Model Compounds for Microbial Iron-transport Compounds. Part IV. Further Solution Chemistry and Mössbauer Studies on Iron(II) and Iron(III) Catechol Complexes

ROBERT C. HIDER*, BRENDAN HOWLIN, JOHN R. MILLER, A. RAHIM MOHD-NOR and JACK SILVER*

Department of Chemistry, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, Essex, U.K.

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Mössbauer spectroscopy and solution studies, together with Evans' Method to measure magnetic moments, on the complexes in the Iron(II)/Iron(III) catechol system are reported. A mechanism previously proposed for the reduction of Iron(III) to Iron(II) is modified by consideration of new evidence for the monoprotonation of the mono-catecholato acid stable iron complex. The mono-protonated catechol moiety is the electron donor to the coordinated iron(III) and thus protonation of iron-containing hexadentate catecholate siderophores is probably essential for the reduction.

Introduction

Catecholic chelating agents have aroused considerable interest both as analytical reagents and as models for siderophores [1–10]. In the course of our work on models for microbial iron transport we have carried out studies on iron(II) and iron(III) complexes from phenolic [7, 9] and catecholic systems [9]. Mössbauer spectroscopy studies provided unambiguous data for the assignment of iron oxidation states, in both the iron/catechol [1] and the related iron/pyridinol systems [11].

We have identified four distinctly different coloured species in the iron catechol system, namely green, blue, purple and red [9]. The first two were found to be iron(II) complexes and the latter two, iron(III) complexes [9].

The determination of formation constants for some of the complexes of the iron/pyridinol system, attracted our attention to an apparent contradiction in the parallel data for the iron catechol system. The reported stability constant for the iron(II)–catechol green species, $k_1 = 10^{20}$ [6], is surprisingly high when considered in the light that ascorbic acid ($k_1 = 10^{9.8}$**, pH 3.0 [12]), removes iron from catecholic ligands in the pH range 3–4.5 [9]. Furthermore, at low temperatures, Cl^- is also able to displace catechol as shown by the Mössbauer data found for frozen solutions of iron(II)–catechol (green species) in the pH range 2.9 to 4.0 [9]. This latter observation is in agreement with the results of Wells *et al.* [13].

We assigned mono-protonated structures to the iron-pyridinol radical species [11], unlike our original structural assignment of the green iron(II) catechol species [9], which had been partially based on structural proposals of Mentasti *et al.* [5] and Avdeef *et al.* [6]. These differences led us to re-examine some of the data in the catecholic iron-system which has been utilised to calculate stability constants. During these studies, we also obtained further evidence for the existence of radical species, by use of Evans' Method, and for the existence of iron(II) catechol species, by use of Mössbauer studies on freeze dried materials.

We report here these new results which have enabled us to modify part of our original pH-dependent reaction scheme, thus contributing to the understanding of the radical reduction and oxidative processes found in these systems.

Experimental

Materials

Catechol (S.L.R. Fisons) was recrystallised before use. Anhydrous iron(III) chloride (S.L.R. Fisons) was used without further purification. Solutions

*Authors to whom correspondence should be addressed.

**This value was determined for the complex resulting from the reaction of ascorbic acid with iron(III).

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were prepared by dissolving weighed amounts of the reagents in deionised water and were stored under an oxygen-free nitrogen atmosphere.

Magnetic Moment Measurements

They were carried out by Evans' Method [14] using an EM-360 NMR Spectrometer.

The iron concentration of the solutions used for the magnetic studies was 6.178×10^{-3} mol dm^{-3}. The ratios of iron to catechol were those necessary to satisfy the stoichiometries of the green, blue, purple and red complexes.

Solution Chemistry

Titration data were obtained by titration of a solution of $FeCl_3$ (2×10^{-3} mol dm^{-3}) with aliquots of catechol (2×10^{-3} mol dm^{-3}) by the mole ratio method [22] using a Perkin-Elmer Coleman 575 spectrophotometer. Stability constants were calculated from the Job's plots reported previously [9] using the computer program of Likussar [15] (which was modified by us for a PDP 10 computer). The program gives a best fit to the Job's plot data in terms of an equilibrium constant and a stoichiometry for the complex formed.

Mössbauer Spectroscopy

Aqueous solutions of catechol (3.22 mol dm^{-3}) and iron(III) chloride (1.074 mol dm^{-3}), were mixed (catechol 1 cm^3; iron salt 0.5 cm^3); the pH was quickly adjusted to the required value using 5 mol dm^{-3} hydrochloric acid or 5 mol dm^{-3} sodium hydroxide. Thus the catechol:iron(III) ratio was fixed at 6:1. All samples from pH 0 to pH 4.5 precipitated a black material; this is only found in the case of concentrated solutions, and is said to be an iron-free polymeric catechol species [7], although we found it to contain iron(III) by Mössbauer spectroscopy (results not reported). The solutions were filtered, freeze dried, and transferred to the Mössbauer sample cell. This consisted of two pieces of PTFE machined to fit into each other, providing a solution space, 2 mm in thickness and 12.55 mm in diameter, with windows 0.5 mm thick. This cell was quick-frozen in liquid nitrogen and transferred to a precooled Harwell MNC 200 cryostat. The Mössbauer spectra were obtained at 80 K using a Harwell spectrometer (Waveform, generator MWG 200, servo-amplifier MSA 200, proportional counter MPC 200, vibrator MV 200), and Canberra (multichannel analyser series 30, HV power supply 3105 Amplifier 2012, pre amplifier 200 BE). The source was cobalt 57 (4 mCi) in rhodium (Radiochemical Centre, Amersham). The spectrometer was operated in a 'saw tooth' mode and the spectra computer fitted. The spectrometer was calibrated with a 25 μm thick natural iron reference absorber. All isomer shifts are referred to this as zero shift.

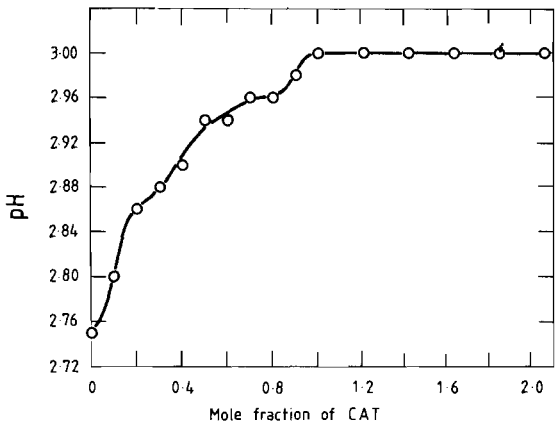


Fig. 1. Plot of mole fraction of catechol added *versus* pH to a solution of $FeCl_3$ 2×10^{-3} mol dm^{-3} fitted by computer using a least squares fit to the observed points.

Results and Discussion

Stability Constants

In the pH range 2.8 to 4.5, the green complex is dominant. We previously identified this compound to be a positively charged 1:1 iron:catechol complex with iron in the (II) oxidation state. It was formulated as $[Fe^{II}(\dot{O}OC_6H_4)(H_2O)_4]^+$ [9]. Avdeef *et al.* [6] report that this complex has a stability constant of 10^{20} and Mentasti *et al.* [5] report an equilibrium constant of 10^{-2} for the reaction in eqn. (1) from data obtained in the pH range 0 to 2 [1, 5, 6, 9].

$$Fe^{3+} + H_2cat \rightleftharpoons [Fe^{II}(cat)]^+ + 2H^+ \quad (1)$$

This leads to a value for the stability constant for the green complex of $10^{8.6}$ if one proton is dissociated and 10^{20} if two protons are dissociated. The finding that this green complex is unstable in the pH range 0 to 2 due to quinone formation eqns. (2 and 3) is difficult to explain if its stability constant is 10^{20}.

$$[Fe^{II}H\dot{c}at]^{2+} \rightleftharpoons Fe^{2+} + H\dot{c}at \quad (2)$$

$$2H\dot{c}at \rightleftharpoons H_2cat + qno \quad (3)$$

In contrast, the lower stability constant readily accounts for its acid lability and the efficient competition by ascorbic acid ($k_1 = 10^{9.82}$) [9] for iron.

The plot of mole fraction of catechol added against pH (Fig. 1) maximises at 1 equivalent of catechol indicating no further change in pH with the addition of excess catechol. This finding is in accord with the loss of only one proton on complexation, as assuming complete formation, *i.e.* K_{eq} for reaction (4) is equal to infinity,

TABLE I. Stability Constants (β_n) for Iron–Catechol Complexes.

Iron valence state	II	II	III	III
Colour	Green	Blue	Purple	Red
Fe: catechol ratio	1:1	1:3	1:2(2:4)	1:3
Log β_n, this work	9.0[a]	20.2[a]	30.5	42.3
Avdeef *et al.* [7]	20[b]		34.7	43.7

[a]Assuming the existence of a monoprotonated species. [b]Assuming the existence of a non-protonated species.

$$CatH_2 + Fe^{3+} \rightleftharpoons [Fe^{II}H\dot{c}at]^{2+} + H^+ \quad (4)$$

$$K_{eq} = \frac{[Fe^{II}H\dot{c}at]^{2+}[H^+]}{[catH_2][Fe^{3+}]}$$

then the resulting pH would be 3.00 for a catechol concentration of 10^{-3} mol dm^{-3}. The same process but assuming dissociation of two protons would give a final pH of 2.70.

In view of these calculations it was decided to directly measure the stability constant for the green complex (and subsequently the other three complexes) by the method of Job (using Likussars program [15]).

This program produces a conditional formation constant which must be modified for the degree of hydrolysis of the iron and the interaction of the ligand with protons by the use of α coefficients as recommended by Ringbom [16].

Assuming a formulation of $[Fe^{II}(\dot{O}OHC_6H_4)(H_2O)_4]^{2+}$, a stability constant of 10^9 was obtained (Table I). This value also fits the data of Mentasti [5].

Using Likussar's program stability constants were also determined for the other three major iron catechol complexes (Table I). The value of 10^{20} for the blue compound readily explains the inability of ascorbic acid to compete for iron. The stability constants for the purple and red species were in good agreement with those reported by Avdeef *et al.* [6].

Magnetism of the Iron Catechol Complexes

The magnetic moments for the iron catechol systems were studied in the pH range 1.4 to 11.5. These data are presented in table II.

The magnetic moments for the iron catechol system at pH 1.4 are of considerable interest. The moment is time dependent showing the instability of the green species at this pH due to quinone formation [5]. A moment of 5.06 BM stabilises after one hour which is equivalent to 4 unpaired electrons. The time dependence mentioned above is probably due to some monoprotonated catechol Hċat) radicals persisting in solution. In contrast at pH 3.5 where the green complex is stable, a value of 5.53 BM is found. This value is similar to that which would be expected for an iron(II) semiquinone complex consisting of a four electron centre (the iron(II) ion) and a one electron centre (the Hcat radical) with little or no coupling between the two centres (see Table legend). This compared with a value of 5.85 BM for 5 unpaired electrons in a solution of $FeCl_3$ at pH 2.5. A solution made from iron(II) and catechol (where no reduction takes place [9]) gives a value of 5.11 BM at pH 4.5.

At pH 5.0 where the blue complex is dominant, the magnetic moment is 5.66 BM indicating, as in the case of the green complex, the presence of a two centre entity containing the iron(II) cation and the associated catechol radical.

TABLE II. Theoretical and Experimental Magnetic Moments of Iron-Catechol Complexes at Various pHs.

pH		Observed magnetic moments (B.M.)	Number of unpaired electrons	Theoretical magnetic moments (B.M.)
4.5 (H_2cat + Fe(II))		5.11 ± 0.05	4	4.90
2.5 Fe(III)		5.85 ± 0.05	5	5.92
1.4 (15 min)	(H_2cat + Fe(III))	5.29 ± 0.05	4	4.90
1.4 (1 hour)	(H_2cat + Fe(III))	5.06 ± 0.05	4	4.90
3.5	(H_2cat + Fe(III))	5.53 ± 0.05	4 + 1	5.2(5.4)[a]
5.0	(H_2cat + Fe(III))	5.66 ± 0.05	4 + 1	5.2(5.4)[a]
8.0	(H_2cat + Fe(III))	5.78 ± 0.05	5	5.92
10.3	(H_2cat + Fe(III))	5.94 ± 0.05	5	5.92
11.5	(H_2cat + Fe(III))	5.94 ± 0.05	5	5.92

[a]5.2 calculated using spin only 4-electron and 1-electron centres. 5.4 is calculated assuming a value of 5.11 for 4 unpaired electrons.

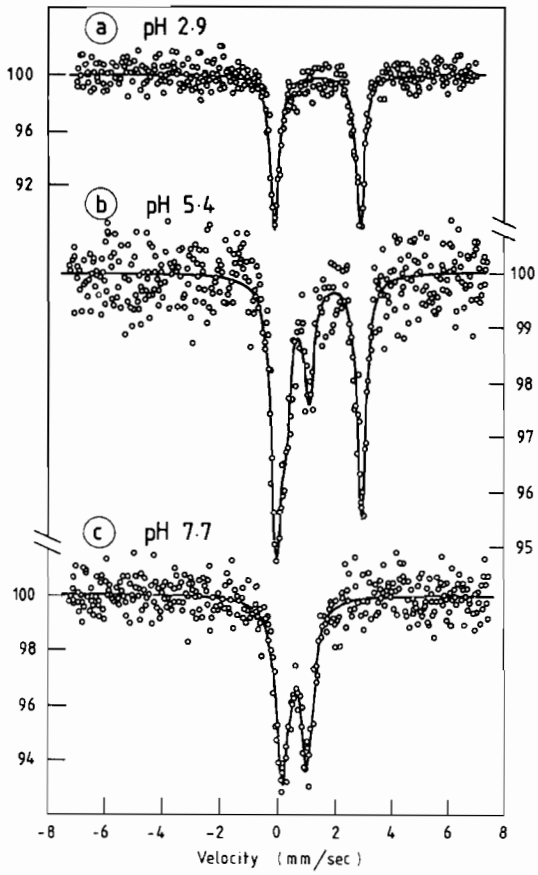


Fig. 2. Mössbauer spectra at 80 K of freeze dried and quenched iron/catechol (1:6). (a) pH 2.9 green complex; (b) pH 5.4 blue complex; (c) pH 7.7 purple complex.

TABLE III. ^{57}Fe Mössbauer Data for Freeze-dried and Quenched Iron-catechol Materials.

Experimental conditions (iron:catechol)	δ	Δ	Γ
pH 2.9			
(1:6)	1.366(6)	2.68(1)	0.184(8)
(+ water)	1.388(7)	3.34(1)	0.175(8)
(1:1)	1.356(5)	3.022(9)	0.177(8)
(+ water)	1.383(6)	3.20(1)	0.257(9)
pH 5.4			
(1:6)	1.365(9)	3.00(2)	0.21(2)
	0.60(2)	0.83(2)	0.19(2)
(+ water)	1.42(1)	3.23(2)	0.23(1)
	0.57(2)	0.82(2)	0.24(2)
pH 7.7			
(1:6)	0.54(2)	0.82(2)	0.31(3)
(+ water)	0.55(2)	0.86(2)	0.27(1)

The parameters for the purple complex at pH 7.7 are different in quadrupole splitting from those we originally reported (Table II, reference 9, for this pH) but are derived from data that do not contain any iron(II) complex (Fig. 2). In contrast, the original data were complicated by the presence of iron(II) and fitting this site as a symmetric doublet may well have biased both the isomer shift and quadrupole splitting separated for the iron(III) site.

At pH 8.0 the magnetic moment of the purple iron catechol complex is 5.78 BM which corresponds to 5 unpaired electrons typical of an iron(III) species. In fact, the magnetic moment for this solution is slightly lower than the theoretical value of 5.92, but as the complex is possibly dimeric [9], some antiferromagnetic interaction between the two iron(III) sites could explain the low value.

At pH 10.3 and 11.5 the magnetic moment of the red complex is 5.94 BM which corresponds to the theoretical value of 5.92 BM for high spin iron(III).

Mössbauer Spectroscopy

Previously reported Mössbauer data [9] for quench frozen green iron(II) catechol solutions at pH 2.9 show a quadrupole splitting of 3.43 mm s^{-1} whereas at pH 0.0, (iron catechol solutions in which the green complex does not exist), the quadrupole splitting is 3.34 mm s^{-1}. It must be noted that even in the event of rapid freezing, kinetically labile equilibria may shift during cooling. In this instance the Mössbauer parameters reflect the structure not of the initial solution but of the solution at the solidification temperature [21].

It has been established by previous workers [17–19] that the quadrupole splitting for the iron(II) cation in aqueous glasses is 3.35 mm s^{-1} when it is surrounded by six water molecules, as in $FeCl_2 \cdot 9H_2O$. In our work, the larger quadrupole splittings of 3.43 mm s^{-1} on annealing at 200 K gave way to much reduced quadrupole splittings of about 1.6 mm s^{-1}. Ruby [17] assigned such a splitting *ca.* 1.6 mm s^{-1} to $FeCl_2 \cdot 6H_2O$ where the iron is surrounded by four water molecules and two *trans* chloride anions. In our work this spectrum appeared on annealed samples at pH 1.6 and above. It would therefore appear that the green iron(II) catechol complex is unstable even in the event of rapid freezing, in which case it dissociates to form $[Fe^{II}Cl_2(H_2O)_4]^{2+}$ octahedra and separate Hcat radicals. Although Ruby *et al.* [17] using phase diagram studies for ferrous chloride in aqueous solution, found that the hexa-aquo species was difficult to grow in the presence of a low percentage of iron, this appears not to be the case in the presence of catechol.

The Mössbauer data for freeze dried iron-catechol complexes are presented in Table III and Fig. 2. The freeze dried material from a green iron (II) catechol solution at pH 2.9, gave Mössbauer data δ = 1.37 mm s^{-1} and Δ = 2.68 mm s^{-1}. The quadrupole splitting is close to that of $FeCl_2 \cdot 2H_2O$ though the δ is higher suggesting the presence of a catechol iron species. On adding a trace of water to this freeze dried material and quenching, the resultant Mössbauer parameters were found to be close to that for $FeCl_2 \cdot 9H_2O$. In contrast to the annealed frozen solution at this pH [9], no trace of $FeCl_2 \cdot 6H_2O$ was detected.

Significantly there was little evidence for iron(III) in the Mössbauer spectrum of this freeze dried sample at pH 2.9.

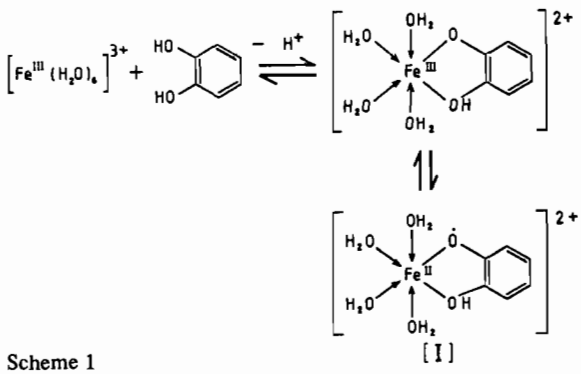
The fact that the chloride anion is able to replace catechol at low temperatures suggests that the stability constants for the iron(II) catechol and iron(II) chloride possess a differential temperature dependence, the chloride anion dominating at low temperatures. As the stability constant for iron(II) chloride is low [20], even with a large relative temperature dependence, it is inconceivable that chloride could effectively compete with the green complex if the latter had a stability constant of 10^{20}. However, the observed competition is consistent with a stability constant of 10^9.

Thus, the combined evidence resulting from the direct determination of stability constants, magnetic moment and Mössbauer studies provides strong evidence for the green iron(II) catechol species containing a monoprotonated catechol radical.

Freeze dried material from solutions at pH 5.4 yielded two Mössbauer sites. One, the major species, possesses an iron(II) site (δ = 1.365 mm s^{-1} Δ = 3.00 mm s^{-1}), and corresponds to the blue iron(II)–catechol species [9], as shown by the addition of water and subsequent quenching. The differences in isomer shift and quadrupole splitting in the freeze dried and frozen solutions for this complex are probably due to slight distortions in the octahedral environment of the complex in frozen solution, compared to solid, *i.e.* the freeze dried material. The other component is an iron(III) site with parameters similar to those reported for the purple species [9]. This iron(III) centre did not change with the addition of water and dominated the spectrum of the freeze dried sample from pH 7.7 solution. Some weak antiferromagnetic interaction, such as suggested above, under magnetism, for the purple iron catechol complex would also explain why the iron(III) doublet spectrum is observed in the Mössbauer spectrum of the frozen solution, rather than a spectrum manifesting magnetic splitting [9]. The above data support the structure previously reported [9].

Conclusions

The major conclusion from the data presented is that whereas the previous structural assignments of the blue, purple and red iron–catechol complexes [1] are confirmed, that of the green iron(II) catechol species is modified by monoprotonation [1]. This proposal leads to a stability constant which is capable of explaining all the reported competition studies involving the green iron(II) catechol complex. Furthermore, it would appear to be more reasonable for only one proton to dissociate at low



Scheme 1 [I]

pH values (Scheme 1), rather than two as previously suggested by ourselves [9], and Avdeef *et al.* [6]. The now well characterised iron(III)–catechol redox system [7–10] may well be the means by which catechol containing siderophores release iron in the cytoplasm of bacteria.

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