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The Reactivity of Different Iron(III) Species in the Formation of Monoacethydroxamatoiron(III) Complex

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The dependence of the rate constant of the monoacethydroxamatoiron(III) complex formation on ionic strength in acid solution at 25 °C has been studied. The observed 'secondary salt effect' strongly supports the assumption that an iron(III) hydroxo complex is the reactive species. Evidence is presented showing that the iron(III) hydroxo dimer ($Fe_2(OH)_2(H_2O)_8^{4+}$*) exhibits approximately equal reactivity toward acethydroxamic acid as the monomer does. This fact, and lack of evidence for 'primary salt effect' strongly confirms the previous findings that the reactive form of ligand is the undissociated rather than dissociated hydroxamic acid. The rate of the reaction of hydroxamic acid with iron(III) hydroxo ion is estimated to be of the same order of magnitude as the rate of water exchange of the same ion.*

Introduction

Reasons for great interest in compounds containing hydroxamic acids lie in their biological activities as antibiotics, growth factors, and chelating agents [1–3]. The most important feature of the hydroxamic acids is their ability of metal ion sequestration, particularly iron(III) ions, which classify them in a group of compounds intimately associated with iron-transport phenomena in living organisms [4]. Different aspects of chemical as well as biochemical behavior of the iron(III) hydroxamates have been studied. However, little attention has been given to the mechanism which operates in their formation [5, 6].

On the basis of simple kinetic rate law it is not possible to distinguish between the mechanism which includes reaction of $Fe(H_2O)_6^{3+}$ with the dissociated acids, and the one including $Fe(H_2O)_5OH^{2+}$ and the undissociated acids, as we have already shown [5]. The purpose of the present work is to describe the reactivity of different iron(III) species in the formation of the hydroxamato complexes. The related evidence has been obtained from indirect data such as medium effect on the rate of formation.

Experimental

Materials

The stock solution of $Fe(ClO_4)_3$ was prepared by dissolving a proper amount of solid perchlorate, $Fe(ClO_4)_3 \cdot 6H_2O$, Alfa Product-Ventron, in 0.0142 *M* $HClO_4$ to prevent hydrolysis, and the exact concentration of iron was determined gravimetrically as Fe_2O_3, and spectrophotometrically with sulfosalycilic acid [7]. This solution was stored for a month without any change in concentration.

Acethydroxamic acid was prepared by the procedure described elsewhere [8, 9] and its reagent solutions were prepared by dissolving the solid in 0.0142 *M* $HClO_4$, immediately before the kinetic measurements were done.

All the experiments were performed in doubly-distilled water, and ionic strength was adjusted by sodium perchlorate.

Procedure

All of the spectrophotometric and potentiometric measurements were performed at 25.0 ± 0.1 °C, using a Cary 16K spectrophotometer and a Potentiograph E 436 Metrohm with a combined glass electrode standardised by two buffers, respectively. The reaction kinetics were in the stopped-flow range and were recorded on a Durrum stopped-flow spectrophotometer, model D-110, measuring an increase in absorbance at 500 nm. The pseudo-first order conditions were ensured by holding one of the reactants in at least 20-fold excess over the other. The first order rate constants were calculated from log $(A_\infty - A_t)$ *vs.* time plots, as an average of the three identical runs. The plots were linear through at least three half lives of the reaction. Except for the data presented in Fig. 3, the contribution of backward reaction on the total rate was negligible, as was confirmed by the spectrophotometrically determined overall equilibrium constant of the formation of

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monoacethydroxamatoiron(III) complex at 25 °C. The values of 132, 118, and 89.0 at 0.05, 0.10 and 0.50 *M* ionic strength agree favorably with reported values [10, 11]. On the other hand, from the kinetic data shown in Fig. 3 it was possible to calculate the equilibrium constant at 1.0 *M* ionic strength, and the value of 87.3 is in agreement with the one determined spectrophotometrically.

The observed rate constants were fitted to the proposed reaction models using a non-linear least squares program* on a Univac 110 system, at the University Computing Center, Zagreb. The reliability of calculated parameters was estimated from the standard deviation of the appropriate parameter.

The total concentration of H^+ ion in the experiments was calculated by summation of added $HClO_4$ an the proton released by Fe(III) hydrolysis, using the hydrolysis constants reported by Milburn and Vosburgh [13].

Results and Discussion

It has been recognised that the formation rates of labile metal ion complexes are, in general, independent of the nature of entering ligand and are characteristic of the central metal ion; this behavior was originally explained by Eigen [14]. According to this mechanism the observed rate constant is a product of an outer sphere association constant, K, and a first order rate constant k*, for exchange of coordinated water molecule and entering ligand.

The kinetics of Fe(III) complex formation have been widely investigated but are still not well understood. In the kinetics of Fe(III) ion, an increase of the ligand basicity causes an increase of the rate of water substitution, and Eigen suggested that this phenomenon could be interpreted in terms of internal hydrolysis. However, Seewald and Sutin [15] drew attention to the fact that the reaction between $Fe(H_2O)_6^{3+}$ and dissociated acid has the same pH dependence as the one between $Fe(H_2O)_5OH^{2+}$ and the undissociated acid. According to the latter mechanism the rate constants of water substitution for most ligands fall in a very narrow range, indicating no dependence of the substitution rate of the nature of the entering ligand. Nevertheless, the pathway including the dissociated acids cannot be ruled out since the related rate constants do not exceed that of the diffusion rate. In addition, the mechanistic picture is complicated by the presence of $Fe_2(H_2O)_8(OH)_2^{4+}$ dimer (scheme 1) which also should be considered as the potential reaction species.

$$Fe(H_2O)_6^{3+} \overset{\beta_1}{\rightleftharpoons} Fe(H_2O)_5OH^{2+} + H^+ \qquad (1)$$

$$2\ Fe(H_2^2O)_6^{3+} \overset{\beta_2}{\rightleftharpoons} Fe_2(H_2O)_8(OH)_2^{4+} + 2H^+ \qquad (2)$$

Scheme 1

In the previous papers [5] we have reported that the reaction of iron(III) ion with hydroxamic acids follows the same kinetic pattern as with other weak acids. Using a value of $pK_a \sim 9$ for acethydroxamic acid, assuming the outer sphere association constant of $\sim 1\ M^{-1}$ between $Fe(H_2O)_6^{3+}$ and mono-negatively charged anions [16], and taking the value of $5.9 \times 10^3\ M^{-1}\ s^{-1}$ for the rate constant of the undissociated acid [5] as the reactive species, one obtains a value of $\sim 10^{10}\ s^{-1}$ for the rate constant of the reaction between iron(III) aquo complex and the dissociated acid. The obtained value does not again exceed that of the diffusion rate constant, therefore the pathway should be considered as possible.

Although it was accepted [16] that on the basis of the ionic strength effect on the rates it is not possible to distinguish between the two reaction pathways (as for example [3], and [4]) related through rapid equilibrium, we would like to point out that under certain conditions, *i.e.* when the equilibrium constant dependences of ionic strength are known, the differentiation is still possible.

If for a moment equilibrium (2) is ignored (*e.g.* at low iron(III) concentration), while the pathways involving the reaction between $Fe(H_2O)^{3+}$ and HL, and $Fe(H_2O)_5OH^{2+}$ and L^- (HL and L^- representing protonated and deprotonated form of acethydroxamic acid, respectively) were already ruled out [5] on the basis of pH-dependence of the rate, the reaction between iron(III) ion and acethydroxamic acid can be sufficiently well described by eqns. (3), (4), and (5), with K_a representing the acid dissociation constant of HL.

$$Fe(H_2O)_6^{3+} + L^- \xrightarrow{k_1} Fe(H_2O)_4L^{2+} + 2H_2O \qquad (3)$$

$$Fe(H_2O)_5OH^{2+} + HL \xrightarrow{k_2} Fe(H_2O)_4L^{2+} + 2H_2O \qquad (4)$$

$$\text{Rate} = \frac{(k_1K_a + k_2\beta_1)[HL]_{tot}[Fe(III)]_{tot}}{[H^+] + \beta_1} \qquad (5)$$

From the charge of the reactants it can be readily predicted that, because of the opposite charge of the reacting species, k_1 for reaction (3) should decrease markedly with increasing ionic strength, but k_2

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*W. L. Chan, *Master thesis,* Ohio State University, 1978, a modification of the program developed by D. L. Leussing [12].

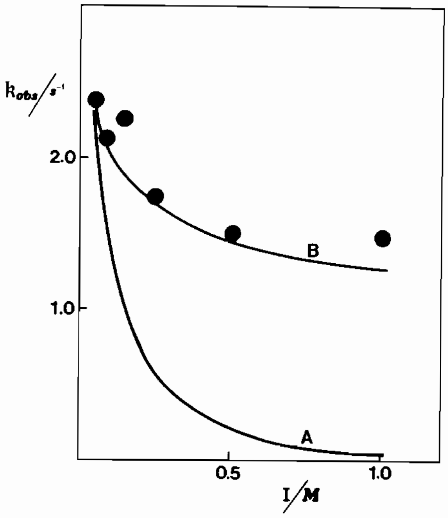

Fig. 1. The effect of ionic strength on the rate constant of formation of monoacethydroxamatoiron(III) complex at 25 °C. The theoretical curve A for pathway (3), B for pathway (4), were calculated as described in the text. The concentrations were: HL = 1 × 10^{-4} *M*, $HClO_4$ = 1.42 × 10^{-2} *M*, and $Fe(ClO_4)_3$ = 2.27 × 10^{-3} *M*.

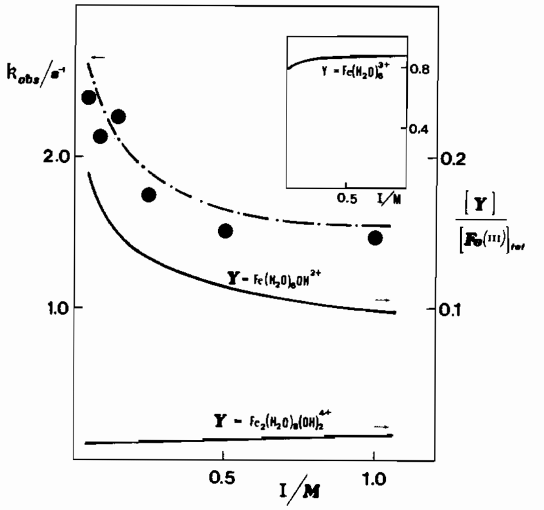

Fig. 2. The effect of ionic strength on the rate constant of formation of monoacethydroxamatoiron(III) complex, and on the fractional distribution of the Fe(III) species at 25 °C. Through the experiments total concentrations were as follows: HL = 1 × 10^{-4} *M*, $HClO_4$ = 1.42 × 10^{-2} *M*, $Fe(ClO_4)_3$ = 2.27 × 10^{-3} *M*. The dashed line represents the theoretical curve calculated according to eqn. (7), using average values of k_2 and k_3, and assuming no 'primary salt effect'.

for the reaction (4) must be relatively independent of ionic strength since HL is an uncharged molecule.

In the rate expression (5), the factor $(k_1K_a + k_2\beta_1)$ decreases because β_1 and k_1 decrease with increasing ionic strength. On the other hand, this factor could increase if K_a tends to increase with increasing ionic strength. However, if the product k_1K_a decreases slightly then the observed decrease in k_{obs} may be attributed to this term. For that reason the acid dissociation constant of acethydroxamic acid was determined by potentiometric titration at 25 °C. The corresponding values of K_a: 4.96 × 10^{-10}, 5.65 × 10^{-10}, 7.17 × 10^{-10}, 7.90 × 10^{-10}, 1.12 × 10^{-9}, and 1.24 × 10^{-9} *M* at 0.0442, 0.0842, 0.144, 0.244, 0.504, and 1.00 *M* ionic strength, respectively, were inserted in eqn. (5) in order to calculate the theoretical curve for the pathway (3). In addition, the k_1 dependence of the ionic strength was calculated employing a charge product of −3 in the equation $k_1^I = k_1^{I^\circ} \times 10^{Z_1Z_2I^{1/2}}$ (I, Z_1, and Z_2 mean ionic strength, the charge of $Fe(H_2O)_6^{3+}$, and the charge of L^{1-}, respectively), with all the calculations standardized by $k_{obs} = 2.39\ s^{-1}$ at 0.0442 *M* ionic strength.

In Fig. 1 the results of investigation of the ionic strength effect on the rate of formation of monoacethydroxamatoiron(III) complex in 0.0142 *M* $HClO_4$ and 2.27 × 10^{-3} *M* $Fe(ClO_4)_3$ are presented. Under these experimental conditions the demand for low content of the dimer is almost completely fulfilled, as is shown by Fig. 2 and Table I. From Fig. 1 it is obvious that if the pathway (3), depicted by the theoretical curve A, had operated only, the obtained decrease of k_{obs} would be much stronger than the observed one.

Milburn and Vosburgh [13] have determined values of equilibrium constant β_1 at different ionic strengths, what enabled us to calculate a decrease in k_{obs} for the case when the reaction path (4) only operates. The calculated decrease is presented by curve B in Fig. 1 and is obviously favorably followed by experimentally determined k_{obs}, most likely as a strict consequence of the decrease of the concentration of total iron(III) present as $Fe(H_2O)_5OH^{2+}$ due to the increase of ionic strength. In other words, the observed decrease of k_{obs} by increasing ionic strength must be attributed to the 'secondary salt effect', expected to operate in path (4), but not to 'primary salt effect' expected to operate in pathway (3). Consequently, path (3) can be ruled out even though the rate law (5) itself fails to distinguish between the alternative pathways (3) and (4), related through the rapid equilibrium.

The observed evidence referred to above provides a strong support to the view that formation of monoacethydroxamatoiron(III) complex proceeds mainly by the path (4) under the described experimental conditions. However, when the fraction of dimer cannot be neglected, *e.g.* in solution containing a higher concentration of $Fe(ClO_4)_3$, the rate increase does not parallel the increase in $Fe(H_2O)_5OH^{2+}$ ion concentration, as is shown by experiments 7 and

TABLE I. Dependence of the Rate Constant of the Monoacethydroxamatoiron(III) Complex Formation on Ionic Strength, and $Fe(ClO_4)_3$ Concentration at 25 °C in 0.0142 *M* $HClO_4$ and 1×10^{-4} *M* acethydroxamic acid.

Exp. No.	I/*M*	$[Fe(ClO_4)_3]$/*M*	$[Fe(H_2O)_5OH^{2+}]$/*M*[a]	$[Fe_2(OH)_2(H_2O)_8^{4+}]$/*M*	k_{obs}/s^{-1}	k_{calc}/s^{-1}
1	0.044	2.27×10^{-3}	0.43×10^{-3}	0.02×10^{-3}	2.39	2.59[b]
2	0.084	2.27×10^{-3}	0.38×10^{-3}	0.02×10^{-3}	2.13	2.39[b]
3	0.144	2.27×10^{-3}	0.34×10^{-3}	0.02×10^{-3}	2.27	2.12[b]
4	0.244	2.27×10^{-3}	0.30×10^{-3}	0.03×10^{-3}	1.75	1.93[b]
5	0.500	2.27×10^{-3}	0.26×10^{-3}	0.03×10^{-3}	1.51	1.69[b]
6	1.00	2.27×10^{-3}	0.22×10^{-3}	0.04×10^{-3}	1.51	1.55[b]
7	0.50	4.54×10^{-3}	0.50×10^{-3}	0.11×10^{-3}	3.25	3.60[c]
8	0.50	9.08×10^{-3}	0.91×10^{-3}	0.37×10^{-3}	9.90	7.68[c]
9	0.50	1.77×10^{-2}	0.15×10^{-2}	0.11×10^{-2}	20.2	15.7[c]
10	0.50	3.54×10^{-2}	0.25×10^{-2}	0.27×10^{-2}	34.8	31.5[c]
11	0.50	5.31×10^{-2}	0.31×10^{-2}	0.43×10^{-2}	53.1	46.0[c]
12	0.50	7.08×10^{-2}	0.36×10^{-2}	0.81×10^{-2}	59.8	73.8[c]
13	1.00	2.27×10^{-3}	0.22×10^{-3}	0.04×10^{-3}	1.53	1.57[d]
14	1.00	4.54×10^{-3}	0.43×10^{-3}	0.13×10^{-3}	4.20	3.50[d]
15	1.00	9.08×10^{-3}	0.77×10^{-3}	0.44×10^{-3}	11.3	7.97[d]
16	1.00	1.77×10^{-2}	0.13×10^{-2}	0.12×10^{-2}	22.4	17.2[d]
17	1.00	3.54×10^{-2}	0.20×10^{-2}	0.30×10^{-2}	39.9	36.1[d]
18	1.00	5.31×10^{-2}	0.26×10^{-2}	0.48×10^{-2}	39.4	53.8[d]
19	1.00	7.08×10^{-2}	0.30×10^{-2}	0.66×10^{-2}	79.2	70.3[d]

[a] Distribution of iron(III) species calculated as described in text, where $[Fe(ClO_4)_3]_{total} = [Fe(H_2O)_5OH^{2+}] + 2[Fe_2(OH)_2(H_2O)_8^{4+}] + [Fe(H_2O)_6^{3+}]$. [b] Rate constants calculated using average values of $k_2 = 5.71 \times 10^3$ M^{-1} s^{-1} and $k_3 = 7.33 \times 10^3$ M^{-1} s^{-1}. [c] Rate constants calculated by fitting the data at I = 0.5 *M* to eqn. (7). [d] Rate constants calculated by fitting the data at I = 1.0 *M* to the eqn. (7).

10, or 14 and 17 in Table I. In these experiments a tenfold increase in the rate constant is observed on a fivefold increase in concentration of $Fe(H_2O)_5OH^{2+}$ ion. This observation can be easily accommodated by invoking the pathway (6), showing that the iron(III) hydroxo dimer is also a reactant in the formation of the Fe(III) complex.

$$Fe_2(H_2O)_8(OH)_2^{4+} + HL \xrightarrow{k_3} Fe(H_2O)_4L^{2+} + Fe(H_2O)_5OH^{2+} \qquad (6)$$

In such a case, and when $Fe(ClO_4)_3$ is in large excess over the hydroxamic acid, the observed rate constant is described by the eqn. (7):

$$k_{obs} = k_2[Fe(H_2O)_5OH^{2+}] + k_3[Fe_2(H_2O)_8(OH)_2^{4+}] \qquad (7)$$

The experimental data in which the concentration of $Fe(ClO_4)_3$ was varied from 2.27×10^{-3} up to 7.08×10^{-2} *M*, for ionic strength 0.5 and 1.0 *M* were fitted separately to the eqn. (7). The calculated value of k_2 are practically identical within the limits of experimental uncertainties, 5760 ± 994, and 5650 ± 864 M^{-1} s^{-1}, respectively (uncertainties mean standard deviations), showing again no salt effect. The calculated values of k_3, 6510 ± 1390, and 8150 ± 1350 M^{-1} s^{-1}, differ quite more, what is probably caused by a smaller accuracy of the rate constant determination at higher $Fe(ClO_4)_3$ concentrations where the fraction of dimer is significant, but where the rates are closer to the limit of experimental technique.

A significant amount of a binuclear Fe_2L dimer was not observed either from spectrophotometric or potentiometric titrations [10, 17]. Therefore, it seems reasonable to suppose a mechanism in which the formed $Fe_2(OH)_2HL$ complex breaks down rapidly after its formation (the charge and water molecules of the complex were omitted for simplicity). A destabilization of the dimer by HL may be due to a fast proton transfer from HL to the hydroxo group in the complex. The calculated values of k_2 and k_3 are almost equal in spite of the different charge of the dimer and monomer, supporting the view that the undissociated acid is the reacting moiety. With our average value of 5.71×10^3 M^{-1} s^{-1} for k_2 (= k_2*K), the reactivity of $Fe(H_2O)_5OH^{2+}$ ion toward

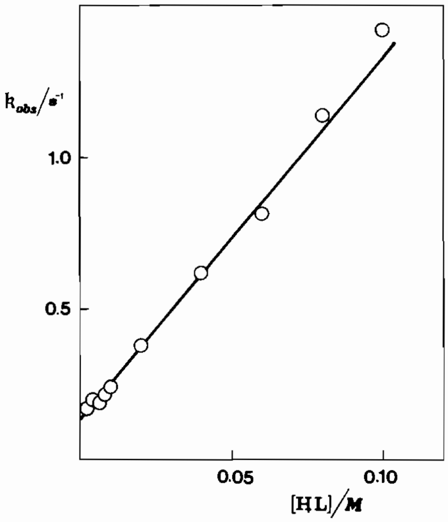

Fig. 3. The dependence of the rate constant of monoacethydroxamatoiron(III) complex formation on concentration of acethydroxamic acid at 25 °C, I = 1 *M*, in 1 *M* $HClO_4$ and 2×10^{-4} *M* $Fe(ClO_4)_3$.

the hydroxamic acid can be estimated and compared with the reactivity toward water molecule. In order to calculate k_2^* it was necessary to define a value of the outer sphere association constant, K, between $Fe(H_2O)_5OH^{2+}$ ion and HL. For that reason we undertook a series of experiments in which HL, in excess over $Fe(ClO_4)_3$, was varied up to 0.1 *M*, at constant ionic strength held by 1.0 *M* $HClO_4$. Perchloric acid was in such high concentration to prevent a formation of bis-, and tris-acethydroxamatoiron(III) complexes. The obtained linearity (Fig. 3) does not allow a calculation of the association constant from the expression $k_{obs} = k_1 f_{FeOH} ([H^+] + K[HL])/K$, where f_{FeOH} stands for the fraction of the total iron(III) present as $Fe(H_2O)_5OH^{2+}$ in 1 *M* $HClO_4$. However, from the HL concentration range it appears that K must be lower than 1 M^{-1}, giving $k_2^* \geqslant 6 \times 10^3$ s^{-1}. This value is slightly lower than the water exchange rate constant for $Fe(H_2O)_5OH^{2+}$ ion, which was estimated [16] as 10^5 s^{-1}, and it seems reasonable since both ligands are neutral molecules.

In conclusion our results show that the pathway involving $Fe(H_2O)_5OH^{2+}$ ion and HL is favored over the pathway involving $Fe(H_2O)_6^{3+}$ and L^- ions in acidic medium. However, at lower acidity and higher $Fe(ClO_4)_3$ concentration, the $Fe_2(H_2O)_8(OH)_2^{4+}$ ion is in competition with $Fe(H_2O)_5OH^{2+}$ ion for acethydroxamic acid, and under certain reaction conditions it may even be the main reactant.

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