Metal Complex Formation of a New Siderophore Desferrithiocin and of Three Related Ligands

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Desferrithiocin shows, in comparison with other iron chelators, a similar but lower tendency to bind iron(III) under physiological conditions although its biological efficiency in animal models is quite promising.

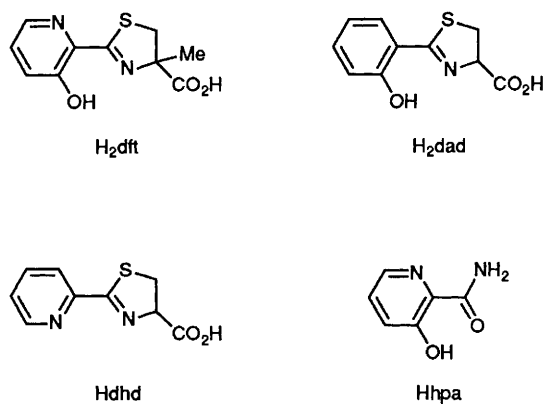
In the course of investigations with a series of *Streptomyces* in relation to the production of new metabolites, Nägeli and Zähner[1] isolated an orange–brown compound containing iron and sulphur which they called ferrithiocin. The iron-free product is secreted from culture media of *Streptomyces antibioticus* Tü 1998 and, on addition of an iron(III) salt, forms the characteristically coloured complex, which can be extracted with organic solvents. The ligand in question belongs to the steadily growing class of iron-complexing natural substances, called siderophores, and could be an important drug for iron overload.

In recent years we have investigated the complexing properties of desferrithiocin [H_2dft; 2-(3-hydroxypyridin-2-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylic acid], and of three other similar ligands, namely deazademethyldesferrithiocin (H_2dad), dehydroxydemethyldesferrithiocin (Hdhd) (obtained from Ciba-Geigy), and hydroxypicolinamide (Hhpa) (obtained from EGA, Steinheim; recrystallized from water).

The protonation and the stability constants were obtained from alkalimetric titrations with 0.1 mol dm^{-3} KOH of solutions of the above acids alone and in presence of the metal nitrate, in a nitrogen atmosphere, at the chosen standard state [I = 0.1 mol dm^{-3} (KNO_3) and 25 °C]. For some stable complexes (Cu^{2+}, Al^{3+}, and Fe^{3+}) exchange reactions with auxiliary ligands (2,2′,2″-triaminotriethylamine[2,3] and ethyl

enediaminetetra-acetic acid,[4] H_4edta) were necessary in order to obtain equilibrium constants using pH measurements with or without spectrophotometric measurements. The equilibrium constants (Table 1) were calculated with our programs[5] in which all species $M_qH_pL_n$ can be considered. The above mentioned ligands decompose in acidic solutions but the reaction rate depends on the pH value and on the metal ion present. In the case of Hhpa the amide group hydrolyses and with the others rupture of the thiazoline ring occurs and the liberation of cysteine can be detected by the development of the thiol odour. For these reasons the solutions for the measurements should be prepared just before the measurements of the equilibria, which sometimes are carried out by using batch procedures.[6] For the ligands H_2dft, H_2dad, and Hhpa it was possible to obtain reliable results. However, for Hdhd, for the reasons mentioned above as well as because of the weakness of the complexes formed, the standard deviation of the values is exceptionally high (Table 1). In the pH range 5.8—7.7, equilibrated solutions may be obtained for H_2dft within 24 h without any appreciable ligand decomposition.

The protonation constants K_p are in accordance with the expectations[7] taking into consideration the different basic groups of the ligand anions. Protonation occurs first at the deprotonated hydroxy group of dft (log K_1 = 9.91), dad (log K_1 = 10.47), and hpa (log K_1 = 7.84). This occurs for dft with the formation of a hydrogen bridge with the N atom of the thiazoline ring, as shown from the *X*-ray structure of H_2dft.[8] Similar H bridges are expected for dad and hpa. For dhd the first protonation involves H bridge formation between the N and one O atom of the thiazoline ring carboxylate group. All other log K_n values are as low as expected for the protonation of aromatic N atoms as well as for carboxylate groups.



Scheme 1

A common feature of the first three ligands is the presence of a 4-carboxylated thiazoline ring connected in the 2 positions with a pyridine (dft and dhd) or a phenol ring (dad) through the 2'-carbon atom. Although a thioether sulphur atom is present, because of its low co-ordinating power, binding through the nitrogen and the carboxylate group of the thiazoline ring seems more effective, also because of the formation of a chelate ring. Indeed the values of log K_1 of glycinate are 2—4 units larger than those of R–S–$CH_2CO_2^-$.[9] The other aromatic ring of dft, dad, and dhd can further contribute to complex formation chelating either through the pyridine nitrogen or through the deprotonated hydroxy group acting as tridentate ligands.

From the data obtained it appears that dft and dad form complexes in which the deprotonated oxygen atom of the hydroxy group is bound. This is particularly evident on comparing their stability constants with those of the bidentate anion of 8-hydroxyquinoline-5-sulphonic acid[10] which has the same charge as dft and dad and contains one nitrogen and the oxygen atom of a deprotonated hydroxy group as donors. The values of log K_1 for the latter ligand are generally lower by 1 (Co^{2+}, Ca^{2+}) to 2.55 (Cu^{2+}) because of the lower denticity and basicity. Another comparable ligand is the tridentate anion of iminodiacetic acid[11] L^{2-}, for which the log K_1 values are lower by 1.5 (Cd^{2+}) to 3.84 (Cu^{2+}) than those of dft. The case of complex formation between Ni^{2+} and dft needs particular mention. It occurs first by co-ordination of both N atoms of each dft with formation of $Ni(HL)_2$ followed by deprotonation to $NiHL_2^-$ and NiL_2^{2-}, HL^- being the anion containing the hydroxy group. From these comparisons it appears that dft has remarkable complexing properties with the metal ions investigated.

In order to compare the role of an iron chelator in the regulation of the free metal ion concentration, one cannot use the constants directly, but it is necessary to consider the experimental conditions. For this purpose two parameters are generally calculated: (a) the conditional constant[12] K_{eff} in presence of free calcium ions, $[Ca^{2+}]$ = 0.001 mol dm^{-3}; (b) the concentration K_{sol} of the iron complex in mmol dm^{-3}

Table 1. Equilibrium constants log K_n of the ligands with H^+ {K_n = $[H_nL]/([H_{n-1}L][H])$} and with the metal ions M^{z+} {K_n = $[ML_n]/([ML_{n-1}][L])$} at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). In parentheses are the standard deviation in the last figure(s). Charges in the equilibrium species are omitted.

	n	dft	dad	dhd	hpa
H^+	1	9.91(4)	10.47(3)	3.39(2)	7.84(1)
	2	3.31(1)	3.83(2)		2.26(2)
	3	1.65(2)			
Ca^{2+}	1	4.65(2)	3.56(6)	2.5(1)	2.68(2)
	2	2.6(1)			
Mg^{2+}	1	5.10(2)	4.19(6)	2.5(1)	2.64(6)
	2	4.06(7)	3.0(2)		
Mn^{2+}	1	7.28(2)	6.35(6)	2.4(1)	3.81(4)
	2	6.43(2)	5.20(17)		3.36(10)
Fe^{2+}	1				5.24(3)
	2			4.24(1)	
	3				4.08(1)
Co^{2+}	1	9.13(8)	8.75		5.68(5)
	2	7.8(1)	6.8(1)	$\log\beta_2$ = 7.6(2)	5.41(3)
Ni^{2+}	1	$\log\beta_2$ = 17.74(8)	9.62(9)		6.59(1)
	2	[a]	7.10(13)	$\log\beta_2$ = 7.9(2)	6.04(2)
	3			3.1(1)	5.52(2)
Cu^{2+}	1	14.47(2)	14.27(3)	4.0(2)	7.66(1)
	2	6.40(6)	5.60(14)	4.0(2)	6.68(8)
Zn^{2+}	1	9.28(5)	8.5(1)	3.8(1)	5.62(8)
	2	7.41(8)	6.1(2)	3.2(1)	5.17(10)
	3				4.2(1)
Cd^{2+}	1	7.40(7)	6.48(8)	3.8(1)	4.77(3)
	2	6.77(4)	5.4(1)	2.9(2)	4.23(6)
Fe^{3+}	1	$\log\beta_2$ = 29.6(1)	$\log\beta_2$ = 31.4(2)		8.70(13)
	2				7.79(8)
	3				6.39(5)
Al^{3+}	1	$\log\beta_2$ = 22.2(1)	12.22(3)		7.54(3)
	2		10.60(3)		6.25(1)

[a] $[NiHL_2]/([Ni][H][L]^2) = 10^{22.49(10)}$; $[NiH_2L_2]/([Ni][H]^2[L]^2) = 10^{26.59(19)}$.

obtained by dissolving α-FeOOH ($K_{so} = 10^{-41}$) in the ligand solution until its excess is 1 mmol dm^{-3}. The values obtained at pH 7.4 in the case of dft ($\log K_{eff} = 15.1$; $\log K_{sol} = 0.07$) are somewhat lower than those for desferrioxamine H_2L ($\log K_{eff} = 16.3$; $\log K_{sol} = 4.40$) by formation of $Fe(dft)_2^-$ and FeL^+, respectively. Investigations on animal models[13,14] have shown that the new ligand increases the iron release albeit with strong toxic secondary effects which can be eliminated by reducing the ligand concentration, whereas the Fe release remains remarkable. Further investigations on Cebus monkeys[15] have shown that dft has significant activity as an oral chelator.

More details on the determination of the equilibrium constants are given in the dissertation of M.R.[16]

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