Chelating Tendencies of *N,N'*-Bis(2-hydroxyphenyl)-ethylenediamine-*N,N'*-diacetic Acid

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The proton association and metal complex formation constants of *N,N'*-bis(2-hydroxyphenyl)ethylenediamine-*N,N'*-diacetic acid (H_4hedda) have been determined utilising potentiometric and spectrophotometric techniques. The ligand is highly selective for the trivalent metal ion Fe^{III}, the formation constant for the Fe^{III}–H_4hedda complex [log K_{FeL} = 40.1 at 25 °C and I = 0.10 mol dm^{-3} (KNO_3)] being one of the highest reported so far for any 1:1 complex involving a multidentate ligand. Binding to the divalent metal ions Mg^{II}, Ca^{II}, Mn^{II}, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}, Cd^{II} and Pb^{II}, and to the trivalent metal ion Lu^{III}, takes place in a stepwise manner, and the order in which the ligand donor groups become co-ordinated to the metal ions has been deduced.

Recently we have become involved in the search for orally active ion(III) chelating agents for the treatment of iron-overload conditions,[1] associated with the treatment of β-thalassaemia.[2] The current drug of choice is desferrioxamine[3–5] whose utility is limited by low oral activity, rapid metabolism and only moderate efficacy. In this paper we report a detailed investigation of *N,N'*-bis(2-hydroxyphenyl)ethylenediamine-*N,N'*-diacetic acid (H_4hedda) and its interaction with a number of metal ions in solution.

Experimental

The ligand H_4hedda was prepared in the manner previously described.[1] Metal nitrate solutions, of concentrations 0.4×10^{-4}–8.0×10^{-4} mol dm^{-3}, were prepared from Aldrich and BDH reagent-grade nitrates, which were standardised where necessary.[6] All solutions were made up to 0.10 mol dm^{-3} ionic strength with KNO_3.

Ultraviolet–visible spectra were recorded on a Philips model PU8700 spectrophotometer, using 1 cm quartz cells. In the spectrophotometric competition experiments, samples (30 cm^3) were allowed to equilibrate for up to 3 weeks. For the determination of the Fe^{III}–H_4hedda formation constant, equilibrium was obtained by approach from three directions. In the first case, the ligand was added to a solution of $Fe(NO_3)_3$ and ethylenediamine-*N,N,N',N'*-tetraacetic acid (H_4edta). In the second case, H_4edta was added to a solution of the other two reagents, and in the final case $Fe(NO_3)_3$ was added to a solution of H_4hedda and H_4edta. The solutions were allowed to stand under an atmosphere of argon, at 25 °C, for up to 3 weeks. Solutions were 2×10^{-4} mol dm^{-3} with respect to Fe^{III}, H_4hedda and H_4edta, and maintained at 0.10 mol dm^{-3} ionic strength with KNO_3. This procedure provided ineluctable evidence as to when equilibrium had been attained.

Potentiometric measurements were made with a Corning model Delta Ion Analyser pH meter, equipped with Corning glass and double-junction reference electrodes, linked to a microcomputer. Over a period of 18 months the electrodes showed no sign of degradation in response. Samples (25 cm^3) were placed in a double-walled, capped titration cell maintained at 25 ± 0.05 °C by a Churchill constant-temperature water-circulating system. All solutions were kept under a positive pressure of argon, which was bubbled through a KOH solution to ensure exclusion of carbon dioxide. The following calibration routine was performed before each set of titrations. Standard buffers [pH 4.008 potassium hydrogenphthalate and pH 6.865 potassium dihydrogenphosphate–disodium hydrogenphosphate (1:1)] were used to adjust the slope and calibrate functions of the pH meter before each titration. The electrodes were allowed to stabilise in the neutral buffer (≈4 h) before the 'calibrate' function adjustment was made, after which the phthalate buffer (immersion for 30 min) was used to set up the slope adjustment. A titration of 0.01 mol dm^{-3} HCl with 0.10 mol dm^{-3} KOH was then performed, such that the hydrogen-ion concentration, not activity, could be calculated. The pH system was connected to a microlitre dispense (Hook and Tucker Instruments Microspenser). The titrant was added in 25–100 μl increments (accuracy ±0.5 μl). Readings of pH were taken with the stirring motor on. In most cases where there were systematic drifts in the pH, equilibrium was defined as having been reached when the drift was less than 10^{-3} pH units min^{-1}. Most of the ligand and ligand–metal titrations exhibited such drift in certain pH ranges (usually 6–9), and on some occasions it was necessary to wait as long as 60 min for a reading.

Results

Proton Association Constants.—Ultraviolet–visible spectra of H_4hedda over a wide pH range are presented in Fig. 1. At low pH values only one band, at 275 nm, is observed, and is assigned to the unionised phenolic groups. As the pH is increased this band shifts towards 298 nm, and increases in intensity, indicating complete conversion of the phenolic hydroxy groups into the phenolate ions. The longer wavelength of the latter is in accord with the greater negative charge, which would be expected to result in a bathochromic shift. The sharp rise in absorbance above pH 10, which represents the degree of association of the two phenolic groups, was used to measure K_1^H and K_2^H.

The potentiometric titration curve for H_4hedda, shown in Fig. 2, corroborates the discussion above. There are two distinct regions of interest. The first, between pH 4 and 7, corresponds to the removal of the protons from the zwitterionic aminocarboxylate groups. The second region, from pH 7 upwards, occurs as a result of the removal of the phenolic protons, but because the protonation reactions involving the phenolic protons are not complete until well above pH 11, where potentiometric measurements become inaccurate, the proton association constants for these phenolic groups cannot be calculated from the potentiometric data. Thus, the determination of the proton

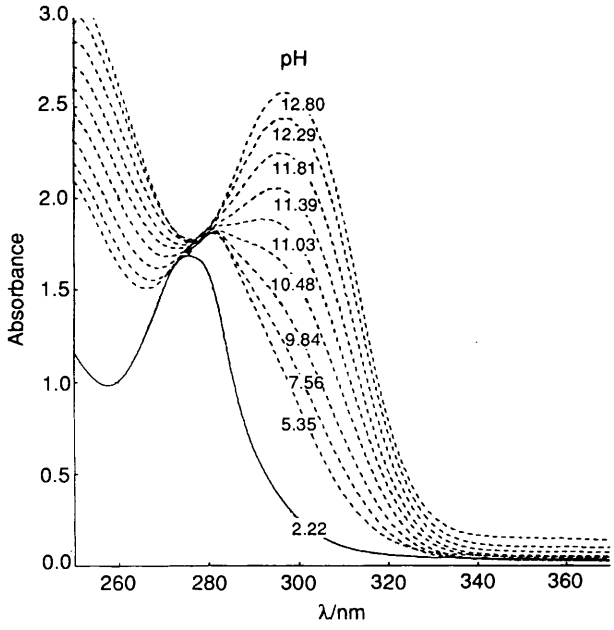

Fig. 1 Ultraviolet–visible spectra of H_4hedda as a function of pH. [H_4hedda] = 4×10^{-4} mol dm^{-3} initially, I = 0.10 mol dm^{-3} (KNO_3), 25 °C, 1 cm cell

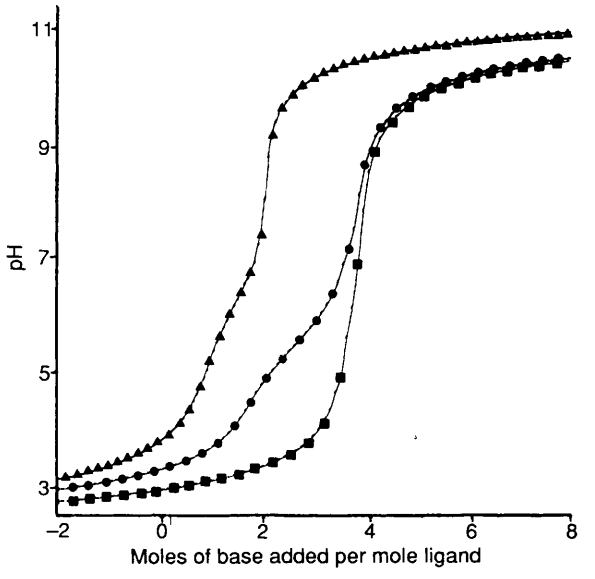

Fig. 2 Potentiometric titration curves of H_4hedda (▼), Cu^{II}–H_4hedda (●) and Fe^{III}–H_4hedda (■). [H_4hedda] = [Cu^{II}] = [Fe^{III}] = 4×10^{-4} mol dm^{-3} initially, using [KOH] = 0.10 mol dm^{-3}, I = 0.10 mol dm^{-3} (KNO_3), 25 °C

Table 1 Comparison of proton association constants

	H_4hedda[a]	H_4bedda[b]	H_4ehpg[b]
log K_1^H	13.5 (0.3)[c]	12.60	11.68
log K_2^H	11.5 (0.1)	11.00	10.24
log K_3^H	8.38 (0.02)	8.44	8.64
log K_4^H	4.47 (0.02)	4.72	6.32

[a] Constants determined at 25 °C and I = 0.10 mol dm^{-3} (KNO_3). [b] Data taken from ref. 7 for 25 °C and I = 0.10 mol dm^{-3} (KCl). [c] The values in parentheses represent the estimated standard deviations (σ) in the corresponding constants.

association constants of H_4hedda is divided into two parts.

(1) The constants K_1^H and K_2^H were determined using spectrophotometric measurements between pH 10.0 and 13.0 at 298 nm. The measured absorbance is the sum of the products of the species H_nL^{n-4} (H_4L = H_4hedda) and the associated molar absorption coefficients ε (H_nL) (n = 0, 1 or 2); the concentrations of H_3L^- and H_4L may be assumed negligible in this pH range. The absorbance, A, and ε (H_nL) must correspond to the same wavelength. The total ligand concentration (c_L) is also known [equations (1) and (2)] where $K_n^H = [H_nL^{n-4}]/[H^+][H_{n-1}L^{n-5}]$ (n = 1–4).

$$A = \varepsilon(H_2L)[H_2L^{2-}] + \varepsilon(HL)[HL^{3-}] + \varepsilon(L)[L^{4-}] = \varepsilon(H_2L)K_1^HK_2^H[H^+]^2[L^{4-}] + \varepsilon(HL)K_1^H[H^+][L^{4-}] + \varepsilon(L)[L^{4-}] \quad (1)$$

$$c_L = [H_2L^{2-}] + [HL^{3-}] + [L^{4-}] = K_1^HK_2^H[H^+]^2[L^{4-}] + K_1^H[H^+][L^{4-}] + [L^{4-}] \quad (2)$$

Dividing equation (1) by (2) and eliminating the term $[L^{4-}]$, one gets equation (3). Using this equation the values of

$$A = \frac{c_L\{\varepsilon(H_2L)K_1^HK_2^H[H^+]^2 + \varepsilon(HL)K_1^H[H^+] + \varepsilon(L)\}}{K_1^HK_2^H[H^+]^2 + K_1^H[H^+] + 1} \quad (3)$$

K_1^H and K_2^H may be determined iteratively, utilising a linear least-squares fitting routine,[8] which matches the experimentally observed absorbance values as a function of pH, at fixed wavelengths, with those calculated with assumed values of K_1^H, K_2^H and ε(HL). The value of ε(L) at 298 nm (8330 dm^3 mol^{-1} cm^{-1}) was estimated from the absorbance at pH 14, where L^{4-} is the dominant species. The value of ε(H_2L) at 298 nm (1338 dm^3 mol^{-1} cm^{-1}) was estimated from absorbance values between pH 7 and 9, where it may be assumed that neither phenolic group is contributing significantly to the total absorbance, *i.e.* where the concentration of HL^{3-} and L^{4-} are negligible and where it may be seen that the absorbance is fairly constant. As a consequence of the low absorbance over this region, any error in the actual value taken, for example due to the presence of some H_3L^-, will have minimal effect when used in equation (3). For the results see Table 1.

(2) The protonation constants K_3^H and K_4^H, involving loss of the amino protons, were determined from potentiometric measurements,[8] using the FORTRAN computer program STBLTY.[9] The results are also shown in Table 1.

Fe^{III}–H_4hedda *Formation Constant*.—The potentiometric titration curve for the 1:1 Fe^{III}–H_4hedda system is shown in Fig. 2, and the single step which occurs when 4 mol of base per mol of H_4hedda have been added clearly indicates that the equilibrium $H_4L + Fe^{3+} \rightleftharpoons [FeL]^- + 4H^+$ ($K_{FeL} = [FeL^-]/[Fe^{3+}][L^{4-}]$) is displaced completely to the right hand side (L^{4-} = tetradeprotonated anion of H_4hedda). As the ratio of Fe^{III} to H_4hedda was reduced, structure appeared in the relevant titration curves, but since this was exactly as expected for the iron-free system this could not be ascribed to intermediate protonated iron(III) complexes. As a consequence of these observations it is evident that the formation constant of the Fe^{III}–H_4hedda complex cannot be calculated from the potentiometric titration curves.

The ultraviolet–visible spectrum of Fe^{III}–H_4hedda, like those of the iron(III) complexes of *N,N'*-ethylenebis[2-(*o*-hydroxyphenyl)glycine] (H_4ehpg)[10] and *N,N'*-bis(2-hydroxybenzyl)-ethylenediamine-*N,N'*-diacetic acid (H_4bedda),[7,11] shows that the pH-dependent phenol band of the ligand (Fig. 1) is replaced by a single band at 277.5 nm which is independent of pH. This is indicative of the complete dissociation of the phenol groups and further establishes their binding to the Fe^{III} ion over the entire range pH 2.5–13.0.

Since the Fe^{III}–H_4hedda complex has such an intense red colour [at λ = 484.6 nm, ε(FeL) = 2360 dm^3 mol^{-1} cm^{-1}], the competition reaction for Fe^{III} between H_4hedda and H_4edta

Table 2 Formation constants[a] of metal ions with H_4hedda

Metal	log $K_{MH_2L}^{M}$	log K_{MHL}^{M}	log K_{ML}^{M}	log $K_{MH_2L}^{Hb}$	log K_{MHL}^{Hb}
Mg^{II}	6.0	10.3	14.4	7.2	9.5
	(0.20)	(0.27)	(0.38)		
Ca^{II}	6.6	10.5	14.7	7.6	9.3
	(0.29)	(0.34)	(0.46)		
Mn^{II}	4.7	9.8	16.0	6.4	7.3
	(0.31)	(0.27)	(0.24)		
Co^{II}	10.0	15.7	19.9	5.8	9.4
	(0.24)	(0.26)	(0.28)		
Ni^{II}	10.5	15.4	19.4	6.7	9.6
	(0.62)	(0.65)	(0.64)		
Cu^{II}	11.2	17.2	23.2	5.5	7.6
	(0.36)	(0.42)	(0.41)		
Zn^{II}	10.1	15.0	19.0	6.7	9.5
	(0.54)	(0.65)	(0.64)		
Cd^{II}	10.6	14.6	18.4	7.6	9.7
	(0.20)	(0.29)	(0.24)		
Pb^{II}	9.9	14.9	18.4	6.5	10.1
	(0.54)	(0.61)	(0.24)		
Lu^{III}	9.3	14.1	19.2	6.8	8.4
	(0.29)	(0.39)	(0.57)		
Fe^{III}	—	—	40.1[c]	—	—

[a] Constants determined at 25 °C and I = 0.10 mol dm^{-3} (KNO_3). All solutions were titrated from low to high pH in the range 2.50–11.0. The numbers in parentheses refer to the standard deviations (σ) of the corresponding constants as given by STBLTY.[9] [b] The values of these constants were calculated using the constants derived from STBLTY.[9] [c] Value determined spectrophotometrically using the iron(III) competition experiment described in the text.

Table 3 Comparison of formation constants of H_4hedda and H_4ehpg complexes

	log K_{ML}^{M}		
Metal	H_4hedda	H_4ehpg*	Δ log K
Mg^{II}	14.4	8.0	6.4
Ca^{II}	14.7	7.2	7.5
Ni^{II}	19.4	19.7	−0.3
Cu^{II}	23.2	23.9	−0.7
Zn^{II}	19.0	16.8	2.2
Cd^{II}	18.4	13.1	5.3
Fe^{III}	40.1	33.9	6.2

* Refs. 7 and 10.

may be studied spectrophotometrically, and the Fe^{III}–H_4hedda formation constant determined. The equilibrium for this reaction is $[FeE]^- + L^{4-} \rightleftharpoons [FeL]^- + E^{4-}$ ($K_x = [FeL^-][E^{4-}]/[FeE^-][L^{4-}]$), where L^{4-} and E^{4-} are the fully deprotonated tetraanions of H_4hedda and H_4edta respectively, both of which will participate in the above equilibrium as the protonated species H_nL^{n-4} and H_nE^{n-4} (n = 1–4). The protonated iron(III) complex species $[Fe(H_2L)]^{n-1}$ and $[Fe\text{-}H_nE]^{n-1}$ (n = 1–4), may however, be neglected. Thus, the total concentrations of H_4hedda (c_L), H_4edta (c_E) and Fe^{III}(c_{Fe}), are given by equations (4)–(8). The term α_E may be calculated from

$$c_L = \Sigma[H_nL^{n-4}] + [FeL^-] = \alpha_L[L^{4-}] + [FeL^-] \quad (n = 1\text{–}4) \quad (4)$$

$$c_E = \Sigma[H_nE^{n-4}] + [FeE^-] = \alpha_E[E^{4-}] + [FeE^-] \quad (n = 1\text{–}4) \quad (5)$$

$$c_{Fe} = [FeL^-] + [FeE^-] \quad (6)$$

$$\alpha_L = 1 + \beta_1^H[H^+] + \beta_2^H[H^+]^2 + \beta_3^H[H^+]^3 + \beta_4^H[H^+]^4 \quad (7)$$

$$\beta_n^H = [H_nL^{n-4}]/[L^{4-}][H^+]^n \ (n = 1\text{–}4) \quad (8)$$

equations analogous to (7) and (8). Since the absorbance is measured at 484.6 nm, and $[FeE]^-$ does not absorb at this wavelength, the measured absorbance A (for a 1 cm path length) is given by equation (9). Thus $[FeL^-]$ may be calculated from

$$A = \varepsilon(FeL)[FeL^-] \quad (9)$$

equation (9), $[FeE^-]$ from (6) and $[E^{4-}]$ from (5). A similar procedure starting with the known $[FeL^-]$ and using equation (4) yields $[L^{4-}]$. These data may then be used to calculate the displacement constant K_x. However, this constant is also defined as K_{FeL}/K_{FeE}, and so by using it with the known value of K_{FeE}[12] the formation constant K_{FeL} can be calculated: log K_{FeL} = log K_x + log K_{FeE} = 15.0 + 25.1 = 40.1.

Divalent Metal Ion–H_4hedda Complexes.—The potentiometric titration curve for the 1:1 Cu^{II}–H_4hedda system is shown in Fig. 2. The two-step behaviour for this ion is typical of those for the other divalent ions listed in Table 2. In general, for these divalent ions the initial reaction with H_4hedda is a two-proton displacement from the ligand by the metal ion to form the diprotonated complex: $M^{2+} + H_4L \rightleftharpoons [M(H_2L)] + 2H^+$. This is followed by two subsequent dissociation reactions, in which the remaining protons are removed, to form the monoprotonated and fully deprotonated complexes, represented by the equilibrium constants $K_{MH_nL}^{H} = [MH_nL^{n-2}]/[MH_{n-1}L^{n-3}][H^+]$ for n = 1 or 2. These are the association constants relating to the above dissociation reactions and are related by the ligand proton association constants to the metal association constants as follows: $K_{MH_nL}^{M} = [MH_nL^{n-2}]/[M^{2+}][H_nL^{n-4}]$ for n = 0, 1 or 2, and $K_{MH_nL}^{H} \cdot K_{MH_{n-1}L}^{M}/K_n^H$ for n = 1] or 2.

The trivalent metal ion Lu^{III} was also studied and found to behave in a similar way to the divalent ions, namely that the initial reaction is to displace two protons from H_4L to give $[Lu(H_2L)]^+$; this is followed by two further dissociations to give [Lu(HL)] and $[LuL]^-$. This behaviour is not unexpected because similar observations have been noted with H_4bedda.[11]

The formation constants for the metal ion–H_4hedda species were determined using the program STBLTY.[9]

Discussion

The formation constants of the metal ion–H_4hedda complexes are given in Table 2. It can clearly be seen that H_4hedda is highly selective for Fe^{III}, the formation constant of the Fe^{III}–H_4hedda complex being one of the highest reported for any 1:1 iron(III) chelate of a multidentate ligand.*

Table 3 presents a comparison between the fully deprotonated complexes of H_4hedda and H_4ehpg, where, for the relevant ions, the expected Irving–Williams order[14] is seen to be followed in each series. A study of Table 3 shows that for all complexes having no crystal-field stabilisation energy (c.f.s.e.) the H_4hedda ligand confers the greater stability. This is probably a function of the higher basicities of its phenolate ligands (Table 1); it is also likely to arise for steric reasons since H_4hedda offers only five-membered rings to the metal ions, whereas with H_4ehpg two out of the five rings which include the metal are six membered. The complexes of Ni^{II} and Cu^{II} are anomalous in that for these ions H_4ehpg confers the greater stability. This is in all likelihood a c.f.s.e. effect which may be attributed to binding by the carboxylate groups, even though these metal ions often prefer a square-planar geometry. When compared against H_4ehpg, the weaker binding of the H_4hedda anion, arising as a

* The values reported in ref. 13 are in fact those for H_4ehpg and not H_4hedda.

Table 4 Comparison of affinities of ligands for Fe^{III}

Ligand	n^a	log K_{FeL_n} [b]	pFe^{3+} [c]	log K_{eff} [d]	log K_{sol} [d]
H_4hedda	1	40.1	29.8	19.28	6.64
H_4ehpg	1	33.91	26.4	15.70	3.06
H_4bedda	1	39.68	31.0	20.89	8.25
H_3nta	2	24.30	—	8.84	−6.80
H_4edta	1	25.00	22.2	8.74	−3.90
H_5dtpa	1	25.00	24.7	8.39	−4.25
H_6ttha	1	26.80	22.6	11.04	−1.61
H_2cat [e]	3	43.8	—	12.33	−6.31
f	1	41.7	28.5	—	—
g	1	45.8	29.4	—	—
Enterobactin	1	≈52	35.5	25.80	13.16
Desferrioxamine	1	30.60	26.6	16.48	3.84
Transferrin	—	≈24	23.6	≈16	—

[a] *n* Represents the number of ligand molecules required to form the iron(III) complex. [b] For all ligands, except H_4hedda (present work) and transferrin (refs. 15 and 16), pK_a values and formation constants were obtained from refs. 7 and 10–12. [c] Calculated for solutions 10 μmol dm^{-3} in ligand, 1 μmol dm^{-3} in Fe^{III}, pH 7.4 and 25 °C. [d] Calculated for solutions 1×10^{-3} mol dm^{-3} in ligand, 5×10^{-4} mol dm^{-3} in Ca^{II}, pH 7.4, 25 °C and pK_w 13.80. [e] H_2cat = Catechol. [f] *N,N′,N″*-Tris(2,3-dihydroxy-5-sulfobenzoyl)-4-azaoctane-1,8-diamine. [g] *N,N′,N″*-Tris-(2,3-dihydroxy-5-sulfobenzoyl)-1,3,5-triaminomethylbenzene.

consequence of the lower basicity of the zwitterionic amino-carboxylate groups (Table 1), implies first that, in addition to the phenolates, the carboxylates do indeed bind to Ni^{II} and Cu^{II} as well as to the other ions, and secondly that the carboxylates are more important in determining the ligand-field strength than are the phenolates.

Although the iron(III) complex-formation constants of many other ligands (Table 4) are exceptionally large, it is nevertheless a fact that, at physiological pH, protons will compete effectively with iron for bonding to many ligand donor groups. This leads to the presence of other protonated metal–ligand species in solution, and hence K_{FeL} alone is clearly not a sufficient standard by which to measure the effectiveness of a potential iron-removal agent at physiological pH. The amount of free Fe^{III} in solution provides a better standard, and can be expressed in terms of pFe^{3+} values (where $pFe^{3+} = -\log[Fe^{3+}]$). Therefore, the concentration of unchelated Fe^{3+} in a solution, under conditions previously defined [15,16] has been calculated and is expressed as pFe^{3+}. The value for H_4hedda is given in Table 4, along with those for other ligands of interest. The comparison between different ligands now takes on a more meaningful significance, since pFe^{3+}, unlike K_{FeL}, takes into account the effects of ligand basicity, chelate protonation and hydrolysis as well as differences in metal–ligand stoichiometries. The value of pFe^{3+} shows that H_4hedda, in addition to having an extremely high overall formation constant K_{FeL}, which is independent of pH, is also one of the most effective ligands for Fe^{III} at the fixed pH pertinent to the living cell.

Despite the fact that many ligands have much higher formation constants and pFe^{3+} values than those of transferrin and ferritin (Table 4), many of them fail to mobilise Fe^{III} *in vivo*. This is because, in biological systems, many metal ions may be partly hydrolysed though still soluble, and these reactions in turn are a function of other metal ions which may be present. To account for possible interferences by other ions, such as Ca^{II} and hydroxide, under physiological conditions, a term known as the 'effective' formation constant, K_{eff}, can be used.[8,17] In principle it is possible to maximise K_{eff} by decreasing the pK_a of the ligand, increasing K_{FeL} and by designing ligands with a low affinity for Ca^{II} and other metal ions. The first of these variables is limited by the necessity to keep the pH ≈7.4 if a neutral or low charged ligand is required under physiological conditions. The values of K_{eff} for various ligands (Table 4) clearly indicate that H_4hedda, according to the above definition, should be among the best of known ligands for binding Fe^{III} under partially simulated biological conditions.

The K_{eff} value of an iron(III) complex takes into account those types of interference that might occur in homogeneous aqueous solutions. In situations where iron(III) hydroxide species may precipitate the picture becomes further complicated, since at equilibrium the concentration of free Fe^{III} is maintained constant by the solid phase. This can be expressed by its solubility constant, K_{sp}. Thus, at any given pH, the concentration of Fe^{III} is fixed. Using this fact, a further term, known as the solubilising constant, K_{sol}, can be utilised.[8,17] The solubilising constant can be considered as expressing the degree of conversion of the free ligand into the metal complex, and is dependent on the number of ligand molecules required to form the iron(III) complex. It is maximised for multidentate ligands that have sufficient donor groups to complex completely the Fe^{III}. Table 4 shows values for complexes of Fe^{III} with H_4hedda and other ligands.

It is evident from the above discussion that formation constants do not represent the only criteria when considering the potential for iron(III) chelation *in vitro* and *in vivo*. The aminocarboxylic acids of the series nitrilotriacetic acid (H_3nta), H_4edta, diethylenetriaminepentaacetic acid (H_5dtpa), and triethylenetetraaminehexaacetic acid (H_6ttha) all have reasonably high formation constants, but their K_{eff} values are not particularly high and they have negative solubilisation constants. Indeed, none of this series is able to dissolve iron(III) hydroxide or displace Fe^{III} from transferrin.[14,15] However, the phenolic aminocarboxylic acids H_4hedda, H_4bedda and H_4ehpg all have high values for all three constants: H_4hedda would appear to be only slightly less effective than H_4bedda, the difference being primarily due to the fact that the former has a greater affinity for Ca^{II} than has the latter.[7,11] Nevertheless H_4hedda has significantly larger values for all three constants than desferrioxamine. Hence, on the basis of thermodynamic criteria alone, it should be able to mobilise Fe^{III} from transferrin to a much greater extent than desferrioxamine can, and is therefore worthy of further investigation.

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