(III) as the rate-determining step. The ligand-substitution reaction of the complex by ethylenediamine-*N,N,N′,N′*-tetraacetic acid depends neither on the entering ligand nor on the hydrogen-ion concentration. The dissociation of a coordinated $\mathrm{Hpdx\text{-}L\text{-}trp^-}$ from $\mathrm{[Fe(Hpdx\text{-}L\text{-}trp)_2]^+}$ is concluded to be the rate-determining step. The reaction mechasnisms are discussed in detail.

Metal complexes containing Schiff bases of pyridoxal and optically active amino acids have attracted great attention as reaction intermediates since the pioneering work by Metzler and Snell[1)] on the nonenzymatic transamination of the Schiff base of pyridoxal and glutamic acid. This is because these complexes play an important role not only in the transamination, but also in the racemization, decarboxylation, and related reactions, of the amino acids. Many studies have been made since that time of the structures of these Schiff-base complexes of various sorts of metal ions and amino acids; an iron(III) complex with pyridoxal and DL-valine has been isolated in the crystalline state, and its structure has been determined by the X-ray diffraction method.[2)] However, little have been known on the reaction kinetics and mechanism as well as on the thermodynamic stability of these complexes.

In the present work, the formation and ligand-substitution reactions of the iron(III) complex with a Schiff base of pyridoxal and L-tryptophan(*N*-pyridoxylidene-L-tryptophan, Hpdx-L-Htrp) were investigated by means of the stopped-flow circular dichroism (CD) method. The iron(III) complex with Hpdx-L-Htrp was chosen here because the Schiff-base ligand forms quantitatively over a wide range of acidities and because the iron(III) complex is stable to coordinated-ligand reactions during a kinetic investigation. The reactions were studied in a $0.50\ \mathrm{mol\,dm^{-3}}$ aqueous sodium chloride solution in order to make it possible to compare the present work's to those in the literature data on *N*-pyridoxylidene-L(and/or D)-amino acids. The reaction mechanisms of the formation and ligand-substitution by ethylenediamine-*N,N,N′,N′*-tetraacetic acid (EDTA, $\mathrm{H_4edta}$) of the iron(III) complex will be discussed in detail.

Experimental

Reagents. The analytical-grade reagents of pyridoxal (as hydrochloride) and L-tryptophan were supplied by the Tokyo Kasei Kogyo Co., Ltd., Tokyo, and the Wako Pure Chemicals Ind., Ltd., Osaka, and by the Kanto Chemical Co., Ltd., respectively. The Hpdx-L-Htrp was prepared by the condensation of pyridoxal and L-tryptophan in an aqueous solution in the usual manner. The ligand was characterized by the elemental analysis. A stock solution of iron(III) was prepared from $\mathrm{(NH_4)Fe(SO_4)_2\cdot 12H_2O}$ and was acidified with the minimum quantity of sulfuric acid necessary to prevent the hydrolysis of iron(III). The absence of traces of iron(II) was checked on the the stock solution by means of a reaction with 2,2′-bipyridine. The other reagents used were of an analytical or equivalent grade.

Measurements. The electronic absorption and CD spectra were recorded on a JASCO spectrophotometer, model UVIDEC-1M, and a JASCO spectropolarimeter, model J-500C. The protonation, stability, and rate constants were determined in a $0.50\ \mathrm{mol\,dm^{-3}}$ aqueous NaCl solution at 25.0 ± 0.1 °C.

The pH of the solution was measured with an Orion Research digital pH meter, model 801A, with glass and saturated calomel electrodes. The hydrogen-ion concentration, $-\log\,[\mathrm{H^+}]$, was calculated from the pH value, $\mathrm{pH_{meas}}$, according to Eq. 1:

$$-\log\,[\mathrm{H^+}] = \mathrm{pH_{meas}} + \log f_{\mathrm{H^+}} \qquad (1)$$

The coefficient, $f_{\mathrm{H^+}}$, was estimated on the basis of the literature procedure[3)] by defining a solution containing $0.0100\ \mathrm{mol\,dm^{-3}}$ of HCl and $0.4900\ \mathrm{mol\,dm^{-3}}$ of NaCl at 25 °C as having $-\log\,[\mathrm{H^+}] \equiv 2.00$.

The kinetic runs were carried out on the JASCO spectropolarimeter, to which were attached a stopped-flow apparatus, model SFC-5, a data processor, model DP-500, and a Watanabe Sokki X-Y recorder, model WX-441. The formation reaction was studied at 600 nm by following the

CD-intensity change with the time under the pseudo-first-order kinetic conditions for the metal-ion concentration. The ligand-substitution reaction of the iron(III) complex with Hpdx-L-Htrp by EDTA was studied at the same wavelength under the pseudo-first-order kinetic conditions used for the iron(III) complex concentration. These conditions satisfied the requirement that the CD intensity depends linearly on the concentration of the iron(III) complex with Hpdx-L-Htrp. This complex was stable enough to show no racemization of the coordinated ligand during the kinetic runs.

The observed rate constant, k_{obsd}, was calculated from the slope of the linear relation, $-\ln(\Delta E_t - \Delta E_\infty)$ vs. t, on the basis of Eq. 2:

$$\ln\{(\Delta E_0 - \Delta E_\infty)/(\Delta E_t - \Delta E_\infty)\} = k_{\text{obsd}}t \qquad (2)$$

where ΔE_0, ΔE_t, and ΔE_∞ stand for the CD intensities at the initial state, at time t, and at equilibrium, respectively. Good straight lines were obtained for the formation and lignd-substitution reactions for more than three half-life periods.

Results and Discussion

Electronic Absorption and CD Spectra. Figure 1 shows the electronic absorption and the CD spectra of the iron(III) complex, with the corresponding values for Hpdx-L-Htrp and the free ligand shown for the sake of comparison. The iron(III) complex gives new absorption bands as shoulders near 18×10^3 and $23\times10^3\,\text{cm}^{-1}$ assignable to the $t_{2g}(O_h)\leftarrow\pi$ and $\pi_2^* \leftarrow e_g(O_h)$ charge-transfer transitions, and a shoulder near $28\times10^3\,\text{cm}^{-1}$ and bands around $35\times10^3\,\text{cm}^{-1}$ assignable to the $\pi_1^* \leftarrow \pi$ (azomethine chromophore) and $\pi_2^* \leftarrow \pi$ (pyridine and indole ring chromophores) transitions of the coordinated ligand.[4—14] The CD spectrum of the iron(III) complex shows new negative and positive bands at 16.6×10^3 and $19.1\times10^3\,\text{cm}^{-1}$ in the $t_{2g}(O_h)\leftarrow\pi$ transition region due to a diastereoisomeric pair of the complex, Δ_{LL}-spiral and Λ_{LL}-spiral,[15] with the meridional (mer) coordination[2,16—19] of Hpdx-L-Htrp. The relatively large magnitude of the $\Delta\varepsilon$ on the CD spectrum indicates that one of the diastereoisomers is present in solution in preference to the other and that the CD spectral pattern in the $t_{2g}(O_h) \leftarrow \pi$ transition region reflects the total concentration of the complex.

Hence, the absorption and CD spectral bands in the wavenumber region below $21\times10^3\,\text{cm}^{-1}$ can be used for the thermodynamic and kinetic studies of the iron(III) complex with Hpdx-L-Htrp.

Stability. The protonation constants of Hpdx-L-Htrp were determined spectrophotometrically for $K_{a(\text{phOH})}$ $(=[\text{Hpdx-L-trp}^-]/[\text{H}^+][\text{pdx-L-trp}^{2-}])$ at the absorption maximum of the pdx-L-trp^{2-} species (394 nm) and for $K_{a(\text{pyNH})}$ $(=[\text{H}_2\text{pdx-L-trp}]/[\text{H}^+][\text{Hpdx-L-trp}^-])$ at that of the Hpdx-L-trp$^-$ species (390 nm), exept for $K_{a(\text{COOH})}$ $(=[\text{H}_2\text{pdx-L-Htrp}^+]/[\text{H}^+][\text{H}_2\text{pdx-L-trp}])$, which was estimated separately (vide infra) because of the very small spectral changes on protonation to the H_2pdx-L-trp sepcies. The data are summarized in Table 1.

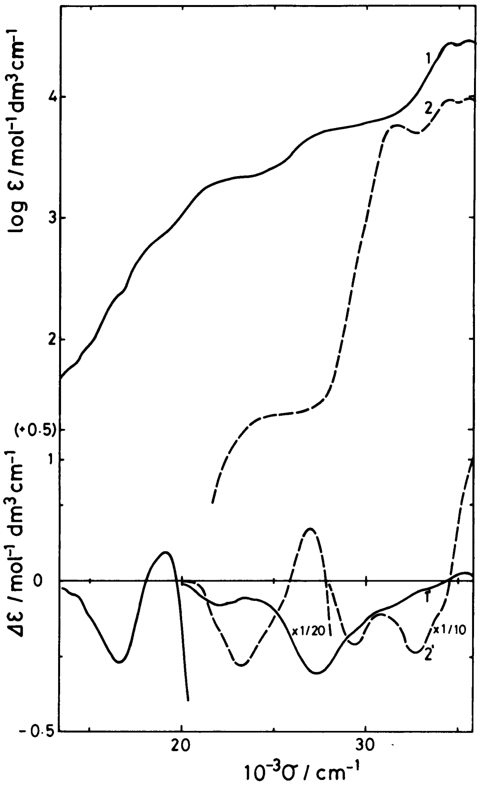

Fig. 1. Electronic absorption and CD spectra of iron(III) complex with Hpdx-L-Htrp in aqueous solution. 1,1′: $[\text{Fe(Hpdx-L-trp)}_2]^+$; and 2,2′: H_2pdx-L-trp species. The complex was used after isolation as a solid (Anal.($\text{C}_{38}\text{H}_{38}\text{N}_6\text{O}_8\text{Cl}_2\text{FeCl}\cdot\text{H}_2\text{O}$) C,H,N,Fe).

Table 1. Protonation and Stability Constants

	$\log K_{a(\text{COOH})}$	$\log K_{a(\text{pyNH})}$	$\log K_{a(\text{phOH})}$
	$\text{mol}^{-1}\,\text{dm}^3$	$\text{mol}^{-1}\,\text{dm}^3$	$\text{mol}^{-1}\,\text{dm}^3$
Hpdx-L-Htrp[a]	2.30±0.05	4.10±0.05	8.24±0.05
	$\log \beta_1$	$\log \beta_2$	
	$\text{mol}^{-1}\,\text{dm}^3$	$\text{mol}^{-2}\,\text{dm}^6$	
$[\text{Fe(Hpdx-L-trp)}_2]^+$	9.65±0.10	18.38±0.10	

0.50 mol dm^{-3} (NaCl), 25 °C. a) The $K_{a(\text{COOH})}$, $K_{a(\text{pyNH})}$, and $K_{a(\text{phOH})}$ of the ligand refer to the protonations at the carboxylate oxygen, pyridyl nitrogen, and phenolate oxygen respectively.

The stability constants of the iton(III) complex with Hpdx-L-Htrp were determined spectrophotometrically at 450 nm under the experimental conditions of $C_L \gg C_M$ (C_L and C_M are the total concentrations of iron(III) and Hpdx-L-Htrp; C_M is of the order of 10^{-4} mol dm^{-3}). By taking into consideration the hydrolysis constant ($\log K^{11}_{OH} = -2.72$ mol dm^{-3} (0.50 mol dm^{-3} (NaClO$_4$), 25 °C; $K^{11}_{OH} = [FeOH^{2+}][H^+]/[Fe^{3+}]$)[20,21] and the chloro complex formation constant ($\log \beta^1_{Cl} = 0.64$ mol^{-1} dm^3 (0.50 mol dm^{-3}(—), 25 °C; $\beta^1_{Cl} = [FeCl^{2+}]/[Fe^{3+}][Cl^-]$)[22] of iron(III), and the protonation constants of Hpdx-L-Htrp, the following relations can be derived[23] for the bis-ligand complex:

$$\log (A_{max} - A)/(A - A_{min}) = 2 \log \{k_{a(phOH)}[H^+] (K_{a(COOH)} [H^+] + 1)\} + \log (1 + K^{11}_{OH} [H^+]^{-1} + \beta^1_{Cl}[Cl^-]) - \log \beta_2 C_L^2 \tag{3}$$

and for the mono-ligand complex:

$$\log (A_{max}' - A)/(A - A_{min}) = \log \{k_{a(phOH)}[H^+] (K_{a(COOH)} [H^+] + 1)\} + \log (1 + K^{11}_{OH} [H^+]^{-1} + \beta^1_{Cl}[Cl^-]) - \log \beta_1 C_L \tag{4}$$

Here, A_{max} stands for the absorbance of a solution in which the bis-ligand complex forms quantitatively: A_{max}', for that of a solution in which the mono-ligand complex forms quantitatively; A_{min}, for that of a solution containing the ligand alone, and A, for that of a solution in which the bis-ligand or the mono-ligand complex and the free ligand coexist.

The experimental data are depicted in Fig. 2. Good linear relations with a slope of unity were obtained above $-\log [H^+]$ of 2.3 (cf. line 1) and below 1.9 (cf. line 2) with a suitable assumption of the value of A_{max}' between the absorbance and the hydrogen-ion-concentration terms of Eqs. 3 and 4, respectively. The stability constants for the bis-ligand complex (β_2) and the mono-ligand complex (β_1) are summarized in Table 1.

The behavior of pyridyl nitrogen during the complex formation should also be discussed. In a weakly acidic aqueous solution, the principal complex formation reaction equilibrium can be expressed as follows ($C_L \gg C_M$):

$$Fe^{3+} + 2\,H_2pdx\text{-L-trp} \rightleftharpoons [Fe(Hpdx\text{-L-trp})_2]^+ + 2H^+ \tag{5}$$

Here, pyridyl nitrogen was assumed still to be protonated in the coordinated ligand, because a reasonable analysis of the experimental data on absorbance vs. the hydrogen-ion concentration based on the formation of $[Fe(pdx\text{-L-trp})_2]^-$ and the liberation of four protons per complex instead of Eq. 5 was unsuccessful. This means that the coordination of

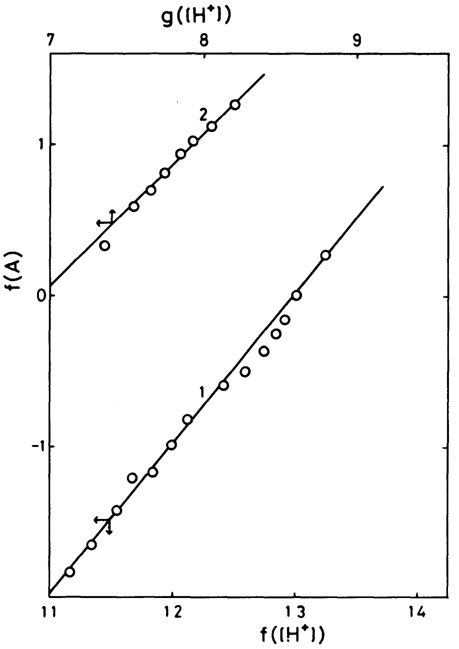

Fig. 2. Dependence of $f(A)$ on $f([H^+])$ and on $g([H^+])$. $f(A) \equiv \log (A_{max} - A)/(A - A_{min})$ or $\log (A_{max}' - A)/(A - A_{min})$, $f([H^+]) \equiv 2 \log \{K_{a(phOH)}[H^+](K_{a(COOH)}[H^+]+1)\} + \log (1 + K^{11}_{OH}[H^+]^{-1} + \beta^1_{Cl}[Cl^-])$, $g([H^+]) \equiv \log \{K_{a(phOH)}[H^+](K_{a(COOH)}[H^+]+1)\} + \log (1 + K^{11}_{OH}[H^+]^{-1} + \beta^1_{Cl}[Cl^-])$, C_L: 2.00×10^{-3} mol dm^{-3}; C_M: 1.25×10^{-4} mol dm^{-3}; 0.50 mol dm^{-3} (NaCl); 25 °C; and λ: 450 nm.

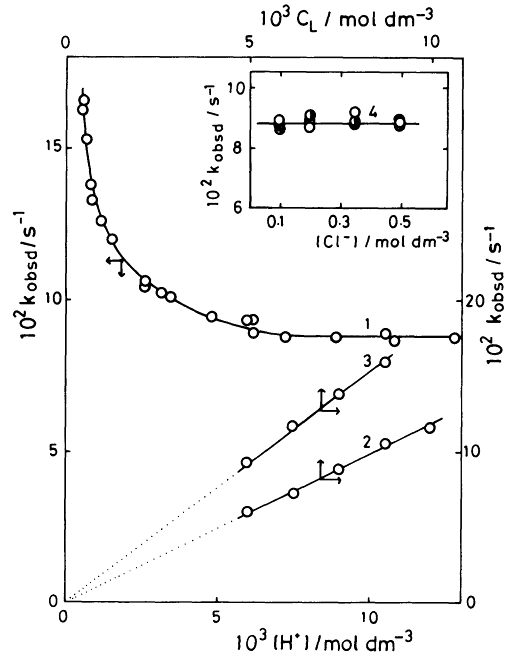

Fig. 3. Dependence of k_{obsd} on $[H^+]$, on C_L, and on $[Cl^-]$. 1: C_L: 7.50×10^{-3} mol dm^{-3}; C_M: 2.50×10^{-4} mol dm^{-3}; 0.50 mol dm^{-3} (NaCl). 2: C_M: 2.50×10^{-4} mol dm^{-3}; $[H^+]$: 1.05×10^{-2} mol dm^{-3}; 0.50 mol dm^{-3} (NaCl). 3: C_M: 2.50×10^{-4} mol dm^{-3}; $[H^+]$: 8.10×10^{-4} mol dm^{-3}; 0.50 mol dm^{-3} (NaCl). 4: C_L: 7.50×10^{-3} mol dm^{-3}; C_M: 2.50×10^{-4} mol dm^{-3}; $[H^+]$: 7.18×10^{-3} (◐), 1.05×10^{-2} (○), and 1.28×10^{-2} mol dm^{-3} (◑); [NaCl]+[NaClO$_4$]: 0.50 mol dm^{-3}. 25 °C; and λ: 600 nm.

Hpdx-L-Htrp to iron(III) has little influence on the basicity of pyridyl nitrogen.

Formation-Reaction Kinetics. Experimental data on the relations between k_{obsd} and $[\mathrm{H}^+]$, k_{obsd} and C_{L}, and k_{obsd} and $[\mathrm{Cl}^-]$ under the conditions of $C_{\mathrm{L}} \gg C_{\mathrm{M}}$ are given in Fig. 3. The linear relation between k_{obsd} and C_{L} at constant $[\mathrm{H}^+]$ and $[\mathrm{Cl}^-]$ values, which can be extrapolated to the point of origin, indicates that the coordination of the first Hpdx-L-Htrp to iron(III) corresponds to the rate-determining step.

In the formation of the mono-ligand complex as the rate-determining step, six reaction pathways contributing Fe^{3+}, FeOH^{2+}, and FeCl^{2+} for the metal ion and $\mathrm{H_2}$pdx-L-Htrp$^+$ and $\mathrm{H_2}$pdx-L-trp for the ligand are possible by taking into consideration the protonation constants of the ligand, the hydrolysis constant of iron(III), and the stability constants of the chloro and the Hpdx-L-Htrp complexes, and the rapid attainment of equilibria for the protolytic processes[3] as compared with the complex formation process. The following rate equation can be derived under the pseudo-first-order kinetic conditions for C_{M}:

$$\mathrm{d}[[\mathrm{Fe(Hpdx\text{-}L\text{-}trp)_2}]^+]/\mathrm{d}t = k_{\mathrm{obsd}}([[\mathrm{Fe(Hpdx\text{-}L\text{-}trp)_2}]^+]_\infty - [[\mathrm{Fe(Hpdx\text{-}L\text{-}trp)_2}]^+]) \tag{6}$$

$$k_{\mathrm{obsd}}(1 + K^{11}_{\mathrm{OH}}[\mathrm{H}^+]^{-1} + \beta^1_{\mathrm{Cl}}[\mathrm{Cl}^-])(K_{\mathrm{a(COOH)}}[\mathrm{H}^+] + 1)\, C_{\mathrm{L}}{}^{-1} = (k_{31} + k_{21(\mathrm{Cl})}\beta^1_{\mathrm{Cl}}[\mathrm{Cl}^-])K_{\mathrm{a(COOH)}}[\mathrm{H}^+] + k_{30} + k_{20(\mathrm{Cl})}\beta^1_{\mathrm{Cl}}[\mathrm{Cl}^-] + k_{21}K^{11}_{\mathrm{OH}}K_{\mathrm{a(COOH)}} + k_{20}K^{11}_{\mathrm{OH}}[\mathrm{H}^+]^{-1} \tag{7}$$

Here, k_{ij} is the rate constant for the reaction pathway of $[\mathrm{Fe(OH)}_{3-i}]^{i+}$ (i=2 and 3), and $k_{2j(\mathrm{Cl})}$, that for the pathway of FeCl^{2+} with $\mathrm{H_2}$pdx-L-$\mathrm{H}_j$trp^{j+} (j=0 and 1).

The experimental data given in Fig. 3 indicate that k_{obsd} is constant in the case of changes in $[\mathrm{Cl}^-]$ within the limits of experimental error and that the k_{obsd} value can be classified by acidity region: that in the higher-acidity region, where the contribution of the pathway of k_{20} can be neglected, and that in the lower-acidity region, where that of the pathways of k_{31} and $k_{21(\mathrm{Cl})}$ can be neglected. Firstly, the data in the higher-acidity region ($-\log\,[\mathrm{H}^+] < 2.4$) were analyzed at a constant $[\mathrm{Cl}^-]$ under the assumption of a suitable numerical value of $K_{\mathrm{a(COOH)}}$ so that a good linear relation could be obtained between $k_{\mathrm{obsd}}\,(1+K^{11}_{\mathrm{OH}}[\mathrm{H}^+]^{-1}+\beta^1_{\mathrm{Cl}}[\mathrm{Cl}^-])(K_{\mathrm{a(COOH)}}[\mathrm{H}^+]+1)\, C_{\mathrm{L}}{}^{-1}$ and $[\mathrm{H}^+]$. Secondly, by using the $K_{\mathrm{a(COOH)}}$ value thus estimated and the intercept, which is equal to the $k_{30}+k_{20(\mathrm{Cl})}\beta^1_{\mathrm{Cl}}[\mathrm{Cl}^-]+k_{21}K^{11}_{\mathrm{OH}}K_{\mathrm{a(COOH)}}$ derived from the above relation, the data in the lower-acidity region ($-\log\,[\mathrm{H}^+] > 2.4$) were analyzed for the linear relation between $k_{\mathrm{obsd}}(1+K^{11}_{\mathrm{OH}}[\mathrm{H}^+]^{-1}+\beta^1_{\mathrm{Cl}}[\mathrm{Cl}^-])(K_{\mathrm{a(COOH)}}[\mathrm{H}^+]+1)\, C_{\mathrm{L}}{}^{-1}$ and $[\mathrm{H}^+]^{-1}$. Finally, $k_{31}K_{\mathrm{a(COOH)}}+k_{21(\mathrm{Cl})}\beta^1_{\mathrm{Cl}}K_{\mathrm{a(COOH)}}\,[\mathrm{Cl}^-]$ and $k_{30}+k_{21}K^{11}_{\mathrm{OH}}K_{\mathrm{a(COOH)}}+k_{20(\mathrm{Cl})}\beta^1_{\mathrm{Cl}}[\mathrm{Cl}^-]$ were plotted against $[\mathrm{Cl}^-]$ to obtain the individual rate constant. Those of k_{31}, $k_{31(\mathrm{Cl})}$, k_{20}, $k_{20(\mathrm{Cl})}$, and $k_{30}+k_{21}K^{11}_{\mathrm{OH}}K_{\mathrm{a(COOH)}}$, the last of which cannot be separated because of the proton ambiguity (vide infra), are summarized in Table 2.

Many kinetic data have been reported[24] for the iron(III) complexes with various sorts of ligands. The rate constant for the pathway of Fe^{3+} with $\mathrm{H_2}$pdx-L-Htrp$^+$, k_{31}, is in close proximity to the constants for the reaction pathways of Fe^{3+} with aliphatic carboxylic acids. This fact indicates that the coordination of Hpdx-L-Htrp to iron(III) proceeds through the donation of the carboxylato group of the amino acid moiety rather than the phenolato group of the pyridoxal moiety, because the analysis of kinetic data by using $K_{\mathrm{a(phOH)}}$ instead of $K_{\mathrm{a(COOH)}}$ gave an extremely small and unreasonable magnitude of k_{31}. Furthermore, this close proximity in the rate constants substantiates the fact that the rate-determining step of the reaction of iron(III) with Hpdx-L-Htrp lines in the coordination of the first ligand to iron(III), because the rate constants for Fe^{3+} with these carboxylic acid ligands have been determined on the mono(carboxylato)iron(III) complexes.[25–32]

Contrary to k_{31}, k_{20} is considerably smaller than the value of FeOH^{2+} with $\mathrm{ClCH_2COO^-}$ (k_{20}: $(4.1\pm0.2)\pm10^4$ mol^{-1} dm^3 s^{-1} (1 mol dm^{-3}($\mathrm{NaClO_4}$), 25 °C).[25] The magnitude of k_{20} reflects the fact that the carboxylato group of $\mathrm{H_2}$pdx-L-trp ligand species may not be free, but hydrogen-bonded. There is a possibility of an intramolecular hydrogen bond between the hydroxyl and the carboxylato groups for the enol imine form and also between the imino hydrogen and the carboxylato group for the keto enamine form of Hpdx-L-Htrp. This role of the intramolecular hydrogen bond is substantiated by a comparison of the rate constant for FeOH^{2+} with $\mathrm{ClCH_2COO^-}$ (k_{20}: vide supra) with that for FeOH^{2+} with the monoprotonated salicylato ligand (k_{21}: 2.9×10^3 mol^{-1} dm^3 s^{-1} (0.1 mol dm^{-3}

Table 2. Rate Constants for the Iron(III) Complex with Hpdx-L-Htrp

Pathway	k_{ij}
	mol^{-1} dm^3 s^{-1}
Fe^{3+}+$\mathrm{H_2}$pdx-L-Htrp$^+$ (k_{31})	10.1±0.3
Fe^{3+}+$\mathrm{H_2}$pdx-L-trp and FeOH^{2+}+$\mathrm{H_2}$pdx-L-Htrp$^+$ ($k_{30}+k_{21}K^{11}_{\mathrm{OH}}K_{\mathrm{a(COOH)}}$)	21.3±0.6
FeCl^{2+}+$\mathrm{H_2}$pdx-L-Htrp$^+$ ($k_{21(\mathrm{Cl})}$)	11.7±0.3
FeCl^{2+}+$\mathrm{H_2}$pdx-L-trp ($k_{20(\mathrm{Cl})}$)	11.5±0.3
FeOH^{2+}+$\mathrm{H_2}$pdx-L-trp (k_{20})	32.5±0.3
$[\mathrm{Fe(Hpdx\text{-}L\text{-}trp)_2}]^+$ (+$\mathrm{H_2O}$) ($k_{-3\bar{1}}$)	2.9±0.3[a]

0.50 mol dm^{-3} (NaCl), 25 °C. a) $k_{-3\bar{1}}$/s^{-1}.

($LiClO_4$), 25 °C[31] and (1.4±0.1) ×10^4 mol^{-1} dm^3 s^{-1} (1.0 mol dm^{-3} (KNO_3), 25 °C[32]). The progress of the reaction through the donation of the carboxylato group indicates that there are no fundamental differences in the kinetics of the enol imine and the keto enamine forms of Hpdx-L-Htrp.

The rate constant, $k_{21(Cl)}$, is very close to k_{31}, as can be expected from independence of k_{obsd} on [Cl^-] at constant C_L and [H^+] values. There have been several papers[33-36] concerning with kinetics of Fe^{3+} with Cl^-; the rate-determining step for the formation of $FeCl^{2+}$ from Fe^{3+} and Cl^- has been concluded to be the release of a water molecule from the primary coordination sphere of iron(III).[33,35] The kinetic data (k/mol^{-1} dm^3 s^{-1} ($Fe^{3+}+Cl^-$): 9.4±1.0[33] and 4.8±0.4;[36] and k/s^{-1} ($FeCl^{2+}+H_2O$): 2.3±0.2[33] and 3.2±0.2[36] (1.0 mol dm^{-3},[33] and 1.5 mol dm^{-3} ($NaClO_4$), 25 °C[36]) and the ^{35}Cl and ^{17}O NMR spectral data[34,35] on the exchange of a water molecule and a chloride ion between the primary coordination sphere of the chloro and aqua iron(III) complex species and the bulk solvent establish that the exchange process, together with the protolytic reaction of $Fe^{3+} \rightleftharpoons FeOH^{2+} + H^+$, is rapid enough for us to regard the process as a reversible pre-equilibrium for the kinetic process of Fe^{3+} with Hpdx-L-Htrp. Our experimental data on the close proximity of $k_{21(Cl)}$ to k_{31} support this deduction: The coordinated chloro ligand makes no intrinsic contribution to the coordination reaction kinetics of Hpdx-L-Htrp to iron(III).

The deduction mentioned above indicates that k_{21} can be estimated from the composite rate constants of $k_{30}+k_{21}K^{11}_{OH}K_{a(COOH)}$ given in Table 2, because k_{30} can reasonably be assumed to be equal to $k_{20(Cl)}$. The value of k_{21} thus calculated was 26±6 mol^{-1} dm^3 s^{-1}.

It can be summarized that the formation of the iron(III) complex with Hpdx-L-Htrp proceeds through the parallel pathways of Fe^{3+}, $FeOH^{2+}$, and $FeCl^{2+}$ with H_2pdx-L-$Htrp^+$ and H_2pdx-L-trp via the coordination of the carboxylato group to iron(III) as the mechanistic rate-determining step. This coordination of the carboxylato group follows the rapid coordination of the azomethine nitrogen and the phenolato oxygen to form an intermediate, Fe(Hpdx-L-trp)$^{2+}$, with five- and six-membered fused chelate rings. The coordination of the second Hpdx-L-Htrp to the intermediate proceeds in just the same way as that of the first one, but in a more rapid way as a result of the labilization[3,37] of the remaining coordinated water molecules in Fe(Hpdx-L-trp)$^{2+}$ to complete the thermodynamically more stable [Fe(Hpdx-L-trp)$_2$]$^+$.

Ligand-Substitution Reaction Kinetics. Fe(Hpdx-L-trp)^2]$^+$ gives a ligand-substitution with EDTA to form Fe(edta)$^-$ quantitatively in an acidic aqueous solution under the experimental conditions of $C_{edta} \gg C_M$ and $2C_L > C_M$. The ligand-substitution reaction was of the first order with respect to the starting complex concentration, as has been described in the Experimental section. The relations between k_{obsd} and C_{edta}, between k_{obsd} and [H^+], and between k_{obsd} and C_L are given in Table 3. The k_{obsd} depends neither on C_{edta}, C_L, nor [H^+]; the ligand-substitution reaction proceeds by means of the dissociation of one of the Hpdx-L-trp$^-$ ligands from [Fe(Hpdx-L-trp)$_2$]$^+$ to form Fe(Hpdx-L-trp)$^{2+}$ as an intermediate as the rate-determining step:

Table 3. Kinetic Data of the Ligand-Substitution Reaction

[H^+]	C_{edta}	C_L	k_{obsd}
10^3 mol dm^{-3}	10^2 mol dm^{-3}	10^3 mol dm^{-3}	s^{-1}
0.575	0.500	3.00	2.87
3.24	0.500	3.00	3.20
3.98	0.500	3.00	2.95
5.89	0.500	3.00	3.03
8.71	0.500	3.00	2.96
9.77	0.500	3.00	3.06
4.91	0.250	3.00	2.87
4.91	0.500	3.00	2.91
4.27	1.00	3.00	3.00
5.01	1.00	3.00	2.96
3.98	1.50	3.00	3.23
4.27	1.70	3.00	2.80
4.57	1.75	3.00	2.77
4.57	0.500	2.00	2.94
4.57	0.500	2.50	3.01
4.57	0.500	3.50	3.07
4.57	0.500	4.00	2.86

C_M: 1.00×10^{-4} mol dm^{-3}, 0.50 mol dm^{-3} (NaCl), 25 °C.

$$-d[[Fe(Hpdx\text{-}L\text{-}trp)_2]^+]/dt = k_{obsd}[[Fe(Hpdx\text{-}L\text{-}trp)_2]^+] \quad (8)$$

$$k_{obsd} = k_{-3\bar{1}} \quad (9)$$

The rate constant, $k_{-3\bar{1}}$, as estimated from Table 3, is given in Table 2.

The ligand-substitution reaction of [Fe(Hpdx-L-trp)$_2$]$^+$ with EDTA belongs to a simple aquation, because the reaction is of zeroth order with respect to the entering and leaving ligand concentrations, which fact rules out the liberation of the second ligand from Fe(Hpdx-L-trp)$^{2+}$ or its mixed-ligand complex with EDTA as an intermediate as the rate-determining step. Furthermore, the pathway of [Fe(Hpdx-L-trp)$_2$]$^+$+H^+ and analogous proton-assisted pathways, which are anticipated from the formation reaction kinetics, are impracticable in the present ligand-substitution reaction. Only the pathway of [Fe(Hpdx-L-trp)$_2$]$^+$ (+H_2O) to form Fe(Hpdx-L-trp)$^{2+}$ and Hpdx-L-trp$^-$, the latter of which will rapidly protonate to form H_2pdx-L-trp and H_2pdx-L-$Htrp^+$, is practicable. The mechanistic rate-determining step may be the dissociation from the central metal atom of the carboxylato group of the coordinated ligand in the thermodynamically more stable [Fe(Hpdx-L-trp)$_2$]$^+$ by taking the kinetic characteristics of the formation and the basicity of the ligating groups on Hpdx-L-Htrp into consideration.

In conclusion, *N*-pyridoxylidene-L-tryptophan forms a 1:2 (metal: ligand) complex with iron(III) of a high thermodynamic stability and lability. The coordination of the ligand proceeds through six parallel pathways of Fe^{3+}, FeOH^{2+}, and FeCl^{2+} with H$_2$pdx-L-Htrp$^+$ and H$_2$pdx-L-trp, with the donation of the carboxylato oxygen to iron(III) as the rate-determining step. This coordinated ligand leaves the central metal atom dissociatively, even in the presence of other multidentate ligands, through the pathway of Fe(Hpdx-L-trp)$^{2+}$ and Hpdx-L-trp$^-$, with cleavage of the carboxylato oxygen–iron(III) bond as the rate-determining step.

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