Kinetics and Mechanism of the Complexation of La^{3+} and Cu^{2+} Ions with *N*-Methylacetohydroxamic Acid and Desferrioxamine B[1]

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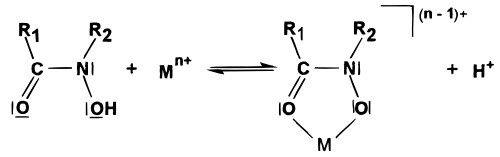
The kinetics and mechanism of the complexation of La^{3+} and Cu^{2+} ions with desferrioxamine B (H_4dfb^+) and *N*-methylacetohydroxamic acid (NMHA) in aqueous medium were studied by stopped-flow and ^1H NMR methods. The equilibrium constants for reactions ($M^{n+} + HA \rightleftarrows MA^{(n-1)+} + H^+$) of NMHA with Cu^{2+} and La^{3+} were determined by the combined pH−spectral titration method at 25 ± 0.1 °C as (1.58 ± 0.02) × 10^{-1} and (3.5 ± 0.2) × 10^{-4}, respectively. In aqueous solution of 2 M ionic strength, the kinetic parameters for complexation of La^{3+} and Cu^{2+} with NMHA were determined as k(25 °C) = 3.0 ± 0.3 s^{-1}, $\Delta H^{\ddagger}$ = 76 ± 3 kJ mol^{-1}, $\Delta S^{\ddagger}$ = 19 ± 7 J K^{-1} mol^{-1}, $\Delta V^{\ddagger}$ = +5.3 ± 0.5 cm^3/mol and k(25 °C) = 3.4 ± 0.2 s^{-1}, $\Delta H^{\ddagger}$ = 69 ± 1 kJ mol^{-1}, $\Delta S^{\ddagger}$ = −3 ± 3 J K^{-1} mol^{-1}, $\Delta V^{\ddagger}$ = +5.0 ± 0.5 cm^3/mol, whereas with H_4dfb^+ they were determined as k(25 °C) = 2.9 ± 0.3 s^{-1}, $\Delta H^{\ddagger}$ = 76 ± 1 kJ mol^{-1}, $\Delta S^{\ddagger}$ = 34 ± 4 J K^{-1} mol^{-1}, $\Delta V^{\ddagger}$ = +5.2 ± 0.5 cm^3/mol; and k(25 °C) = 3.0 ± 0.4 s^{-1}, $\Delta H^{\ddagger}$ = 72 ± 1 kJ mol^{-1}, $\Delta S^{\ddagger}$ = 5 ± 2 J K^{-1} mol^{-1}, $\Delta V^{\ddagger}$ = +3.4 ± 0.2 cm^3/mol, respectively. The rotation about the C−N hydroxamate bonds in NMHA and H_4dfb^+ is characterized by k(25 °C) = 11 ± 2 s^{-1}, $\Delta H^{\ddagger}$ = 76 ± 5 kJ mol^{-1}, $\Delta S^{\ddagger}$ = 31 ± 16 J K^{-1} mol^{-1}, $\Delta_r V_{(cis \rightleftarrows trans)}$ = +1.5 ± 0.8 cm^3/mol, $k_{trans \rightarrow cis}$(25 °C) = 2.8 ± 0.5 s^{-1}, $\Delta V^{\ddagger}_{trans \rightarrow cis}$ = +12 ± 4 cm^3/mol and by k(25 °C) = 9 ± 1 s^{-1}, $\Delta H^{\ddagger}$ = 69 ± 6 kJ mol^{-1}, $\Delta S^{\ddagger}$ = 6 ± 18 J K^{-1} mol^{-1}, $\Delta_r V_{(cis \rightleftarrows trans)}$ = +0.6 ± 0.3 cm^3/mol, $k_{trans \rightarrow cis}$(25 °C) = 2.6 ± 0.3 s^{-1}, $\Delta V^{\ddagger}_{trans \rightarrow cis}$ = +5 ± 2 cm^3/mol, respectively. The results suggest that the slow rotation around the hydroxamate C−N bond is the rate-determining step for the complexation reactions.

Introduction

Hydroxamic acids are weak organic acids (pK_a ~9) with a variety of applications in extractive metallurgy, in pharmaceutics, as food additives, etc.[2−6] Their importance and applications primarily originate from their ability to form stable metal ion complexes, i.e., they act as the metal binding sites.[7] The hydroxamate group is a bidentate ligand, forming the extremely stable five-membered ring by coordination of a central metal ion through the two oxygen atoms. Complexation is accompanied by simultaneous dissociation of one proton per coordinated hydroxamate group, as illustrated in eq 1.



(1)

The complexes between some naturally occurring hydroxamic acids and Fe(III), called *siderophores*, play a significant role in the microbial iron transport phenomenon.[7] Desferrioxamine B (**I** represents the fully protonated structure of this trishydroxamic acid in neutral aqueous media) forms with Fe(III) a siderophore complex called ferrioxamine B, $Fe(Hdfb)^+$.[7]

The results obtained in two independent laboratories[8,9] show that the hydrolysis of ferrioxamine B proceeds in four steps. It

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(1) Taken in part from M.G's. Ph.D. Thesis, submitted to the University of Zagreb. A part of this work was reported at the Inorganic Reaction Mechanisms Meeting, held in Le Bischenberg (France) on Jan 4−6, 1996: Gabričević, M.; Biruš, M. *Inorganic Reaction Mechanisms Meeting*, **1996**, L19.

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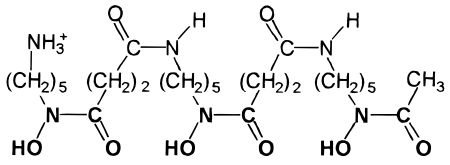
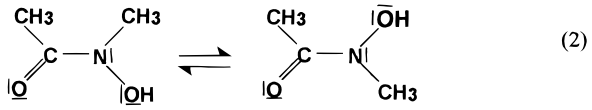


I = H_4dfb^+

was suggested that dechelation of the second hydroxamate group in $Fe(Hdfb)^+$ proceeds in two steps, due to a slow rate of rotation about the middle hydroxamate C−N bond in the partially unwrapped complex.[10,11] The hypothesis was based on the NMR data for both NMHA (structures **II**) and desferrioxamine B. The kinetics and equilibrium between the **IIa** and **IIb** isomers have been established by observation of the temperature-dependent line-broadening in the ^1H NMR spectra of NMHA.[10]



(2)

IIa = *cis*-NMHA **IIb** = *trans*-NMHA

It was recently suggested that the rate of trans → cis isomerization is possibly the rate-determining step in the Cu(II)− and La(III)−desferrioxamine B complex-formation kinetics.[11] In this paper we present some relevant data that unambiguously support the hypothesis of the slow rotation around the C−N bond in NMHA and in desferrioxamine B. Our conclusions are based on kinetic parameters obtained for the complexation of La^{3+} and Cu^{2+} with NMHA and H_4dfb^+, as well as for the rotation rate about the hydroxamate C−N bond in these two hydroxamic acids.

Selection of the metal ions was determined by the water exchange rates, which must be fast enough not to interfere with the rotation. Since for our experimental conditions the Eigen−Fuoss[12] equation predicts outer-sphere association constants between positively charged metal ions and neutral (NMHA) or positively charged (H_4dfb^+) entering ligands much smaller than 1, the water exchange rate constant must greatly exceed the rate of rotation. The selected metal ions satisfy these requirements.[13]

The present work augments the previously reported study[11] on desferrioxamine B, and it extends the investigated system by including NMHA as well. The results strongly confirm that, under certain circumstances, the rotation around the C−N bond is the rate-determining step in the formation of hydroxamate complexes.

Experimental Section

Materials. All water used was deionized and then twice distilled in an all-glass apparatus, first from an alkaline solution of $KMnO_4$. A 1 M stock solution of NaOH was prepared in CO_2-free water from TITRISOL (Merck), whereas 3 M acetic acid and 4.299 M perchloric acid stock solutions were prepared from the concentrated acids (Merck, p.a.) and standardized to a phenolphthalein end point against standard NaOH. HEPES, $LaCl_3$ and *m*-cresol purpur were reagent grade from Merck and were used without further purification. A 1 M stock solution of $Cu(ClO_4)_2$ was prepared by mixing equivalent amounts of $Ba(ClO_4)_2$ (Aldrich, p.a.) and $Cu(SO_4)\cdot 5H_2O$ (Merck, p.a.) followed by filtration through Millipore (filter type HV 0.45 mm). The concentration of Cu(II) in the solution was determined by titration with edta in the presence of murexide as indicator. The methanesulfonate salt of desferrioxamine B (Desferal) was kindly supplied by the Ciba-Geigy Corp. The salt was recrystallized from methanol and was stored in a vacuum desiccator over P_4O_{10} (mp 149−151 °C). A stock solution of $NaClO_4$ was prepared from anhydrous $NaClO_4$, standardized by passage through a DOWEX 50W-X8 strong acid cation exchange column in the H^+ form, and titrated against standard NaOH to the phenolphthalein end point.

Ligand Preparation. *N*-Methylacetohydroxamic acid was prepared by reacting *N*-methylhydroxylamine with ethyl acetate in basic methanol as already described and was characterized by ^1H NMR spectroscopy.[14] Its purity (98%) was determined by a conductometric titration with NaOH.

Physical Measurements. Metal−ligand binding constants for La(III) and Cu(II) with NMHA were determined by spectrophotometric pH titrations. A peristaltic pump was used to bring the solution into the 1 cm cuvette of a HP 8452 diode array spectrophotometer, where spectral measurements were made. Absorbance vs pH data were analyzed using a 2.09 version of the SPECFIT program. An Orion 701 pH meter and a glass electrode calibrated with standard Merck buffers of pH 1.00, 4.00, and 7.00 were used for pH measurements.

Kinetic studies were performed on a Durrum D-110 or an Applied Photophysics stopped-flow spectrophotometer coupled to an on-line data acquisition system. The kinetic traces were evaluated using the KINFIT (Olis, Bogart, GA) set of programs for the Durrum instrument. Measurements at high pressures (up to 1600 bar) were performed on a homemade high-pressure stopped-flow instrument.[15] An average of 5−7 runs were carried out for each experimental point reported. The kinetics of the La(III) complexation was studied by following the absorbance change at 250 nm, or by using *m*-cresol purpur indicator for monitoring (at 576 nm) the attendant pH decrease that occurred when the buffered solutions of $LaCl_3$ were mixed with the buffered solutions of hydroxamic acids. Solutions were lightly buffered with HEPES, which ensured a small pH change (less than 0.1 pH units) and in turn a small indicator-absorbance change (∼0.1). The latter method was used in the determination of the activation volume because of the spectral limitation of the high-pressure stopped-flow spectrophotometer. The results obtained by the two different methods only differ within the experimental error limits from each other. The kinetics of the Cu(II) complexation was studied at constant ionic strength (I = 2, $NaClO_4$) by measuring an increase in absorbance at 400 nm due to the formation of colored Cu(II) complexes. Solutions were buffered with acetate buffer to maintain a constant pH. The temperature dependence was studied by varying temperature within the range 15−50 °C, keeping it constant within ±0.1 °C.

NMR spectra were recorded on a Varian Gemini 300 and on a Bruker AVANCE DRX 400 WB NMR spectrometer equipped with a superconducting BC-94/89 magnet system. Measurements for the temperature dependence were performed with an acquisition time of 6.82 s. The pressure dependence was carried out on the 400 MHz Bruker spectrometer using a home-built high-pressure probe described elsewhere.[16] In addition to the published design a separate deuterium lock channel was implemented to stabilize the magnetic field. The magnetic field homogeneity over the sample volume was improved, resulting in a resolution of 3×10^{-9}. The sample coil was used in a double-tuned manner. The pressure dependence measurements were carried out with

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a pulse width of 8.3 ms and a relaxation delay D1 = 1 s at a temperature of 298 K. A spectral width of about 4006 Hz was used with 16K data points. The corresponding acquisition time was about 2.04 s; 32 scans were accumulated for each spectrum using the digital quadrature detection mode.

A line-broadening analysis was done using a method described in the literature and a program adopted for VECTRA 486/33n.[17] For both ligands, two independent high-pressure cycles were performed and the mean values for the obtained parameters are reported, vide infra (for H_4dfb^+ one of the cycles was carried out at 12 °C; for NMHA the mean values for the rate constants along with the corresponding standard deviations are shown).

The observed rate constants (k_{obs}) calculated from the line-shape analysis exhibit a fairly small individual standard deviation (usually less than a few percent), but independent series of measurements, carried out under the same experimental conditions, resulted in larger deviations. We could roughly estimate this error to be at least 20−30% of the actual value of the calculated rate constants, at the higher temperatures the errors being larger.

The results were less consistent at the upper end of the temperature range used, probably due to the limit for the rate constant when exchange still affects the band shape. The limit is a function of the frequency difference ($\Delta\delta_{cis/trans}$) between two exchanging sites, and for the observed $\Delta\delta_{cis/trans}$ = 10 Hz and [**IIb**]/[**IIa**] ≅ 3, as well as for a required exchange broadening of 0.5 Hz, the limit rate constant is below 200 s^{-1}.

Results

The equilibrium data for complexation of La^{3+} and Cu^{2+} with desferrioxamine B are available from the literature.[18] The equilibrium constants for reactions 3 and 4 were determined experimentally.

$$Cu^{2+} + CH_3C(O)N(OH)CH_3 \overset{K(3)}{\rightleftharpoons} Cu(CH_3C(O)N(O)CH_3)^+ + H^+ \quad (3)$$

$$La^{3+} + CH_3C(O)N(OH)CH_3 \overset{K(4)}{\rightleftharpoons} La(CH_3C(O)N(O)CH_3)^{2+} + H^+ \quad (4)$$

An aqueous solution of NMHA and the appropriate metal ion salt was titrated against 0.1 M $HClO_4$, and the spectral changes were recorded as the shift in equilibrium occurred (Figures S1 and S2 deposited as Supporting Information). A molar excess of the metal ion over NMHA assured the formation of only monohydroxamate complexes in solution. The measurements yield $K(3) = (1.58 \pm 0.02) \times 10^{-1}$ and $K(4) = (3.5 \pm 0.2) \times 10^{-4}$ at 25 °C and I = 2.0 ($NaClO_4$).[19]

From the values of the equilibrium constants it is seen that under our experimental conditions the equilibria are shifted toward the reaction products. Estimated contributions of the backward rates are less than 10% of the overall rates for the complexation reactions.

The complexation reactions were studied under pseudo-first-order conditions, maintaining the proton concentration constant by $HClO_4$ or the sodium hydroxide−acetic acid (or −HEPES) buffer system, with the metal ion in excess. In addition, the latter condition ensured the formation of only monohydroxamate complexes. In all cases single-exponential kinetics were observed, which were preceded by a much faster step, too fast to be followed by the stopped-flow technique. The absorbance change of the measured kinetics was much smaller than the expected value based on the equilibrium spectral measurements. The values of the ("lost-amplitude"/measured amplitude) ratio were calculated for $Cu^{2+}-H_4dfb^+$, Cu^{2+}−NMHA, and La^{3+}−NMHA complexations as (0.09/0.18), (0.06/0.09), and (0.1/0.2), respectively. Each value was calculated as a difference between the absorbance change expected from the spectral measurements and the absorbance change observed in the kinetic stopped-flow experiment.

A possible effect of the buffer concentration on the rate of complexation reactions was examined by measuring the rate constants as a function of the buffer total concentration at constant proton and metal ion concentrations, and at a constant mole ratio of the buffer components. At pH 4.55, the rate of complexation of NMHA is independent of the buffer concentration (Figure S3).

On the basis of the previously published results for the complexation of various hydroxamic acids with the numerous different metal ions,[20−22] the complexation reactions of Cu^{2+} and La^{3+} with NMHA and H_4dfb^+ were expected to be first order in each of the reactants. We have already reported an independence of the reaction rates on the metal ion concentration for the complexation rate of desferrioxamine B with Cu(II) and La(III) in molar excess.[11] For the complexation of NMHA, variation of the initial concentration of metal ions also has no observable effect on the rate constants (Figures S4 and S5). Similarly, a lack of k_{obs} dependence on pH was observed for all of the complexation reactions (Figures S6 and S7).

These results indicate an absence of any role that the metal and hydronium ions might possibly play in the rate-determining step of the complexation kinetics within the ranges investigated.

From the intercept and slope of the Eyring plots for complexation of Cu^{2+} and La^{3+} with NMHA and H_4dfb^+ (Figures S8 and S9), the activation parameters presented in Table 1 were calculated. The pressure dependence of the complexation reactions was also studied, and the results are summarized in Figure 1. All of the reactions exhibit a deceleration on increasing pressure, and the activation volumes calculated from the slopes ($=-\Delta V^{\ddagger}/RT$) of the plots are included in Table 1. Within the experimental error limits we can conclude that all of the complexation reactions are characterized by small positive $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$ values, confirming that the metal ions play no role in the rate-determining step.

Observation of an unequal pair of singlets in ^1H NMR spectra of the C-methyl group due to the presence of trans and cis forms of the hydroxamate functionality was already reported for NMHA[10] and H_4dfb^+[11] in aqueous solution. According to the ^1H NMR data for aceto- and *N*-methylacetohydroxamic acids in $CDCl_3$ and CD_2Cl_2 solvents, the resonance peak of the C-methyl group of the cis isomers is attributed to the downfield peak compared to the trans isomers.[23] The same assignment of the ^1H NMR peaks was argued for the hydroxamate C-methyl

(17) A program adapted for Vectra 486/33n based on the following: Saunders, M. In *Magnetic Resonance in Biological Systems*; Ehrenberg, A., Malmstroem, B. G., Vaenngard, T., Eds.; Pergamon Press: New York, 1966; p 85.

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(19) The titration of the Cu(II)−NMHA system with perchloric acid was also performed in 0.4 M acetate. From the obtained value (1.30 ± 0.10) × 10^{-2} for the apparent stability constant it can be estimated that in 0.4 M acetate, at the lowest Cu(II) concentration and pH used throughout the kinetics, equilibrium 3 is completely shifted toward the formation of the product.

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Table 1. Reaction Parameters[a] at 25 °C for Cis−Trans Rotation about the Hydroxamate C−N Bond in NMHA and H_4dfb^+ as Well as for Complexation of Cu^{2+} and La^{3+} with NMHA and H_4dfb^+

	L = NMHA				L = ^+H_4dfb			
reaction	k, s^{-1}	$\Delta H^{\ddagger}$, kJ mol^{-1}	$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	$\Delta V^{\ddagger}$, cm^3 mol^{-1}	k, s^{-1}	$\Delta H^{\ddagger}$, kJ mol^{-1}	$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	$\Delta V^{\ddagger}$, cm^3 mol^{-1}
cis ⇄ trans rotation	11(2)	76(5)	+31(16)		9(1)	69(6)	+6(18)	
trans → cis rotation[b]	2.8(5)			+12(4)	2.6(3)			+5(2)
Cu^{2+} + L	3.4(2)	69(1)	−3(3)	+5.0(5)	3.0(4)	72(1)	+5(2)	+3.4(2)
La^{3+} + L	3.0(3)	76(3)	+19(7)	+5.3(5)	2.9(3)	76(1)	+34(4)	+4.6(3)

[a] Figures in parentheses are single standard deviations of the parameters expressed in terms of the last significant digit reported for the particular parameter. [b] Calculated according to the equation $k_{trans\rightarrow cis} = k_{cis\rightleftharpoons trans}(1 + K)^{-1}$.

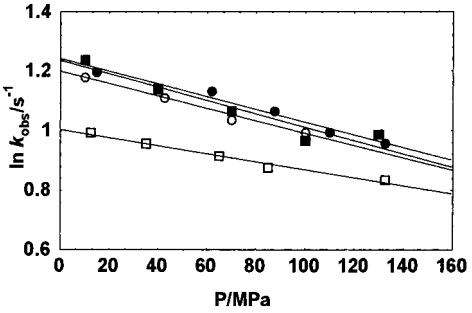

Figure 1. Pressure dependence of ln k_{obs} for complexation of La(III) with NMHA (open squares) and H_4dfb^+ (solid squares), and for complexation of Cu(II) with NMHA (open circles) and H_4dfb^+ (solid circles) at 25 °C. Conditions for the La(III) complexation: [La(III)] = 0.02 M, [NMHA] = 0.002 M, [HEPES] = 0.005 M, pH = 7.55, I = 2 M ($NaClO_4$) and [La(III)] = 0.0045 M, [H_4dfb^+] = 0.00015 M, [HEPES] = 0.001 M, pH = 7.55, I = 2 M ($NaClO_4$). Conditions for the Cu(II) complexation: [Cu(II)] = 0.04 M, [NMHA] = 0.004 M, [HAc] = 0.4 M, pH = 4.35, I = 2 M ($NaClO_4$) and [Cu(II)] = 0.1 M, [H_4dfb^+] = 0.005 M, [HAc] = 0.1 M, pH = 4.35, I = 2 M ($NaClO_4$).

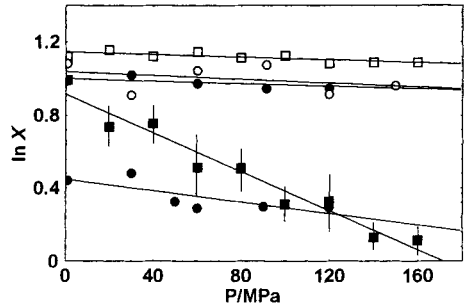

Figure 2. Plot of ln X vs pressure (where for open and solid symbols X represents K and $k_{trans\rightarrow cis}$, respectively) for rotation about the hydroxamate C−N bond in NMHA (squares) and the C-terminal hydroxamate C−N bond in H_4dfb^+ (circles) at 25 °C. For H_4dfb^+ the results obtained at 12 °C are represented by the lower straight line.

group of H_4dfb^+ ion in water[11] and could also be argued for NMHA in water. The peak intensities indicate higher stability of the trans isomers of H_4dfb^+ and NMHA in water in accordance with a recent theoretical calculation for hydroxamic acids.[24] The isomerization equilibrium constant, $K_{isomerization}$ = [trans]/[cis] = 2.8 ± 0.3, was calculated from the peak areas in the ^1H NMR spectrum of NMHA. This value is very close to the analogous equilibrium constants determined for the hydroxamate groups of H_4dfb^+,[11] and except for the reverse assignment of the signals, it agrees well with the previously reported value for the same equilibrium constant.[10] It should be noted that the earlier calculations favor the cis over the trans isomer with about 15 kJ/mol, mainly due to the intramolecular hydrogen bonding. However, recent density functional theory calculations on *N*-methylacetamide (NMA)[25] suggested a cooperative effect of hydrogen bonding in *trans*-NMA complexes, in which three water molecules attach to NMA. The H-bond energies of these three-water complexes were found larger (i.e., more negative) than the sum of the H-bond energies of the corresponding single-water complexes. Since due to the steric hindrances no cooperative H-bonding was found in the three-water cis isomer, the trans isomer was found to be the more stabile form in water. If the similar cooperativity found for the peptide bond applies to the hydroxamate bond, and in the cis isomer steric hindrances prevent the cooperativity, a higher stability of trans-NMHA in water compared to the cis isomer can be expected.

The hindered rotation around the C−N bond, which gives k_{obs} = 11 ± 2 s^{-1} at 25 °C, is based on the temperature-dependent ^1H NMR spectrum of NMHA in D_2O. From the Eyring plot for this rotation along with the analogous plot of ^1H NMR data for the C-terminal methyl group of desferrioxamine B (Figure S10) the corresponding activation parameters were calculated and are summarized in Table 1.

The shape of the ^1H NMR spectra of NMHA and H_4dfb^+ at 298 K also depends on the applied pressure. From the obtained linearity of ln $k_{trans\rightarrow cis}$ and ln K vs applied pressure up to 1600 bar (Figure 2), the activation volumes for the trans → cis isomerization and reaction volumes for reaction 2 of NMHA and H_4dfb^+ were calculated as $\Delta V^{\ddagger}$ = +12 ± 4 cm^3/mol and $\Delta_r V$ = +1.5 ± 0.5 cm^3/mol and as $\Delta V^{\ddagger}$ = +5 ± 2 cm^3/mol and $\Delta_r V$ = +0.6 ± 0.3 cm^3/mol, respectively. The trans forms of these ligands have a slightly higher partial molar volume than their cis forms.

Discussion

The rate of complexation reactions $M(H_2O)_m^{n+}$ + HA → $MA(H_2O)_{m-2}^{(n-1)+}$ + H^+ + $2H_2O$ (M = Cu(II), La(III); HA = NMHA, H_4dfb^+) is expected to be first order in both the metal ions and the ligands. Surprisingly, our kinetic results reveal that the complexations are zero order in the metal concentrations and also independent of pH. Since the water exchange rate constant estimated for the Cu^{2+} ion is ~10^9 s^{-1} and for lanthanide ions is ~10^8 s^{-1},[26] it is obvious that the rate constants for complexation with H_4dfb^+ and NMHA are far below the limits imposed by the water exchange kinetics. This fact permits a possibility that the slowest kinetic step in the complexation reactions is not related to the water exchange process, and that

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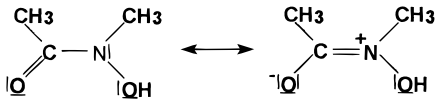
the structural changes which occur in the rate-determining step may be entirely related to the transformation of the ligands.

The crystal structure data reveal that the hydroxamate bond is almost planar.[27,28] In the trans (*E*) configuration, the hydroxamate bond has R_C and R_N at diagonally opposite corners of the hydroxamate plane. In this configuration two oxygens are improperly oriented for the coordination of a metal ion. However, one end could be rotated 180° to bring two oxygens closer together, forming a cis (*Z*) configuration, which is functional for coordination. The observed ratio of two closely spaced signals in the ^{1}H NMR spectra of H_4dfb^+ and NMHA, related to the C−C*H*$_3$ protons, shows[10,11] that this cis configuration is slightly less favorable when R_N is an alkyl group.

The line-broadening NMR measurements reveal that the room temperature rotation about the C−N bond, either of the NMHA- or of H_4dfb^+-hydroxamate groups, is characterized by the rate constant of ca. 10 s^{-1}. The magnitude of this rate constant indicates a partial double bond character of the hydroxamate C−N bonds, because a single-bond rotation is expected to occur on a much shorter time scale. The partial double bond character responsible for the slow rate of rotation about the hydroxamate C−N bond can be explained by two extreme resonance forms of hydroxamic acids shown by structures **IIIa** and **IIIb**.



IIIa **IIIb**

A partial positive charge on the **IIIb** nitrogen may be either stabilized or destabilized by an electron-donating or -withdrawing substituent at that position. In both ligands, desferrioxamine B and NMHA, electron-donating alkyl substituents increase the double bond character in the C−N bond and, therefore, decrease the rate of rotation.

The above value of the rate constants refers to the rate constants defined as a sum of the forward (e.g., $NMHA_{cis} \rightarrow NMHA_{trans}$) and backward ($NMHA_{trans} \rightarrow NMHA_{cis}$) rate constants. The estimated value of the isomerization equilibrium constant, $K = [\mathbf{IIb}]/[\mathbf{IIa}] \cong 3$, indicates that the value of $k_{trans \rightarrow cis}$ equals ~3 s^{-1}. Assuming that the rate-determining step of the metal complexation reactions in the measured (slower) kinetic step involves transformation of the ligand from the trans into the cis form, i.e., for the coordination a structurally inappropriate into an appropriate form, the expected value of the complexation rate constant for both metal ions should equal ~3 s^{-1}. As shown in Table 1, the values of the complexation rate constants obtained at 25 °C for Cu^{2+} and La^{3+} are very close to the expected value. This suggestion is also in perfect agreement with the small positive $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$ values reported for the complexation reactions in Table 1. Isomerization of the ligand from trans to cis can be expected to involve a small increase in partial molar volume on reaching the transition state due to the sweeping out of solvent molecules during this rotation.

The simplest plausible mechanism that explains the obtained results could comprise depletion of the initial pool of the free-ligand cis isomer in a very fast kinetic step which cannot be measured by the stopped-flow technique ("lost" amplitude). Once the initial pool of cis isomer is depleted, the uncoordinated trans ligand begins to transform into the cis form (appropriate for coordination) at the rate of trans → cis conversion, which is dictated by the rotation about the hydroxamate C−N bond. Since the reaction of this new cis isomer with the metal ions is much faster than the trans → cis conversion, this should result in a metal- and proton-independent pathway for the complex-formation process.

On the other hand, a two-step mechanism for coordination of monohydroxamic acids (such as NMHA) to metal ions (such as Fe(III) and Al(III)[20,21]) was proposed, offering a possibility of a certain interaction between the reactants before the rotation and final coordination occur. In this mechanism, the first step is attachment of the ligand to the central metal ion via hydrogen bonding of a coordinated water molecule to the hydroxyl group of hydroxamic acid, followed by coordination of the carbonyl oxygen. Since for the latter step the trans form of the ligand is again inappropriate, in a rate-determining step the trans isomer should be transformed into the appropriate cis form and subsequently be coordinated to the central metal ion.

In order for this mechanism not to conflict with the experimental results, most of the ligand must be hydrogen bonded to the metal ions. Unfortunately, there is no evidence that such a complex exists to a sufficient extent. Our preliminary efforts to observe the magnitude of hydrogen bonding between the metal ions and NMHA, by measuring the carbonyl ^{17}O NMR chemical shift in solutions of reactants under conditions when no complex was formed, gave no evidence which would support it. Therefore, at present it seems reasonable to consider only the former mechanism to be plausible.

The obtained activation enthalpies for the complexation of the two metal ions do not conflict with the proposed reaction mechanism. The obtained $\Delta H^{\ddagger} = 76$ kJ mol^{-1} for the trans−cis isomerization of NMHA compares favorably to $\Delta H^{\ddagger} = 69$ kJ mol^{-1} for the complexation of NMHA with Cu(II), and $\Delta H^{\ddagger} = 76$ kJ mol^{-1} determined for the complexation with La(III). The similar values of the activation enthalpy obtained for these metal ions may indicate a common complexation mechanism in which the rate-determining step is independent of the nature of the metal ions.

The obtained values for the activation entropies would indicate a different reaction mechanism. However, as on many occasions before, it should be stressed that uncertainties in the determination of this parameter, from a far-removed intercept of the Eyring plot, make it less reliable as a mechanistic criterion. The $\Delta V^{\ddagger}$ data quoted for the complexation in Table 1 could be determined more accurately, and clearly demonstrate the operation of a common mechanism in the rate-determining step.

The obtained complexation activation entropies should also be compared to the obtained isomerization $\Delta S^{\ddagger}$ values. However, $\Delta S^{\ddagger}$ for the rotation reaction is related to the overall rate of isomerization and therefore may differ from $\Delta S^{\ddagger}$ of the trans → cis transformation alone. Our experimental results reveal that at the lower temperature range, where the two peaks are nicely separated, integration of peaks gives $K = 2.8 \pm 0.3$, depending on the integration method used. On the other hand, at the highest temperature when only one peak is observed, $K = 3.3$ was calculated from the chemical shifts using the equation $(\delta_{cis} - \delta_{peak})/(\delta_{peak} - \delta_{trans})$. This indicates *no*, or a very weak, temperature dependence of the isomerization equilibrium constant, which in turn argues for $\Delta H^{\circ} \cong 0$. The assumed temperature independence of *K* would still result in ΔS° ($= R \ln K$) $\cong +10$ J K^{-1} mol^{-1}, which would involve the interpretation of the activation entropies. On the other hand, assuming that the observed change in the *K* value was not just an experimental error, the calculated ΔH° would be only about

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−3 kJ/mol, which makes activation enthalpies for the forward and backward isomerization almost equal to the value calculated from the Eyring plot for k_{obs}. However, the latter assumption would result in an even larger calculated value for ΔS^{o} ($\cong$+20 J K^{-1} mol^{-1}), again complicating the discussion of the activation entropies.

Therefore, the line-broadening experiments were performed as a function of pressure at 298 K. Integration of the spectral peaks was easily achieved, making determination of K (and hence $k_{trans\rightarrow cis}$) at each pressure more straightforward than from the chemical shifts at the higher temperature.

The obtained activation volumes for the trans → cis isomerization of NMHA and H_4dfb^+ differ in their size, the obtained $\Delta V^{\ddagger} = +5 \pm 2$ cm^3/mol for the trans → cis isomerization of H_4dfb^+ being equal to the activation volumes found for the complexation reactions. The obtained value for the NMHA isomerization, $\Delta V^{\ddagger} = +12 \pm 4$ cm^3/mol, is rather close to the calculated value of a toroidal volume which must be emptied of solvent molecules during the rotation around the C−N bond. Such a toroidal volume is determined by the size of the rotating carbonyl and methyl groups, and if defined by sphere of a ∼2 Å radius it can be calculated to be ca. 13 cm^3/mol.

The obtained $\Delta V^{\ddagger}$ values for the complexation reactions, determined in H_2O, are less positive than for the isomerization of NMHA which was determined in 5% methanol in D_2O. The obtained difference of ca. 7 ± 4 cm^3/mol between the obtained activation volumes for the trans → cis isomerization of free NMHA and the complex-formation reaction could be caused by a difference in the nature of the two solvents used. As mentioned above, the rotation about the C−N bond results in sweeping out solvent molecules, causing the solvent structure to break up around the ligand. This may affect the obtained activation volumes for essentially the same reaction determined in these two solvents.

Finally, it should be emphasized that all of these conclusions are related to the slower kinetic step, whereas the above mentioned faster kinetic step (not measured) may correspond to the coordination of the cis form of either NMHA or desferrioxamine B. Since this enantiomer represents the proper form for coordination, isomerization is no longer necessary, and the complexation rate will be limited only by the water exchange kinetics at the metal ion centers, as already suggested[11] for the complexation of numerous metal ions with hydroxamic acids in molar excess, when there is enough cis isomer to coordinate all of the metal ions present in solution.

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Supporting Information Available: Figures S1−S10 reporting rate constant data as function of concentration, pH, and temperature. This material is available free of charge via the Internet at http://pubs.acs.org.

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