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Journal of Inorganic Biochemistry 83 (2001) 107–114

JOURNAL OF Inorganic Biochemistry

www.elsevier.nl/locate/jinorgbio

Interaction between iron(II) and hydroxamic acids: oxidation of iron(II) to iron(III) by desferrioxamine B under anaerobic conditions

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Received 16 June 2000; received in revised form 19 September 2000; accepted 25 September 2000

Abstract

Interaction between iron(II) and acetohydroxamic acid (Aha), α-alaninehydroxamic acid (α-Alaha), β-alaninehydroxamic acid (β-Alaha), hexanedioic acid bis(3-hydroxycarbamoyl-methyl)amide (Dha) or desferrioxamine B (DFB) under anaerobic conditions was studied by pH-metric and UV–Visible spectrophotometric methods. The stability constants of complexes formed with Aha, α-Alaha, β-Alaha and Dha were calculated and turned out to be much lower than those of the corresponding iron(III) complexes. Stability constants of the iron(II)–hydroxamate complexes are compared with those of other divalent 3d-block metal ions and the Irving–Williams series of stabilities was found to be observed. Above pH 4, in the reactions between iron(II) and desferrioxamine B, the oxidation of the metal ion to iron(III) by the ligand was found. The overall reaction that resulted in the formation of the tris–hydroxamato complex $[Fe(HDFB)]^+$ and monoamide derivative of DFB at pH 6 is:

$$2Fe^{2+} + 3H_4DFB^+ = 2[Fe(HDFB)]^+ + H_3DFB\text{–monoamide}^+ + H_2O + 4H^+$$

Based on these results, the conclusion is that desferrioxamine B can uptake iron in iron(III) form under anaerobic conditions.

Keywords: Desferrioxamine B; Hydroxamic acid; Fe(II)/Fe(III); Anaerobic oxidation; Complex

1. Introduction

Microbial siderophores are relatively low molecular weight compounds synthesized in order to solubilize and transport iron(III) into the cells in the necessary concentrations [1,2]. Most of the hydroxamate-type siderophores contain three metal-binding groups ($-CONHO^-$) and are able to complete the coordination sphere of the iron(III) in very stable 1:1 complexes [3]. Siderophores are known to have much lower affinity for complexation with the other common oxidation state of iron in aqueous solutions, iron(II). As a consequence, the reduction of the metal center in the iron(III)–siderophore complexes results in much less stable and, in addition, more labile complexes which might play crucial role in the mechanisms for iron release at the cell membrane or interior [3]. It means that the characterization of the interaction between iron(II) and siderophores or hydroxamate based siderophore model compounds and determination of the stability of complexes formed in such systems may provide valuable contributions to a better understanding of these biologically important processes. Iron(II), however, can be easily oxidized to iron(III) by atmospheric oxygen in aqueous solution which makes solution equilibrium studies on iron(II) systems very difficult. The rate of oxidation highly depends on pH (it is much higher under basic than under acidic conditions) and on the nature of coordinating ligands. It is facilitated by iron(III) complexing agents, e.g. EDTA, NTA, citrate, phosphate [4] ligands which can stabilize the iron(III) form in stable complexes. In contrast, the iron(II) oxidation state is completely stabilized in the presence of 2,2-bipyridine or 1,10-phenantroline [5]. The oxidation problem might be the reason why only very few equilibrium studies on iron(II)–hydroxamic acid systems have been done so far [6]. In the present work an attempt is made to determine the stability constants of iron(II)–hydroxamate complexes formed in some selected systems. For this reason, after the construction of an suitable equipment suitable for equilibrium measurements under

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0162-0134/01/$ – see front matter
PII: S0162-0134(00)00197-5

anaerobic conditions, interactions between iron(II) and acetohydroxamic acid (Aha), α-alaninehydroxamic acid (α-Alaha), β-alaninehydroxamic acid (β-Alaha), hexanedioic acid bis(3-hydroxycarbamoyl-methyl)amide (Dha) or desferrioxamine B (DFB) have been studied. pH-potentiometric and UV–Vis spectrophotometric methods have been used to determine the stability constants of the complexes formed in the above systems and to study the redox reaction occurring in the iron(II)–DFB system.

2. Experimental

2.1. Chemicals

Dha was prepared as previously described [7], a standard procedure was used to prepare α-Alaha and β-Alaha [8], Aha was purchased from Sigma and DFB was obtained from CIBA Geigy. The purity of the ligands and the concentrations of the stock solutions were determined by Gran's method [9].

The metal ion stock solutions were prepared from $CoCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$ and from iron dissolved in a known amount of HCl under purified, strictly oxygen-free argon atmosphere. The iron(II) solution was then filtered and stored under anaerobic conditions. KSCN solution was used to test the absence of iron(III) traces in the stock solution. The concentrations of the cobalt(II) and manganese(II) stock solutions were determined gravimetrically via precipitation of the quinolin-8-olates, while the concentration of the iron(II) solution was determined by titrimetry using $KMnO_4$ as titrant under acidic conditions. The HCl concentration of the iron(II) solution was determined by pH-potentiometry. Argon overpressure was used when iron(II) was added to the samples.

2.2. Potentiometric and spectroscopic studies

The pH-potentiometric and spectrophotometric measurements were carried out at an ionic strength of 0.2 M (KCl). Carbonate-free KOH solutions of known concentrations (ca. 0.2 M) were used as titrant and also to maintain the pH in pH-stat measurements. The samples were in all cases completely deoxygenated by bubbling a stream of argon for ~20 min before iron(II) was added.

The pH-metric titrations were performed throughout the approximate pH range 2.0–11.0 (or below precipitation) in samples of 10.00 ml. For the metal–ligand systems, the ligand concentrations varied in the range of 4×10^{-3}–8×10^{-3} M; the metal–ligand ratios ranged from 1:1 to 1:8. Measurements at five or six different ratios were performed. Radiometer pHM 84 instrument equipped with a Metrohm 62104130 combined electrode was used to collect the experimental data. The titrant was added from a Metrohm 715 Dosimat autoburette. The electrode system was calibrated by the method of Irving et al. [10] so that the pH-meter readings could be converted into hydrogen ion concentrations. The pK_w calculated from strong acid–strong base titrations was 13.76 ± 0.01. The experimental results were used to establish the stoichiometry and to calculate the stability of the complexes formed. The calculations were performed with the computer program PSEQUAD [11]. The volumes of the titrant were fitted and the accepted fittings were below 1×10^{-2} ml.

Radiometer TTT60 Titrator, Radiometer REA 270 pH-stat module, REA 160 Titrigraph and ABU 80 autoburette instruments and a pHM 84 pH-meter equipped with a Metrohm 62104130 combined electrode were used in the pH-stat measurements on the iron(II)–DFB system. These measurements were performed at pH 5.5 ± 0.1.

The reaction between iron(II) and DFB was also studied in a special reaction vessel which was designed to keep the reactants separated until the reaction was triggered. In this case, first the reactants were measured into the isolated pockets of the reactor under anaerobic conditions, then the vessel was tightly closed and the reactants were mixed. MES (2-morphine-ethane-sulphonate) was added to the samples as buffer (pH 6). The closed vessel was kept in a box filled with argon. After a time the vessel was opened. The oxidation reaction was quenched by adding 2,2′-bipyridine (bpy) which would also prevent the oxidation of the remaining iron(II) by molecular oxygen. The concentration of DFB varied in the range 1×10^{-3}–6×10^{-3} M and metal to ligand ratios ranged from 2:1 to 1:2. Basic solution of 1,2,3-trihydroxy-benzene (pyrogallol) was used to test whether oxygen traces had got into the vessels.

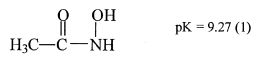
Following the necessary dilutions ($c_{Fe} \approx 1\times10^{-4}$ M), UV–Visible measurements were performed to determine the concentration of iron(II)–bpy and iron(III)–DFB complexes. Both $[Fe(HDFB)]^+$ and $[Fe(bpy)_3]^{2+}$ have characteristic absorption spectra in the region of 330–600 nm. A HP8453 spectrophotometer was used to record the spectra. The molar absorption values $\epsilon_{[Fe(HDFB)]^+}$ and $\epsilon_{[Fe(bpy)_3]^{2+}}$ were determined in independent measurements. Least-square fittings of the measured spectra using the equation

$$A = \epsilon_{[Fe(HDFB)]^+} \cdot c_{Fe(III)} + \epsilon_{[Fe(bpy)_3]^{2+}} \cdot c_{Fe(II)}$$

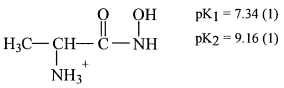
were made to determine the concentrations of Fe^{2+} and Fe^{3+}. The standard deviations of calculated concentrations were below 0.5%. In some cases tandem cuvette was used to record the spectra and determine the concentration of $[Fe(HDFB)]^+$.

2.3. GC measurements

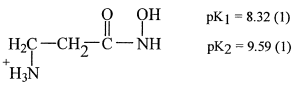
GC measurements were used to check whether $H_{2(g)}$ had evolved in the reaction. Samples were taken out and injected directly into a GC. The conditions for gas chromatographic analysis were as follows: A gas chromatograph HP 5890 Series II equipped with column 1.8 m×2 mm stainless steel was used.



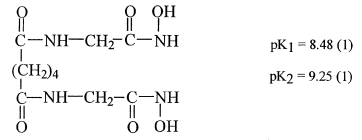
Acetohydroxamic acid (Aha)



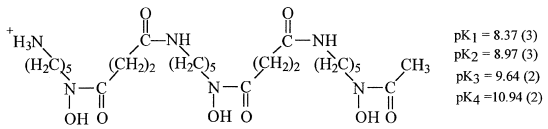
α-Alaninehydroxamic acid (α-Alaha)



β-Alaninehydroxamic acid (β-Alaha)



Hexanedioic acid bis(3-hydroxycarbamoyl-methyl)amide (Dha)



Desferrioxamine B (DFB)

Scheme 1.

Conditions — packing: molecular sieve 13×, mesh size 60/80; oven temperature: 30°C; carrier gas N_2: 80 ml min^{-1}; injection method: syringe; volume of sample: 100 μl; detector: TCD at 120°C; injector temperature: 80°C.

Calibration — standard mixtures in gas sampling bulbs: 0–1 v/v% hydrogen in argon. Under the given experimental conditions, the measured retention times (t_r) of the test mixture (1 v/v% hydrogen and 10 v/v% air in argon) were as follows: H_2: 0.29 min (positive peak); Ar: 0.45 min (negative peak); O_2: 0.48 min (positive peak).

3. Results and discussion

3.1. Iron(II)–Aha/α-Alaha/β-Alaha/Dha systems

The formulae of the protonated ligands together with their dissociation constants are shown in Scheme 1. The dissociation constants were already published in our previous papers [12,13] and identical values were obtained in the present work.

pH-metric measurements were performed under the conditions given in the Experimental section. Titration curves for Aha and iron(II)–Aha systems are shown as representative examples in Fig. 1. For comparison, inset shows titration curves for the Aha and iron(III)–Aha systems.

As Fig. 1 shows, all titration curves run together up to

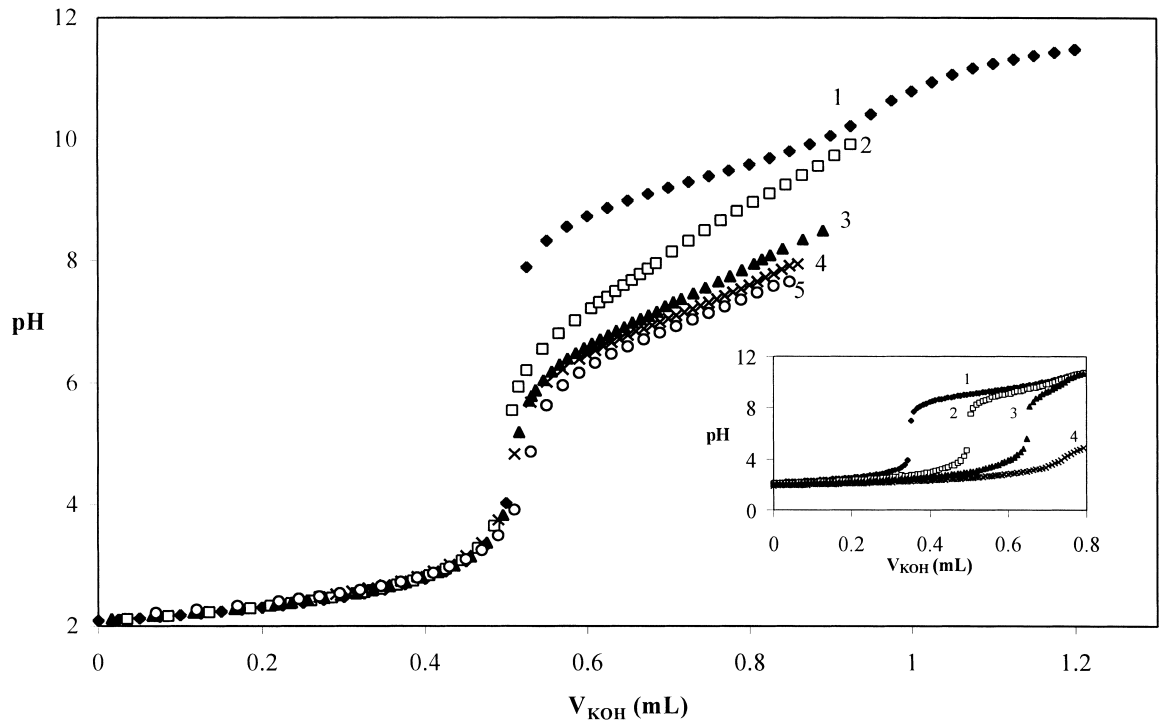


Fig. 1. pH-potentiometric titration curves for the Aha (1) and for the iron(II)–Aha systems at (2) 1:8, (3) 1:4, (4) 1:2 and (5) 1:1 metal to ligand ratios. Inset presents titration curves for the iron(III)–Aha system. (1) Aha, (2) 1:8, (3) 1:4, and (4) 1:2 iron(III)–Aha; $c_{Aha} = 8 \times 10^{-3}$ M.

pH ≈4, which means that in the iron(II)–Aha system there is no measurable complex formation below this pH. In contrast, complex formation starts below pH 2 in the iron(III)–Aha system [12]. Moreover, iron(II)-hydroxide precipitates even if high ligand excess is used (titration curves stop at these points in Fig. 1) but the hydrolysis of iron(III) is very much suppressed by the excess of Aha. The pH-metric experimental findings for the other iron(II)-containing systems are similar and therefore are not presented in figures in the paper.

PSEQUAD program was used to fit the pH-metric titration curves (250–300 experimental points/system). The equilibrium models and calculated stability constants, together with the corresponding data of complexes formed with other $3d^5$–$3d^{10}$ metal(II) ions, are collected in Table 1.

As Table 1 shows [MA], [MA_2] and [MA_3] complexes are formed in stepwise processes in the iron(II)–Aha system. Their stability constants are much lower than those of the corresponding iron(III) complexes which are 11.09, 20.69 and 28.80 [12], respectively. Protonated iron(II) complexes are also formed with α-Alaha, β-Alaha and Dha. The amino group of the coordinated ligand obviously contains the dissociable proton in the protonated complexes formed with α-Alaha, β-Alaha, while one of the hydroxamates is protonated in the species [MAH] formed with Dha.

The normal trend for the stepwise stability constants is a decrease as the number of coordinated ligands increases. However, if we calculate the stepwise constants we can see abnormal sequences in some cases, e.g. for α-Alaha complexes. The log K value for [MA] + A = [MA_2] process is higher than that for M + A = [MA] {$\log(K_1/K_2) = 4.57 - 4.73 = -0.16$}. The unexpectedly high value of $\log K_2$ most probably suggests that the [MA_2] is actually the mixed hydroxo species, [$MA_2H(OH)$].

Very few stability constants for iron(II)–hydroxamate complexes were published in former papers (only data for [MA] and [MA_2] complexes formed in the iron(II)–Aha system were previously determined and the log β values are 4.5 and 8.5, respectively [6]). Taking this into account, we compared our calculated values to the corresponding data of complexes formed with other $3d^5$–$3d^{10}$ metal(II) ions. The well-known Irving–Williams sequence of stability of complexes of 3d metal ions with ligands containing nitrogen or oxygen donors (clear dependence on crystal field stabilization energies) may help to evaluate the calculated values [14]. This is the reason why Table 1 shows results not only for the iron(II) complexes but also for the complexes of all $3d^5$–$3d^{10}$ metal ions. Except for the data for the manganese(II)–Aha/β-Alaha/Dha and cobalt(II)–Aha/β-Alaha complexes which were determined in the present work, all others were taken from our previous papers [12,15]. An evaluation of the results in Table 1 shows the validity of the 'Irving–Williams order' of the stability constants for the complexes formed with Aha, α-Alaha, β-Alaha and Dha. This is demonstrated in Fig. 2 where the stability orders of metal ion–Aha complexes are shown. This supports the validity of the stability constants of the iron(II) complexes reported here. A comparison between these constants and those for the corresponding iron(III) complexes [12,16] confirms the much lower stability of the iron(II) complexes in all the cases studied.

3.2. Iron(II)–DFB system

The natural siderophore, DFB differs from the above ligands in the number of its chelating moieties. It has three hydroxamate groups and its in vivo role is to coordinate iron(III) ion in the very stable 1:1 ferrioxamine B complex ($\log \beta_{[Fe(DFB)]} = 30.4$ [14]). Much work on iron(III)–DFB system has already been done [1,2,14], but only some electrochemical (CV) studies of iron(III)–DFB system have provided stability data for iron(II)–DFB complex. The much lower stability of the latter complex ($\log \beta_{Fe(II)-DFB} = 10$) was proved [17]. In our studies, iron(II) and DFB were directly reacted and it was found that the interaction between these reaction partners occurred only above pH 4. A further increase in the pH, however, results in a continuous pH decrease, making the subsequent equilibrium measurements impossible. Precipitation does not occur in the samples and the pH decrease stops where the interaction between iron(II) and DFB does not exist anymore. These findings are demonstrated in Fig. 3.

Parallel with the pH decrease the characteristic color of the tris-chelated iron(III)–DFB complex (λ_{max} is ≈430 nm and ϵ_{max} is ≈2600 $M^{-1}\,cm^{-1}$) appears. As can be seen in Fig. 4, the tris-chelated $[Fe(HDFB)]^+$ containing the terminal amino group in protonated form exists exclusively in the iron(III)–DFB system in the pH range in question (The calculation of the concentration distribution curves is based on our recent results [12]).

The above experimental results strongly suggest that iron(II) is oxidized to iron(III) under anaerobic conditions. The formation of the Fe(III)–DFB complex in the reaction was proved unambiguously by a spectrophotometric method using tandem cuvette when the spectra as a function of time at pH 6 (the buffer was MES) were recorded. Some of the recorded spectra are shown in Fig. 5. (In a parallel experiment Aha was used as ligand and the iron(III)–Aha complexes were not formed at all).

The finding that oxidation occurs only in the pH-range where the interaction between iron(II) and DFB exists proves the initial formation of the iron(II)–DFB complex prior to the oxidation.

Different types of measurements were performed during the subsequent studies on the iron(II)–DFB system. (1) pH-potentiometric measurements were carried out at constant pH-values using pH-stat technique (pH was

Table 1
Stability constants (log β) for the complexes formed between $3d^5$–$3d^{10}$ M(II) ions and Aha, α-Alaha, β-Alaha or Dha at 25.0±0.1°C and I=0.20 M (KCl)

Metal(II) ion	Complex	Ligand			
		Aha pK_1=9.27	α-Alaha pK_1=7.34; pK_2=9.16	β-Alaha pK_1=8.32; pK_2=9.59	Dha pK_1=8.48; pK_2=9.25
Mn^{2+}	[MAH]	–	10.92[b]	12.51(3)	12.68(2)
	[MA]	3.81(1)	3.47[b]	3.67(3)	5.28(5)
	[MAH_{-1}]	−7.27(9)	−5.99[b]	–	–
	[MA_2H_2]	–	–	24.6(2)	–
	[MA_2H]	–	14.30[b]	15.49(5)	–
	[MA_2]	6.75(1)	5.97[b]	–	–
Fe^{2+}	[MAH]	–	11.99(3)	13.47(2)	13.82(7)
	[MA]	4.56(1)	4.57(4)	–	7.06(3)
	[MAH_{-1}]	–	–	–	−3.43(6)
	[MA_2H_2]	–	–	26.58(3)	–
	[MA_2H]	–	–	18.67(2)	–
	[MA_2]	8.18(1)	9.3(1)	9.20(4)	–
	[MA_2H_{-1}]	–	–	−1.31(5)	–
	[MA_3]	10.1(1)	–	–	–
Co^{2+}	[MAH]	–	12.12[b]	13.45(2)	13.61[a]
	[MA]	4.83(1)	4.74[b]	5.28(2)	6.87[a]
	[MAH_{-1}]	–	−2.64[b]	–	–
	[MA_2H_2]	–	–	26.5(1)	–
	[MA_2]	8.71(2)	9.39[b]	–	–
	[MA_2H_{-1}]	−3.1(1)	1.59[b]	–	–
	[MA_3]	11.15(6)	–	–	–
	[M_2A_3]	–	17.69[b]	–	19.0[a]
Ni^{2+}	[MAH]	–	–	14.12[b]	13.89[a]
	[MA]	5.15[a]	6.76[b]	–	7.44[a]
	[MAH_{-1}]	−4.35[a]	–	–	–
	[MA_2H]	–	–	20.26[b]	–
	[MA_2]	9.18[a]	14.13[b]	11.57[b]	–
	[MA_2H_{-1}]	–	5.47[b]	1.99[b]	–
	[MA_3]	11.68[a]	–	–	–
	[M_2A_3]	–	–	–	20.94[a]
Cu^{2+}	[MA]	7.89[a]	10.89[b]	12.85[b]	
	[MA_2]	14.06[a]	19.87[b]	–	P
	[MA_2H_{-1}]	4.44[a]	9.98[b]	–	R
	[$M_2A_2H_{-1}$]	–	20.89[b]	–	E
	[$M_5A_4H_{-4}$]	–	–	46.66[b]	C[1]
Zn^{2+}	[MAH]	–	12.27[b]	16.16[b]	13.98[a]
	[MA]	5.18[a]	5.29[b]	–	7.73[a]
	[MAH_{-1}]	−3.40[a]	−2.26[b]	–	–
	[MA_2H_2]	–	–	27.59[b]	–
	[MA_2H]	9.45[a]	–	19.65[b]	–
	[MA_2]	–	9.32[b]	10.85[b]	–
	[MA_2H_{-1}]	11.57[a]	–	1.05[b]	–
	[MA_3]	–	–	–	–
	[M_2A_3]	–	18.77[b]	–	22.01[a]

[a] Ref. [12].
[b] Ref. [15].

5.5±0.1), when the base consumption to maintain the pH at 5.5 was measured. The reaction was followed until the change in the pH was stopped and the equilibrium was reached. Depending on the analytical concentrations and metal to ligand ratios it took 10–15 h and the base consumption was twice as much the iron concentration at 1.5:1 DFB to iron ratio and somewhat less at lower ratios. Basic solution of pyrogallol was used to check whether oxygen traces could get into the samples during the preparation of the sample and/or during the reaction time. This check proved that a small amount of oxygen could interfere with the reaction. Most probably the titrant KOH contained oxygen traces, which were, however, very small compared to the amount of iron(II). However, we had to

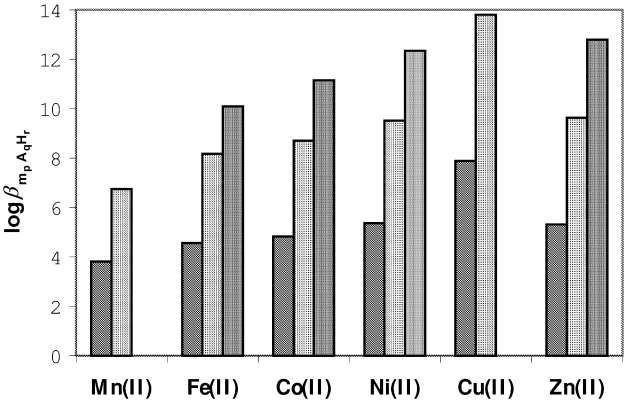


Fig. 2. Trends of stability constants (log β) of the complexes formed in $3d^5$–$3d^{10}$ metal(II) ion–Aha systems.

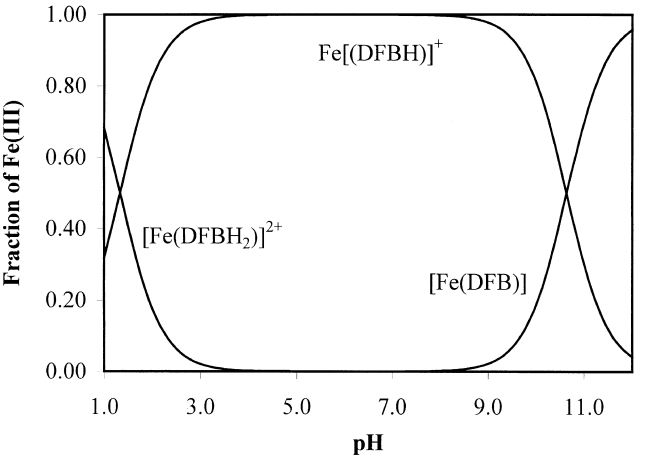


Fig. 4. Concentration distribution curves for the complexes formed in the iron(III)–DFB system at 1:1.5 metal-to-ligand ratio; $c_{DFB}=4\times10^{-3}$ M.

assume that in these measurements, a very small percentage of iron(II) was oxidized by molecular oxygen according to the reaction:

$$4Fe^{2+} + O_2 + 4H^+ = 4Fe^{3+} + 2H_2O \quad (1)$$

This is the reason why a quantitative evaluation of the pH-stat results was not made.

(2) In a second series, the individual components were reacted in a closed, deoxygenated reaction vessel placed in a special box under argon atmosphere. Pyrogallol tests continued for 24 h showed that oxygen could not get into the samples. To maintain the pH at 6, MES was used (the inertness of the buffer was checked). In these samples the reaction was quenched after different times by injecting excess bpy into the vessel. 1:3 complex between iron(II) and bpy was immediately formed and after the adequate dilution, the UV–Visible spectrum was recorded. As both the $[Fe(HDFB)]^+$ and $[Fe(bpy)_3]^{2+}$ have characteristic spectra in the range 300–600 nm, least-squares fit of each spectrum (see equation in Experimental section) was used to calculate the concentrations of $[Fe(bpy)_3]^{2+}$ and $[Fe(HDFB)]^+$. Some details are demonstrated in Fig. 6. (Molar absorbances for the two binary systems were determined from independent measurements. We also did measurement where iron(III)–DFB and iron(II)–bpy were mixed and checked whether the spectra changed in an hour. All of the spectra were recorded within 1 h after the reaction was quenched. We found that the original ratio of iron(III) and iron(II) had not changed during this time).

Summarizing all the findings, we concluded that iron(II) was oxidized by one of the components in our system under anaerobic conditions — either by the water (solvent) or by the ligand. Theoretically both are possible oxidizing agents of the iron(II). The redox potential for Fe(III)–DFB/Fe(II)–DFB system is -0.482 V [17], lower than that for the reaction $2H^+ + 2e^- = H_2$, which is ca. -0.4 V at pH used in our measurements [6]. Consequently, the

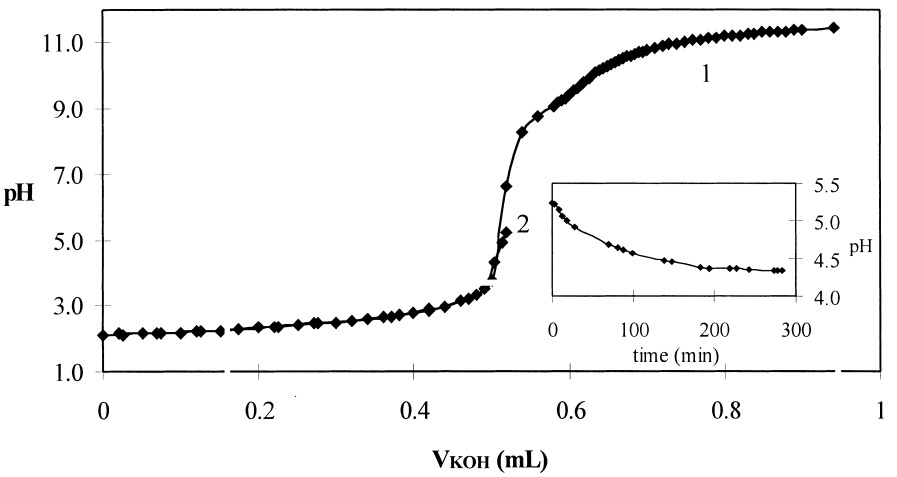


Fig. 3. pH-potentiometric titration curves for the DFB (1) and for the iron(II)–DFB systems at (2) 1:1.5 metal-to-ligand ratio. Inset shows pH decrease as a function of time; $c_{DFB}=4\times10^{-3}$ M.

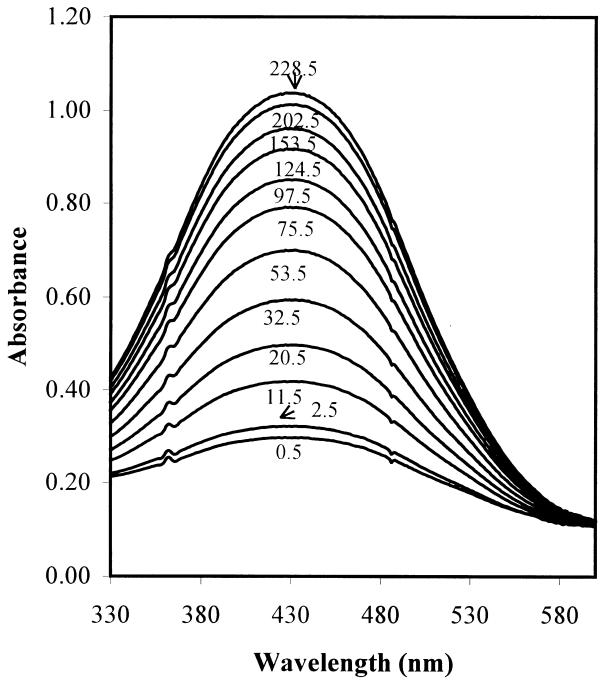


Fig. 5. Absorbance spectra of the iron(II)–DFB system as a function of time. The time in minutes is shown on the individual spectra; $c_{DFB} = 4 \times 10^{-3}$ M.

oxidation by H^+ up to ca. neutral pH, at least theoretically, is possible by the following reaction:

$$2Fe^{2+} + 2H_2O = 2Fe^{3+} + 2OH^- + H_2 \quad (2)$$

If, however, the oxidation of iron(II) occurred according to reaction (2), $H_{2(g)}$ would have to evolve. To find $H_{2(g)}$ we did the following experiments: First we proved that there was no detectable amount of molecular hydrogen in the used inert gas, argon, then $H_{2(g)}$ was injected through septum into the reaction vessel containing the reactants in separated spaces (they were not mixed). We were able to detect the injected $H_{2(g)}$ even 24 h after the injection. Finally, we made a GC analysis of the gas phase of some iron(II)–DFB samples where we could not detect any $H_{2(g)}$ in the gas phase during the monitoring time (24 h). All these results support the view that water is not able to oxidize the metal ion in this system. The final conclusion from all the above findings is that the ligand is responsible for the oxidation of iron(II)–DFB to iron(III)–DFB under anaerobic conditions. If DFB is the oxidizing agent, the hydroxamic acid moiety(ies) should undergo oxygen abstraction and the corresponding amide is formed. Similar reaction was found with V(III), V(IV) and with Mo(V) where the metal ions were oxidized by various mono- and dihydroxamic acids to V(V) and Mo(VI) and the hydroxamic acid moieties were reduced to amides and diamides [18]. The reactive behaviour of hydroxamic acids was also described in a recent paper which involved conversion of hydroxamic acid to carboxylic acid in ruthenium(II) complex [19]. Consequently, the most probable redox reaction in the iron(II)–DFB system can be summarised by the following simplified equation:

$$2Fe^{2+} + {-}CONHOH + 2H^+ = 2Fe^{3+} + {-}CONH_2 + H_2O \quad (3)$$

We can see from Eq. (3) that each hydroxamic moiety oxidizes two iron(II) ion, however, we cannot see how many hydroxamic moiety of DFB takes place in the oxidation. In order to determine the stoichiometry of our reaction, the molar fraction of $[Fe(HDFB)]^+$ formed in the

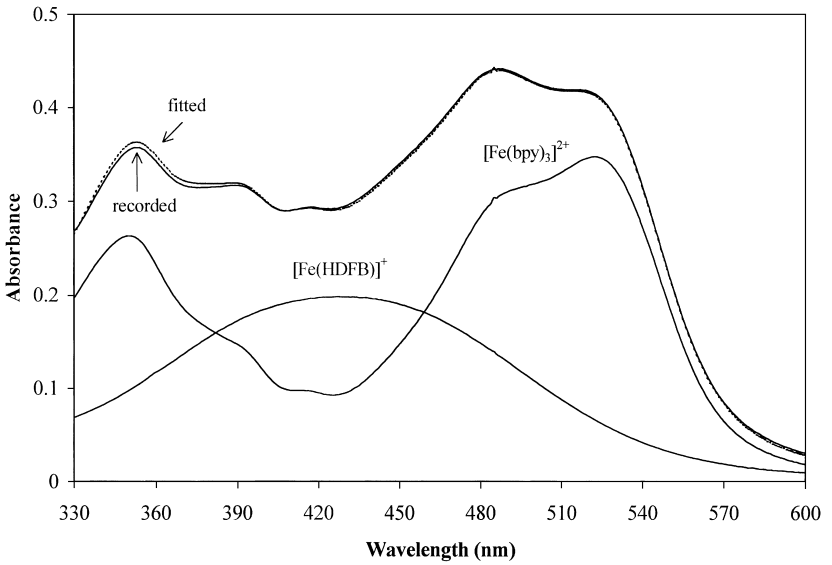


Fig. 6. Recorded spectrum of the iron(II)–DFB system (continuous line), fitted spectrum (dashed line) and its components ($[Fe(HDFB)]^+$ and $[Fe(bpy)_3]^{2+}$); $c_{DFB} = 6 \times 10^{-4}$ M metal-to-ligand ratio = 1:1.5.

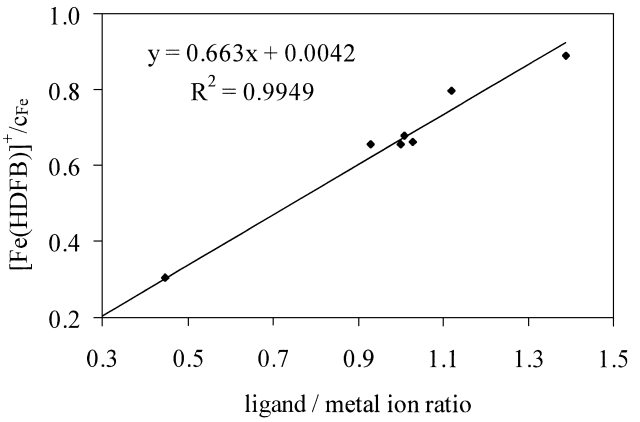


Fig. 7. Molar fraction of the complex $[Fe(HDFB)]^+$ plotted as a function of DFB/c_{Fe} ratio.

samples quenched after 10 h or more were plotted as a function of ligand/metal ratio (Fig. 7).

Fig. 7 shows that a straight line with excellent correlation and with a slope of $0.663 \pm 0.01 \approx 2/3$ was found which strongly supports the Fe/DFB = 2:3 stoichiometry in the overall reaction. The protonation constants of DFB (see Scheme 1) show unambiguously that this ligand exists in its fully protonated form at pH 6. This means that the overall reaction in fact is:

$$2Fe^{2+} + 3H_4DFB^+ = 2[Fe(HDFB)]^+ + H_3DFB\text{–monoamide}^+ + H_2O + 4H^+ \quad (4)$$

In summary, we can conclude that our experiments detailed above prove that the siderophore, desferrioxamine B is able to uptake iron in iron(III) form even under anaerobic conditions. Studies on a method adequate for the detection of the monoamide derivative of DFB and on kinetical aspects are now in progress in our laboratory.

Acknowledgements

This work was carried out within the framework of the COST D8 collaboration and was supported by the Hungarian Scientific Research Fund (OTKA T 23612).

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