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Kinetic and Equilibrium Thermodynamic Description of the Interaction of Desferrioxamine B and Acethydroxamic Acid with Iron(III) in Acid Aqueous Perchlorate

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Some activation parameters of the stepwise formation and hydrolysis of the iron(III) complexes with desferrioxamine B and acethydroxamic acid, as well as the enthalpy and entropy of the overall reactions are reported.

The mechanism of the formation and hydrolysis of the iron(III)-hydroxamate complexes is disscused in view of the obtained data.

INTRODUCTION

Hydroxamic acids (HA) are weak acids of the general formula R_1—C(=O)—N(—OH)—R_2 with pK_a values not much different from nine. One hydroxamato group, after dissociation of one proton from the hydroxyl group, has two oxygen atoms capable of donating free electron pairs to the metal ions. Therefore, three such hydroxamato groups may form a very strong octahedral complex with high-spin iron(III) preventing its hydrolysis, and ensuring its solubility. This feature of hydroxamic acids has been used by numerous microorganisms which produce different hydroxamic acids for iron(III) solubilization and transport.[1] Such compounds are referred to in literature as siderophores.[2]

Desferrioxamine B (*I*) is such a siderophore, currently used in medicine as a drug of choice in the treatment of patients suffering from acute or chronic iron overload.[3] This makes the study of the interaction between iron(III) and hydroxamates even more interesting.

$$H_3N^+\text{-}(CH_2)_5\text{-}[N(\text{-}OH)\text{-}C(=O)\text{-}(CH_2)_2\text{-}C(=O)\text{-}N(H)\text{-}(CH_2)_5]_2\text{-}N(\text{-}OH)\text{-}C(=O)\text{-}CH_3$$

$$I = H_4DFB^+$$

$$H_3C\text{—}C(=O)\text{ — }N(\text{—}OH)\text{ — }H$$

$$II = HA$$

Recently, Crumbliss and coworkers investigated the mechanism of ligand substitution on iron(III) by a series of simple, synthetic monohydroxamic acids examining the influence of R_1 and R_2 substituents of the hydroxamato group on the kinetics and stability of mono(hydroxamato)iron(III) complexes.[4] They found that isokinetic relationship ($\Delta H^{\ddagger}$ *vs.* $\Delta S^{\ddagger}$) holds for this (»model«) system suggesting a common reaction mechanism for the hydroxamates studied, with strongly expressed electronic effects of R_1 and R_2 on the kinetics and stability of iron complexes. They proposed application of the reaction model to the interaction of iron(III) with desferrioxamine B,[5] but no thermodynamic data for this system have been reported so far.

On the other hand, we have extended investigations of the hydroxamato-iron(III) interaction beyond only the first step, *i. e.* formation/hydrolysis of the mono(hydroxamato)iron(III) complex, by including also bis- and tris-(acethydroxamato)iron(III) complexes.[6]

In this paper the thermodynamics of iron(III) interaction with desferrioxamine B is reported (resulting in the formation of ferrioxamine B and diferrioxamine B complexes) as well as with acethydroxamic acid (*II*) in 2 M $H/NaClO_4$.

EXPERIMENTAL

Materials

Iron(III) perchlorate was obtained by dissolution of freshly prepared $Fe(OH)_3$ in perchloric acid, recrystallized twice from dilute perchloric acid. Stock ferric perchlorate aqueous solutions were standardized by the spectrophotometric method ($\varepsilon = 4.16 \times 10^3$ M^{-1} cm^{-1} at 240 nm).[7] Perchloric acid solutions were prepared by dilution of 70% perchloric acid. Stock $NaClO_4$ solutions, used to maintain constant ionic strength of 2.0 M, were standardized by passing an aliquot through the cation exchange column in the acid form and titrating the liberated H^+ to the phenolphtalein end point. In all experiments, doubly distilled water was used as solvent. Desferrioxamine B was kindly supplied by Ciba as its methanesulfonate salt Desferal,^{R} which was recrystallized from methanol prior to use (M. p. = 149—151 °C). Acetohydroxamic acid (Sigma) was recrystallized from ethyl acetate.

Equilibrium Measurements

The interaction of iron(III) with desferrioxamine B was investigated by the spectrophotometric titration method, using a Cary 16K UV/VIS spectrophotometer. Stability constants of ferrioxamine B and diferrioxamine B hexadentate complexes were determined according to the published procedure[8] at five different temperatures in the range from 6.4 to 44.6 °C. Two types of experiments were performed; the first where [Fe(IIII)]/[ligand] ratio varied at a fixed H^+ concentration, and the second where the [Fe(III)]/[ligand] ratio was constant while H^+ concentration changed (0.008—0.8 M H^+).

Kinetic Measurements

The iron(III)-desferrioxamine B interaction was studied by means of hydrolysis and formation reactions using the Durrum D-110 stopped-flow apparatus and Carry 219 spectrophotometer for very fast/fast and slow reactions, respectively. All experiments were performed at different temperatures, as previously reported for 25.0 °C,[5,9,10,11] in the range of 6.4—44.6 °C.

The first two stages of hydrolysis of the tris (acethydroxamato)-iron(III) complex were studied at 6.2, 18.0 and 35.0 °C, using the stopped-flow method as described elsewhere.[6]

RESULTS AND DISCUSSION

The procedure of investigation of the iron(III) interaction with H_4DFB^+ and HA has already been reported and was in this work repeated at different temperatures. The obtained experimental data were treated according to the previously proposed reaction schemes[6,11] shortly reproduced here (eq. 1—9) in order to make the paper more readable.

$$\mathrm{Fe(HDFB)^{+} + H^{+} \rightleftarrows Fe(H_2DFB)^{2+}} \qquad k_1/k_{-1} \tag{1}$$

$$\mathrm{Fe(H_2DFB)^{2+} \rightleftarrows Fe(H_2DFB)^{2+*}} \qquad k_2/k_{-2} \tag{2}$$

$$\mathrm{Fe(H_2DFB)^{2+/2+*} + H^{+} \rightleftarrows Fe(H_3DFB)^{3+}} \qquad k_3/k_{-3} \tag{3}$$

$$\mathrm{Fe(H_3DFB)^{3+} + H^{+} \rightleftarrows Fe^{3+} + H_4DFB^{+}} \qquad k_4/k_{-4} \tag{4}$$

$$\mathrm{Fe(H_3DFB)^{3+} \rightleftarrows Fe(OH)^{2+} + H_4DFB^{+}} \qquad k_5/k_{-5} \tag{5}$$

$$\mathrm{Fe(H_2DFB)^{2+} + Fe^{3+} \rightleftarrows Fe_2(HDFB)^{4+} + H^{+}} \qquad k_6/k_{-6} \tag{6}$$

$$\mathrm{Fe(H_2DFB)^{2+} + Fe(OH)^{2+} \rightleftarrows Fe_2(HDFB)^{4+}} \qquad k_7/k_{-7} \tag{7}$$

$$\mathrm{Fe(A)_3 + H^{+} \rightleftarrows Fe(A)_2^{+} + HA} \qquad k_1/k_{-1} \tag{8}$$

$$\mathrm{Fe(A)_2 + H^{+} \rightleftarrows Fe(A)^{2+} + HA} \qquad k_2/k_{-2} \tag{9}$$

Thermodynamics of the iron(III)-HA interaction was determined from the kinetics of tris(acethydroxamato)iron(III) hydrolysis, while in the case of the H_4DFB^+—Fe(III) interaction hydrolysis and formation kinetics were studied. The stability of ferrioxamine B and diferrioxamine B hexadentate complexes was also determined by spectrophotometric titrations at different temperatures. The calculated values of thermodynamic parameters are summarized in Table I.

TABLE I

Thermodynamic Parameters[a] Corresponding to the Scheme

Hydrox. acid	$\Delta H_1^{\ddagger}$	$\Delta H_{-1}^{\ddagger}$	$\Delta H_2^{\ddagger}$	$\Delta H_{-2}^{\ddagger}$	$\Delta H_{-4}^{\ddagger}$	$\Delta H_{-5}^{\ddagger}$	$H\Delta_6^{\ddagger}$	$\Delta H_{-6}^{\ddagger}$	$\Delta H_7^{\ddagger}$	$\Delta H_{-7}^{\ddagger}$
H_4DFB^+	14.4	16.8		13.8	15.4	12.3	16.4	16.5	9.1	20
HA	10.4	15.9	12.7	12.2						
	$\Delta S_1^{\ddagger}$	$\Delta S_{-1}^{\ddagger}$	$\Delta S_2^{\ddagger}$	$\Delta S_{-2}^{\ddagger}$	$\Delta S_{-4}^{\ddagger}$	$\Delta S_{-5}^{\ddagger}$	$\Delta S_6^{\ddagger}$	$\Delta S_{-6}^{\ddagger}$	$\Delta S_7^{\ddagger}$	$\Delta S_{-7}^{\ddagger}$
H_4DFB^+	2	7		—10	—4	—1	—3	—15	—13	—3
HA	—2	10	—1	—2						

[a] Units are kcal/mol and cal/mol·K (e. u.) for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, respectively (1 cal= =4.185 J).

In Table I the parameters corresponding to paths 2, 3, —3, 4 and 5 of Fe(III)—H_4DFB^+ interaction are missing because of large uncertainties in their determination. Namely, these paths were difficult to determine, as already pointed out,[11] due to mutual coupling through the reaction rates and/or amplitudes as well as due to very complicated expressions used for calculations of microconstants.

Comparison of the paths —1 is of particular interest because the final closure of hexadentate complexes occurs in this stage. There is disagreement[5,11] whether for H_4DFB^+ this stage involves seven coordinated intermediate complex species. The obtained similarity of activation parameters for H_4DFB^+ and HA may suggests that this step corresponds to the tetradentate-hexadentate conversion, in a dissociative manner since no kinetic evidence of the heptadentate intermediate complex in the case of HA has been found.[6]

A slightly lower value of activation enthalpy change $\Delta H_1^{\ddagger}$ than $\Delta H_2^{\ddagger}$ (see Table I) for hydrolysis of $Fe(A)_3$ and $Fe(A_2)^+$, respectively, is in accordance with the proposed I_d mechanism for these paths.[6] One may expect that after dissociation of the first ligand from $Fe(A)_3$, the remaining two are bound more tightly to the central metal ion which now bears increased positive charge. The observed[4a] $\Delta H^{\ddagger} = 6.9$ kcal/mol for proton catalyzed hydrolysis of the last ligand from $Fe(A)^{2+}$ is much lower than our values of the first two stages. However, I_a mechanism is proposed[4a] for this reaction path and this enthalpy change is therefore associated with a new bond formation, in transition state, between the central metal ion and the entering water molecule. Along the same line, the value of $\Delta H^{\ddagger} = 9.2$ kcal/mol is reported for spontaneous dissociation of the ligand from mono(acethydroxamato)iron(III) complex.[4a] This value is somewhere in-between the values for the first two and the third proton catalyzed hydrolysis stages, and interestingly, the authors have proposed a partial associative character for this path. The calculated activation entropy changes may be interpreted in a similar way. For the first two steps negligible entropy changes were obtained, while for the proton catalyzed third step large negative activation entropy change of —40 e. u. is reported. Again, the parameter for the spontaneous hydrolysis is much closer to the values of the first two steps ($\Delta S^{\ddagger} = -3.5$ e. u.). Obviously, $\Delta S^{\ddagger}$ of —40 e. u. is a consequence of a strong bond formation process in transition state associated with the character of I_a mechanism.

The activation parameters (Table I) of paths —4, —5, —6, 6, 7 and —7 were inserted in the »isokinetic plots« of the model system[4], as shown in Figure 1. Namely, all these paths correspond to the coordination of one hydroxamate group to uncoordinated iron(III) (in the form of Fe^{3+} or $Fe(OH)^{2+}$) or dissociation from mono(hydroxamato)iron(III) complexes, respectively.

The obtained linearity confirms the hypothesis that the model system of simple synthetic monohydroxamic acids may be used for prediction of the behaviour of hydroxamate-based siderophores. In addition, it shows that previous coordination of two hydroxamate groups of H_4DFB^+ to iron(III) does not significantly influence electronic properties and, therefore, coordinating ability of the third uncoordinated one. This agrees with the finding[12] that proton dissociation of individual hydroxamate group of H_4DFB^+ also occurs independently with the equal enthalpy change for each ionization.

Our results suggest a common reaction mechanism for the hydroxamates and this is even more emphasized by the obtained linearity in the plot of logarithm of rate constants of spontaneous *versus* proton catalysed hydrolysis of mono(hydroxamato)iron(III) complexes and differrioxamine B (Figure 2). It is also obvious that the charge of the protonated terminal amino group of H_4DFB^+, separated from hydroxamate group by five $—CH_2$-groups, does not significantly induce electron-density shift. For example, data for betainehydroxamic acid, which has positively charged nitrogen separated by only

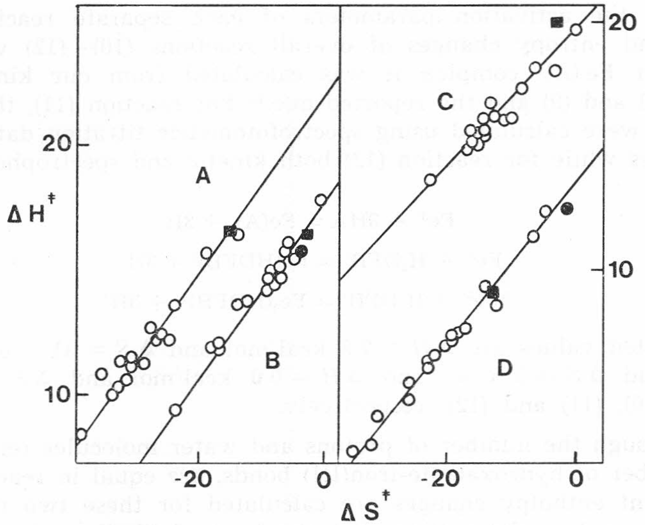

Figure 1. Isokinetic plots for the formation and dissociation of bidentate bond iron. A — formation involving $Fe(H_2O)_6^{3+}$ ion; B — proton catalyzed hydrolysis; C — spontaneous hydrolysis; and D — formation involving $Fe(OH)(H_2O)_5^{2+}$ ion. Key: ○, data points taken from ref. 4; ●, data for bidentate bond iron in ferrioxamine B; ■, data for the iron bidentate bond in diferioxamine B. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are expressed in kcal mol^{-1} and cal mol^{-1} K^{-1}, respectively.

one —CH_2-group from hydroxamate moiety, deviates strongly from the theoretical straight line calculated for neutral hydroxamic acids (Figure 2).

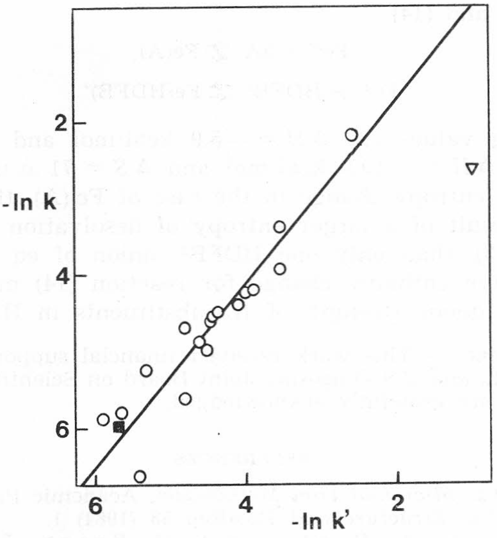

Figure 2. Plot of logarithms of the rate constants of proton catalyzed (k) *versus* spontaneous (k') hydrolysis of bidentate complexes at 25 °C. Key: ○, data points taken from ref. 4; ■, data for the bidentate bond iron of differioxamine B; and ▽, data for betaine hydroxamic acid taken from ref. 14 (in 1 M ($H/NaClO_4$)).

Besides the activation parameters of each separate reaction step, the enthalpy end entropy changes of overall reactions (10)—(12) were also calculated. For $Fe(A)_3$ complex it was calculated from our kinetic data for reactions (8) and (9) and the reported one.[4a] For reaction (11), thermodynamic parameters were calculated using spectrofotometric titration data at different temperatures while for reaction (12) both kinetic and spectrophotometric data were used.

$$Fe^{3+} + 3HA = Fe(A)_3 + 3H^+ \qquad (10)$$

$$Fe^{3+} + H_4DFB^+ = Fe(HDFB)^+ + 3H^+ \qquad (11)$$

$$2Fe^{3+} + H_4DFB^+ = Fe_2(HDFB)^{4+} + 3H^+ \qquad (12)$$

The calculated values are $\Delta H = 7.3$ kcal/mol and $\Delta S = 31$ e. u., $\Delta H = -3.1$ kcal/mol and $\Delta S = 3$ e. u., and $\Delta H = 0.0$ kcal/mol and $\Delta S = 30$ e. u. for reactions (10), (11) and (12), respectively.

Eventhough the number of protons and water molecules released, as well as the number of hydroxamate-iron(III) bonds, are equal in reactions (11) and (12), different enthalpy changes are calculated for these two reactions. This confirms an earlier observation that the heat of binding of a hydroxamate group to the iron is dependent on the number of previously bound hydroxamates.[12] Whereas in ferrioxamine B (eq. 11) the third hydroxamate group is to be bound to the iron already coordinated by two hydroxamate groups, in diferrioxamine B (eq. 12) the third hydroxamate group is to be bound to the uncomplexed iron.

The enthalpy and entropy changes of proton dissociation of H_4DFB^+ and HA are available in literature[12,13] and may be combined with the thermodynamics parameters of eqs. (10) and (11) to calculate thermodynamic parameters of eqs (13) and (14).

$$Fe^{3+} + 3A^- \rightleftarrows Fe(A)_3 \qquad (13)$$

$$Fe^{3+} + HDFB^{2-} \rightleftarrows Fe(HDFB)^+ \qquad (14)$$

The corresponding values are $\Delta H = -5.9$ kcal/mol and $\Delta S = 112$ e. u. for reaction (13) and $\Delta H = -19.8$ kcal/mol and $\Delta S = 71$ e. u. for reaction (14). The more positive entropy change in the case of $Fe(A)_3$ than ferrioxamine B is probably the result of a larger entropy of desolvation of three A^- anions included in eq. (13), than only one $HDFB^{2-}$ anion of eq. (14). On the other hand, more negative enthalpy change for reaction (14) may reflect a larger inductive electron donor strength of R_2-substituents in H_4DFB^+ than in HA.

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SAŽETAK

Kinetičko i termodinamičko istraživanje djelovanja desferioksamina B i acethidroksamne kiseline na željezo(III) u kiseloj vodenoj otopini perklorata

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Određeni su aktivacijski parametri nastajanja i hidrolize kompleksa željeza(III) s desferioksaminom B i acethidroksamnom kiselinom koji se odigravaju u višestepenoj reakciji. Određena je entalpija i entropija sveukupnih reakcija.

Na temelju dobivenih podataka raspravlja se o mehanizmu istraživanih reakcija.