New synthetic, selective, high-affinity ligands for effective trivalent metal ion binding and transport

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Abstract

A series of new ligands containing 3-hydroxy-2-pyridylmethyl donor groups has been synthesized which form complexes of iron(III) having thermodynamic stabilities comparable to and greater than those of natural catechol-type siderophores. When this functional group is substituted on the triazacyclononane framework the resulting hexadentate ligand has an affinity for iron(III) which exceeds that of the natural siderophore, enterobactin, at physiological pH. Ethylenediamine and diethylenetriamine derivatives containing hydroxypyridyl and acetate donors also show high affinity for Fe(III) and other trivalent metal ions. The stabilities of the iron(III) chelates of the new ligands, particularly the triazacyclononane derivatives, are compared with those of other highly effective iron chelators published in the literature, such as the trishydroxamates and triscatecholate ligands.

Introduction

The development of synthetic high-affinity and highly selective chelating agents for Fe(III) has long been an objective of coordination chemists. The amino polycarboxylates such as nitrilotriacetic acid (NTA) (**1**), ethylenediaminetetraacetic acid (EDTA) (**2**) and diethylenetriaminepentaacetic acid (DTPA) (**3**) are relatively ineffective for Fe(III) since they do not prevent the precipitation of ferric hydroxide in the weakly alkaline range (pH 8–9) [1]. The sexadentate chelating agents ethylenebishydroxyphenylglycine (EHPG) (**4**) and bishydroxybenzylethylenediaminediacetic acid (HBED) (**5**) have two phenolic groups replacing two of the carboxylates of EDTA and have very high affinities for Fe(III) with stability constants for *racemic* EHPG of 10^{35} and for HBED 10^{39} [1, 2]. These ligands were developed especially for Fe(III) sequestration and the methyl ester of HBED is now being tested for the removal of iron from the body of patients with Cooley's anemia [3]. A variation on the structure of HBED is indicated by the formula **6**, bispyridoxylethylenediaminediacetic acid (PLED), which has much less basic pseudo phenolic (hydroxypyridyl) groups derived from vitamin B_6. It has a lower affinity for iron than HBED but the lower stability is partly compensated for by the lower p*K*s of the phenolic groups. This ligand has relatively low toxicity, is a fairly good iron chelator, and should also be tested for treatment of iron overload [4, 5]. The many catechol containing ligands of Raymond *et al.* [6] are represented by MECAMS (**7**), in which the three catechols are connected in one molecule through an organizing group, in this case a mesityl function. A cryptand described recently by Raymond and co-workers [7] is bicapped TRENCAM (**8**) in which three catechol groups are arranged in such a way as to coordinate Fe(III) strongly and are connected together by capping groups derived from TREN, triaminotriethylamine. A cryptand with mesityl capping groups was also described by Vogtle and co-workers [8, 9] who claimed very high stability, 10^{59}, for its iron(III) chelate. This has since been corrected to about 10^{43} [7]. Upto the present time enterobactin (**9**), a natural siderophore, containing three catecholate groups bound covalently together through a cyclic cycloserine-trimeric ester was assigned a very high stability constant of 10^{52} by Raymond *et al.* [6] and was the strongest chelator for Fe(III) known. Another natural siderophore which should be mentioned at this point is desferriferrioxamine-B (**10**). This ligand is a trishydroxamic acid which has a very high affinity for the ferric ion and is used medicinally for treatment of iron overload in cases of Cooley's anemia and other diseases of iron excess [4].

In order to explore the possibility of obtaining additional high-affinity chelators for Fe(III), variations of the structure of PLED have been attempted. In PLED it is seen that a 2-methyl group is present in the aromatic rings which would provide considerable steric

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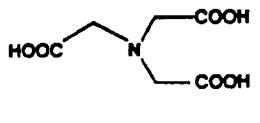
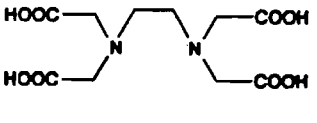


1 Nitrilotriacetic acid (NTA)



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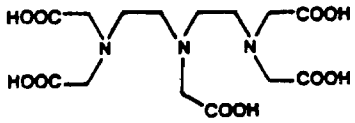
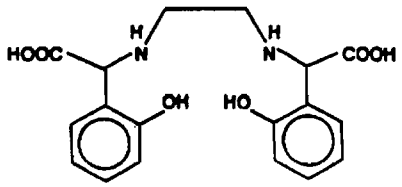
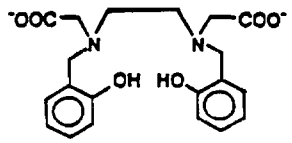


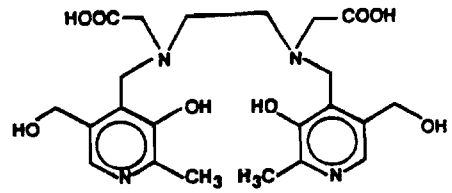
3 Diethylenetriaminepentaacetic acid (DTPA)



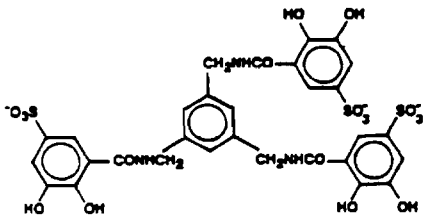
4 Ethylenebishydroxydroxyphenylglycine (EHPG)



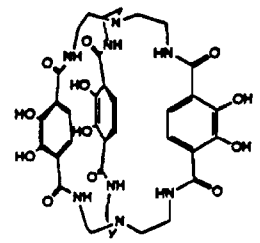
5 Bis-hydroxybenzylethylenediaminediacetic acid (HBED)



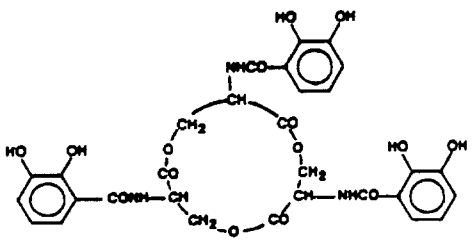
6 Bis-pyridoxylethylenediaminediacetic acid (PLED)



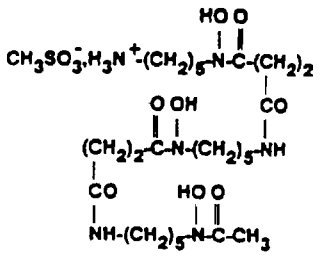
7 MECAMS



8 Bicapped TRENCAM



9 Enterobactin



10 Desferriferrioxamine-B (DFB)

hindrance to complex formation. A method has been developed [10, 11] for the synthesis of analogs of PLED through the introduction of three hydroxypyridyl groups in which the nitrogen is adjacent rather than *para* to the covalent linkage to the polyamino acid or polyamino backbone structure. The compounds thus obtained do not contain a methyl group *ortho* to the phenolic donor but most of the compounds obtained have a methyl group adjacent to the pyridine nitrogen to eliminate byproducts in the Mannich reaction. The compounds described in the present paper are hydroxypyridyl derivatives of ethylenediaminediacetic acid, diethylenetriaminetriacetic acid and triazacyclononane, obtained by the Mannich reaction involving formaldehyde and 3-hydroxy-6-methylpyridine. These ligands, *N,N'*-bis(3-hydroxy-6-methyl-2-pyridylmethyl)ethylenediamine-*N,N'*-diacetic acid, ENDA-HP; *N,N''*-bis(3-hydroxy-6-methyl-2-pyridylmethyl)diethylenetriamine-*N,N',N''*-triacetic acid, DTTA-HP; and *N,N',N''*-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclononane, TACN-HP; are illustrated by formulas **11**, **12** and **13**, respectively. The synthesis and characterization of these ligands has been described by Sun *et al.* [11]. The present paper describes the physicochemical studies of their affinities for the ferric ion and other trivalent metal ions by potentiometric, spectrophotometric and redox measurements. The Fe(III) complex of TACN-HP has been described briefly [10] as being very stable in solution.

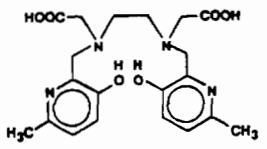
Experimental

Synthesis of ligands

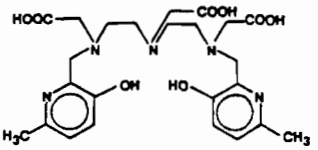
The ligands described in this paper, ENDA-HP (**11**), DTTA-HP (**12**) and TACN-HP (**13**), were synthesized and described in a recent publication by Sun *et al.* [11].

Potentiometric determinations

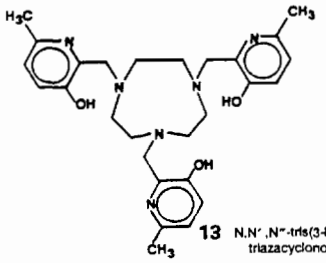
Equilibrium potentiometric determinations of the ligand protonation constants and its metal binding constants for complexes in ligand to metal ratio of 1:1



11 N,N′-bis(3-hydroxy-6-methyl-2-pyridylmethyl)ethylenediamine-N,N′-diacetic acid, ENDA-HP



12 N,N″-bis(3-hydroxy-6-methyl-2-pyridylmethyl)ethylenetriamine-N,N′,N″-triacetic acid, DTTA-HP



13 N,N′,N″-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclononane, TACN-HP

were carried out by the glass electrode method at 25.0 °C, 0.100 M (KCl), and with the use of the program BEST [12]. Details of the potentiometric determinations are found in ref. 13.

The potentiometric apparatus consists of a glass jacketed titration cell, a temperature bath (Haake VWR model 1140, 25.0 °C), glass, reference electrodes, and a 10 ml capacity Metrohm piston buret, for which the buret tip was sealed in the cap of the titration cell with a clamp and O-rings. The electrodes were calibrated in a thermostated cell with standard acid and base to read p[H] directly ($p[H] = -\log[H^+]$). The ionic strength was adjusted to 0.100 M with KCl. Atmospheric CO_2 was excluded from the titration cell with a purging stream of purified argon gas. The log value of K_w ($=[H^+][OH^-]$) determined for this system is -13.78.

Spectral determinations were made with a Perkin-Elmer model 553 fast-scan spectrophotometer equipped with 1.000 ± 0.001 cm cells at 25.0 ± 0.1 °C. The concentrations for spectrophotometry were generally 1×10^{-4} M in each component. The formation constants for gallium TACN-HP, ferric DTTA-HP, and ferric ENDA-HP were found spectrophotometrically. Their stabilities were determined by spectrophotometry as the $MH_2L^{(+,0)}$ or MH_3L^{3+} complexes were formed to different extents depending on the concentration of acid present, giving rise to a change of absorbance with p[H]. The equilibrium in strongly acid solution is simplified by the fact that MH_2L or MH_3L are the only gallium or iron species present. The stability constants, log K_{ML}, were calculated from the data by the use of short, in-house, programs written in BASIC utilizing mass balance and known equilibrium constants while minimizing the least-squares absorbance fit to the observed spectral curves. Each such determination was made on from 5 to 15 spectra.

The Ga(III) complexes of DTTA-HP and ENDA-HP do not dissociate at low p[H]. However, the novel high p[H] method developed by Motekaitis and Martell involving competition by OH^- for Ga(III) to form $Ga(OH)_4^-$, resulting in dissociation of the metal chelate GaL^-, was used to determine the formation constant with these two ligands while monitoring the position of the equilibrium potentiometrically [5, 14].

Indium complexes were all determined directly by potentiometry, since sufficient uncomplexed indium was generally present at the start of the titration with each of the three ligands studied.

Standard reduction potential of Fe(III)/Fe(II) couple

Into an Ar-protected, thermostated cell, equipped with a magnetic stirring bar and with Pt and calomel electrodes, was placed a 50.00 ml solution containing 0.1088 mmol Fe^{3+}, 5.00 mmol KCl and 0.4705 mmol HCl. On top of this solution was floated a small boat containing crystalline 0.1207 mmol $FeSO_4 \cdot 7H_2O$. The cell was sealed and purged with deoxygenated Ar while it was stirred for 6 h to expel any residual oxygen. The boat was tipped by increasing the stirring rate and the measured potential was monitored as the ferrous sulfate slowly dissolved. An additional 1.5 h was allowed during which time the potential remained constant. The practical standard potential determined under the experimental conditions is thus 473.5 mV which includes interactions of the ionic medium with the metal ions.

Stability constant

The Ar purged solution of 0.190 mmol of TACN-HP was prepared in 5.00 ml 0.0941 M HCl and was quantitatively added by means of a syringe to the measuring cell. The rate of formation of FeH_3L was observed to be very slow in that the cell potential dropped only 70 mV during the first 4 h, during which

time it was changing at the rate of about 9 mV/h. A total of 14 days was allowed until the potential stabilized at −97.0 mV. Concentration of free ferric ion was calculated from this potential relative to the above practical standard potential. A spectrum was obtained to determine more accurately the exact amount of total ferric ion present as FeH_3L and hence a small correction was applied to adjust for the amount of spurious oxidation of ferrous ion to ferric complex. Since the free ferric ion concentration is limited by the equilibrium constant for the reactions

$$Fe(III) + H_nL \rightleftharpoons FeH_3L + (n-3)H^+$$

where $n = 7$, 6, 5, 4, all of the previously measured known protonation equilibria were combined in order to solve for $[H_3L]$ in the reaction

$$Fe(III) + H_3L \rightleftharpoons FeH_3L$$

The p[H] used in all these determinations was calculated from the total HCl present plus that amount of acid present in the ligand which was released during the ferric complexation. From this equilibrium constant taken together with the potentiometrically determined chelate and the ligand protonation constants, the normal stability constant was then computed

$$(Fe^{3+} + L^{3-} \rightleftharpoons FeL) \qquad K = [FeL]/[Fe^{3+}][L^{3-}]$$

For further details on the determination of the stabilities of the complexes of In(III), Ga(III) and Fe(III) see ref. 5.

Results

Protonation constants

The protonation constants of ENDA-HP (**11**), DTTA-HP (**12**) and TACN-HP (**13**) were determined by the potentiometric method described above and are listed in Table 1. The first protonation constant in each case was determined by spectrophotometry. While formally an H_4L type ligand, ENDA-HP possesses eight possible protonation sites: two carboxylates, two phenol-like oxygens, two aliphatic nitrogens of the ethylenediamine and two pyridyl nitrogens, it was found that six of these sites could be protonated in the accessible pH range of potentiometry while a seventh could be confirmed spectroscopically. Apparently the net positive charge build-up (of 3+) prevents protonation of the eighth site. DTTA-HP is an H_5L type ligand and possesses ten sites for possible protonation, but only eight were observed to participate in the protonation process. The formula for the latter species is H_8L^{3+}. TACN-HP is an H_3L type ligand possessing no acetate substituents but has nine possible sites for protonation. However, only seven protonation steps could be measured accurately. The heptaprotonated ligand is the species H_7L^{4+}. However in the presence of strong acid, where ionic strength is out of control, a species H_8L^{5+} was detected spectroscopically as shown in Fig. 1.

TABLE 1. Protonation constants of TACN-HP, ENDA-HP and DTTA-HP measured at $t = 25.0$ °C and $\mu = 0.100$ M KCl

Equilibrium quotient	ENDA-HP	DTTA-HP	TACN-HP
$HL/L \cdot H$	11.79	12.12	12.09
$H_2L/HL \cdot H$	11.03	10.95	10.86
$H_3L/H_2L \cdot H$	7.50	8.90	9.73
$H_4L/H_3L \cdot H$	5.61	6.21	7.00
$H_5L/H_4L \cdot H$	3.53	5.19	5.35
$H_6L/H_5L \cdot H$	2.27	3.49	4.28
$H_7L/H_6L \cdot H$	1.14	2.25	1.99
$H_8L/H_7L \cdot H$		1.46	

Figure 1 provides some further insight into the protonation reactions for TACN-HP. The spectra shown are representative in that the other two ligands show similar peak positions and heights. Judging from the orderly progressions of absorbance intensities near 225, 250 and 300 nm it is found that the pyridyl chromophore partakes in the protonated species H_3L^0 through H_8L^{5+}. If this were not so, the spectral changes would not be so orderly. The fact that at 250 and 300 nm the spectral absorbances for H_2L^- and HL^{2-} and L^{3-} are nearly identical serves to point out that the pyridyl substituents do not take part in these interconversions and therefore the focus is on the aliphatic nitrogens of the triazacyclononane moiety. Near the final absorption at 210–220 nm there is a concomitant bathochromic shift upon deprotonation of these two nitrogens. It is this difference which was useful in the evaluation of the highest protonation constants by spectroscopy.

All electronic spectra of the iron(III) chelates are of interest and are reported here. The triprotonated chelate of TACN-HP absorbs at 435 nm (7400 M^{-1} cm^{-1}). Upon total deprotonation the neutral chelate ML experiences a long bathochromic shift and considerable attenuation, 465 nm (3375 M^{-1} cm^{-1}). Similarly the DTTA-HP MH_2L iron chelate absorbs at 425 nm (2900 M^{-1} cm^{-1}) whereas its deprotonated counterpart absorbs at 460 nm (2100 M^{-1} cm^{-1}). The iron chelate MH_2L of ENDA-HP is characterized by a moderately strong absorbance at 430 nm (4100 M^{-1} cm^{-1}), however, its deprotonated counterpart was not measured, since most of the spectroscopy taken in this work was related to stability constant determination.

The UV spectra of gallium(III) and indium(III) chelates of TACN-HP (MH_3L) were determined to be at 312 (27 000 M^{-1} cm^{-1}) and 320 (25 000 M^{-1} cm^{-1}) nm, respectively. The corresponding ML spectra were not determined since they were not necessary for stability constant work. The indium(III) spectra studied as a

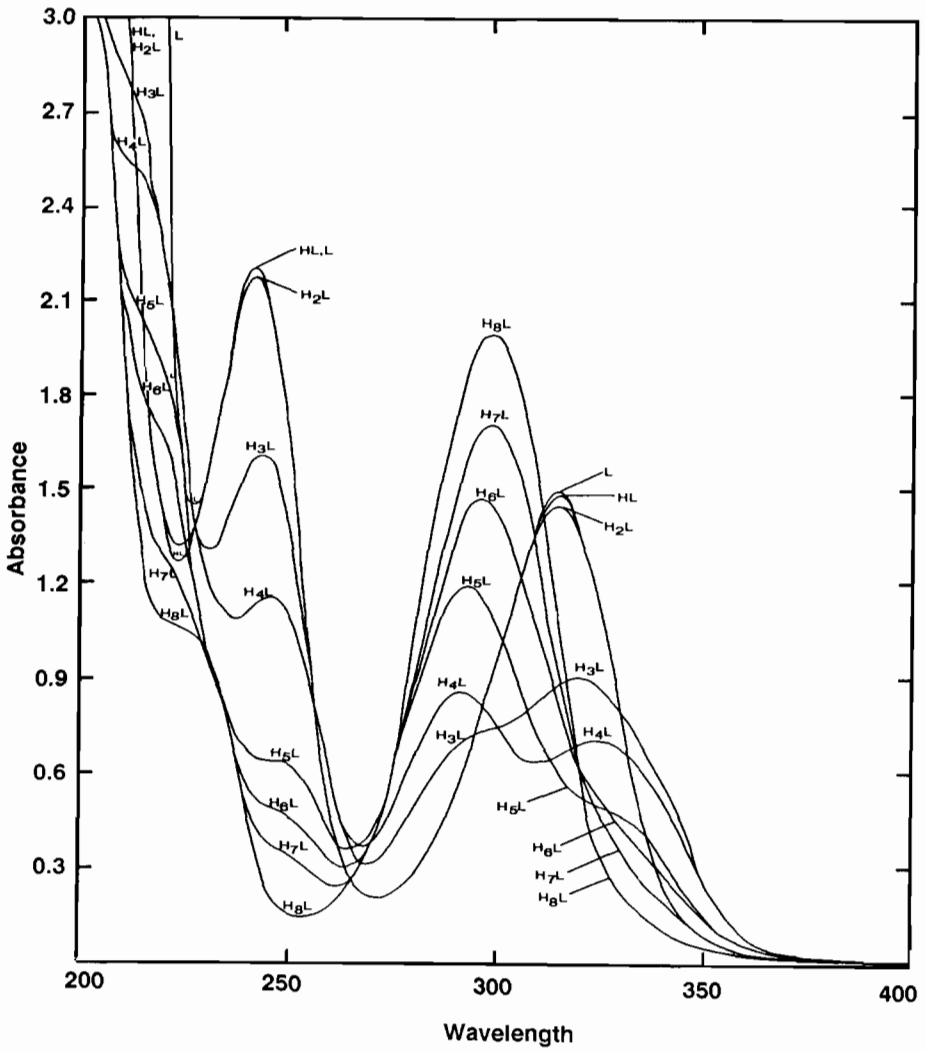


Fig. 1. Electronic spectra of TACN-HP for the various species measured at 25 °C, $\mu = 0.100$ M (KCl). The spectra were obtained at p[H] values taken midway between corresponding log protonation constant values. Spectra labeled H_8L and L were obtained using a large excess of HCl and KOH, respectively. $T_L = 0.845 \times 10^{-4}$ M.

function of low p[H] were consistent with indium dissociation as calculated from pure pontentiometry.

Metal ion affinities

Metal ion affinities of ENDA-HP, DTTA-HP and TACN-HP are listed in Tables 2, 3 and 4, respectively. The formation constants of the iron chelates and their protonation and hydrolysis constants were used to calculate the distribution curves shown in Figs. 2, 3 and 4, respectively. The simplest set of distribution curves was obtained for the ethylenediaminetetraacetate analog, ENDA-HP, for which only the metal chelate and

TABLE 2. Metal ion affinities of ENDA-HP, *N,N'*-bis(3-hydroxy-6-methyl-2-pyridylmethyl)ethylenediamine-*N,N'*-diacetic acid, 25.0 °C, $\mu = 0.100$ M KCl

Equilibrium quotient	Fe^{3+}	In^{3+}	Ga^{3+}
ML/M·L	35.08	28.02	29.18
MHL/ML·H	5.86	5.98	5.94
MH_2L/MHL·H	4.84	4.85	4.85
MH_2L/M·H_2L	22.93	16.03	17.15

TABLE 3. Metal ion affinities of DTTA-HP, *N,N''*-bis(3-hydroxy-6-methyl-2-pyridylmethyl)diethylenetriamine-*N,N',N''*-triacetic acid, $t = 25.0$ °C, $\mu = 0.100$ M KCl

Equilibrium quotient	Fe^{3+}	In^{3+}	Ga^{3+}
ML/M·L	32.7	25.70	31.02
MHL/ML·H	7.8	8.87	7.08
MH_2L/MHL·H	5.6	5.55	5.65
MH_3L/MH_2L·H	4.3	4.42	4.52
MH_4L/MH_3L·H	3.0		2.27
MH_5L/MH_4L·H			~1.9
MOHL·H/ML			−9.77

TABLE 4. Metal ion affinities of TACN-HP, *N,N',N''*-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacyclononane, t = 25.0 °C, μ = 0.100 M KCl

Equilibrium quotient	Fe^{3+}	In^{3+}	Ga^{3+}
ML/M·L	49.98	28.02	45.6
MHL/ML·H	6.14	5.93	5.85
$MH_2L/MHL \cdot H$	5.09	5.13	5.42
$MH_3L/MH_2L \cdot H$	4.51	4.50	4.14
ML/M(OH)L·H	10.45	10.42	10.14
$MH_3L/M \cdot H_3L$	33.04	10.93	19.84

1:1 ENDA-HP:Fe(III)

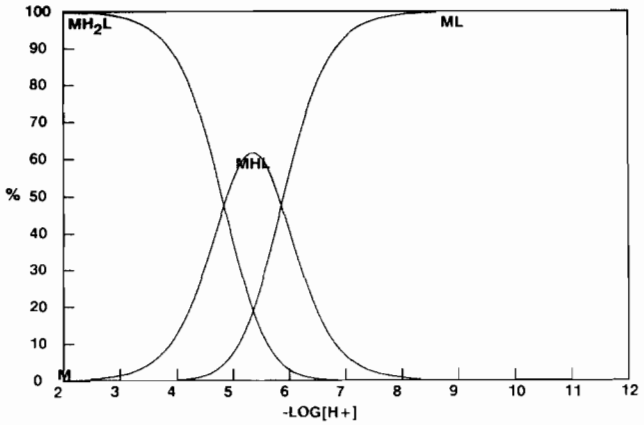


Fig. 2. Species distributions as a function of p[H] for 1.00×10^{-3} M Fe(III) chelate of ENDA-HP at 25.0 °C and μ = 0.100 M (KCl).

1:1 DTTA-HP:Fe(III)

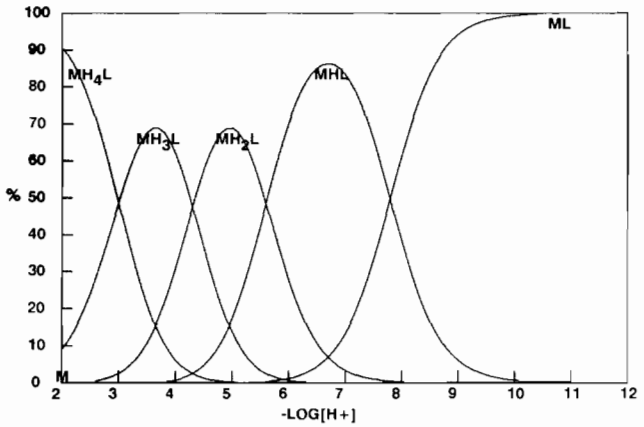


Fig. 3. Species distributions as a function of p[H] for 1.00×10^{-3} M Fe(III) chelate of DTTA-HP at 25.0 °C and μ = 0.100 M (KCl).

two protonated forms were found between p[H] 2 and 12, the limits of potentiometric determinations. The diprotonated form, MH_2L, is converted to the monoprotonated chelate at p[H] ~4.8 which is in turn converted at p[H] ~6 to the deprotonated form, ML.

1:1:1 TACN-HP:Enterobactin:Fe(III)

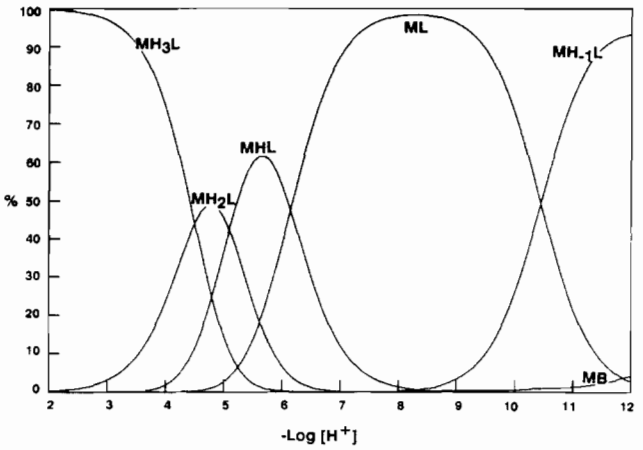


Fig. 4. Species competition diagram depicting the relative concentrations of each species as a function of p[H] when equimolar quantities of TACN-HP, enterobactin and Fe(III) are present at 1×10^{-3} M in aqueous solution. Enterobactin, a natural siderophore possessing three catechol groups is no match for the synthetic-pyridyl compound.

Above p[H] 7 ML is the predominant form of the metal chelate in aqueous solution. The distribution curves (Fig. 3) obtained for the iron chelate of DTTA-HP show a larger number of protonated forms in conformity with the fact that the ligand is octadentate in this case rather than hexadentate as in the case of ENDA-HP. Thus we have a series of four protonated ion chelate species which are finally converted to the unprotonated form about p[H] 8. The Fe(III) chelate of TACN-HP shows the formation of three protonated forms of the metal chelate as well as a hydrolyzed species at high p[H] containing one hydroxyl group (Fig. 4). The triprotonated form of the Fe(III) chelate is the predominant species at very low p[H] and upto p[H] 4. This is converted at higher p[H] successively into the di- and monoprotonated forms and the completely deprotonated species predominates above p[H] 6. At p[H] ~10 the hydroxo species begins to grow in and predominates about p[H] 11 and above.

Discussion

It is apparent from the distribution curves that all of the hydroxypyridyl ligands form several protonated metal chelates at low p[H], while maintaining high stability of the coordination sphere. The additional protons are believed to become covalently attached to the pyridine nitrogen atoms of which there are two in both ENDA-HP and DTTA-HP. The formation of three protonation species is a characteristic of TACN-HP, as would be expected from the presence of three hydroxypyridyl groups. Although the ligands are written

with completely protonated hydroxyl species in the pseudo phenolic form it is recognized that in the free ligands the most basic nitrogen atoms will first accept the protons and this applies to the two aliphatic nitrogens atoms in ENDA-HP and similar basic nitrogens in DTTA-HP. In addition the nitrogens of the pyridine rings are more basic than the pseudo phenolic oxygens and will accept the protons first.

For the Fe(III) chelates of the hexadentate ligands the protonated forms of the metal chelates are clearly obvious in the distribution curves as well as the corresponding constants obtained and listed in Tables 2–4. For the ENDA-HP iron chelate there are two hydroxypyridyl groups and there are correspondingly two protonated species, MH_2L and MHL. For the other hexadentate ligand, TACN-HP, there are three hydroxypyridyl groups and there are a corresponding number of protonated species in which the protons are bound to the pyridine nitrogens. In the case of DTTA-HP with eight donor groups it is impossible to tell from potentiometric methods alone whether six or seven coordinate iron will leave a carboxyl group or a hydroxypyridine group free of coordination. It is believed that the former is the case and we offer the tentative structure of a dangling carboxyl group with two coordinated hydroxypyridines, three aliphatic nitrogens, and a carboxyl group bound to the Fe(III) ion. With this structure or one which is analogous and close to it three aliphatic nitrogens are bound to the ferric ion, which accounts for a somewhat lower stability constant of the Fe(III) chelate than that of the EDTA analog.

While TACN-HP and ENDA-HP behave ideally for all three metal ions, the other ligand shows further protonation beyond the number of pyridine groups present. Thus DTPA-HP forms species MH_3L and MH_4L with both iron(III) and gallium(III) and MH_3L with indium(III).

It is of interest to compare the affinity of ENDA-HP and the nearly isomeric ligand PLED for the Fe(III) ion. PLED differs mainly from the ENDA-HP ligand in that the nitrogen of the pyridine ring is *para* to the ethylenediamine backbone while the ligand involved in the present investigation has the pyridine nitrogen *ortho* to the covalent linkage. In addition PLED has a methyl group *ortho* to the pseudo phenolic group which is absent in the ligand described in the present paper, in addition to a presumably inactive hydroxymethyl group which, however, imparts water solubility to the complex. The Fe(III) chelate of the ENDA-HP ligand is around $10^{35.8}$ compared to $\sim 10^{30.7}$ for the PLED–Fe(III) complex [5]. This difference in stabilities of the Fe(III) chelate is somewhat mitigated by the difference in proton affinities which is about two orders of magnitude higher for the ENDA-HP ligand. This competition which hydrogen ions would be of importance at physiological pH and at any pH approximately equal or lower than the p*K*s involved. This still leaves, however, an increase of three orders of magnitude in the ENDA–Fe(III) chelate, which is presumably due to the absence of the sterically demanding methyl group in a position *ortho* to the coordinating pseudo phenoxide group. There are not enough data, however, to generalize about the comparison of metal ion affinities of the two ligands especially since the trend observed for the ferric isomer is absent in the gallium complex. It will be of some interest to compare the stabilities of a series of complexes for both ligands but the appropriate data are not yet available.

The stabilities of a number of Fe(III) chelating agents are compared in Table 5, including TACN-HP, several catechol ligands including bicapped TRENCAM, MECAM, TRENCAM itself, and hydroxamate ligands, in the order of decreasing stability. It is seen that the last three synthetic ligands in Table 5 are too close to, or lower than, the stability of the transferrin complex when one looks at the effectiveness of the ligand with the p*M* values (p*M* = −log of metal ion concentration in equilibrium with a ten-fold excess of the ligand). The Fe(III) chelates of the remainder of the ligands in Table 5 including DFB are stable enough to maintain their integrities in competition with transferrin in the serum, which explains the use or potential use of some of them for removal of Fe(III) from the body in cases of iron overload disease such as Cooley's anemia [4]. Of special interest is the new ligand describe in this paper, TACN-HP (**12**) and its comparison with enterobactin which was previously described by Raymond *et al.* [6] as the most effective iron chelator known. The value of the stability constant for enterobactin was

TABLE 5. Selected formation constants and p*M* values for iron(III) sequestering agents

Ligand	log β	Σ p*K*	p*M*[a]
TACN-HP[b]	49.98	51.3	40.3
TACN-TX[c]	51.3	50.9	33.0
Enterobactin[d]	~49[e, f]	~58[e, f]	33.5
HBED[d]	39.7	36.85	31.0
Bicapped TRENCAM[d]	43.1	54.0	30.7
MECAM[d]	46	58.5	29.1
TRENCAM[d]	43.6	66.21	27.8
DFB[g]	30.7	26.83	27.0
Trishydroxamate cryptand[h]	29.12	29.16	23.1
Transferrin[i]	20.2, 39.3[j]		21.2
DTPA[g]	28.0	27.73	24.7
DMHP[k]	35.88	13.45	19.3

[a]p*M* = −log[M] at p[H] 7.4, 10^{-6} M [M] and 10^{-5} M [L]. [b]This work; ref. 10. [c]Ref. 16; 75% ethanol, 25% water. [d]Ref. 7. [e]Estimated. [f]Ref. 15. [g]Ref. 17. [h]Ref. 18. [i]Conditional constant at p[H] 7.4. [j]2M:1L conditional constant. [k]3:1 constant, refs. 19 and 20.

estimated because the three highest p*K*s could not be measured. Previously Raymond *et al.* [6] used *N*,*N*-dimethyl-2,3-dihydroxybenzoylamide as a model for enterobactin protonation and gave an estimated value of 10^{52} for the stability constant of enterobactin. On this basis the distribution plot of ref. 10 (p. 1749) was determined. Table 5, however, uses a more recent estimate of the stability constant of enterobactin, 10^{49}, which is based on MECAM as a model. Various reasons which cannot be described here but which are available on the reading of the pertinent literature [15] explain why MECAM is a better model for enterobactin. In any case, use of the data in Table 5 results in the distribution curves indicated in Fig. 4 for a system in which TACN-HP, enterobactin and iron(III) are present in equal concentrations. The competition between these two ligands for ferric ion is seen to be completely in favor of TACN-HP. In fact the affinity between the ferric ion and enterobactin does not appear until one reaches very high p[H], considerably above physiological p[H]. On the basis of this evidence, therefore, TACN-HP turns out to be the most effective chelator for iron found thus far.

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