Equilibria of Some α-Amino-acids containing Sulphur Atoms in the Chain with Metal(II) Ions. Part 1. DL-4,4′-Dithiobis(2-aminobutyric acid) with Manganese(II), Cobalt(II), and Nickel(II) in Aqueous Solution

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The formation constants of the complexes formed between H^+, Mn^{2+}, Co^{2+}, and Ni^{2+} and DL-4,4′-dithiobis(2-aminobutyric acid) (dtba) have been measured potentiometrically at 25 °C and $I = 0.1$ mol dm^{-3} KCl. Titration curves of the ligand in the presence of Mn^{2+}, Co^{2+}, and Ni^{2+} ions did not reveal appreciable complexing in acidic media. At intermediate pH values (*ca.* 6—8) the equilibria, after neutralization of two COOH groups, have been shown to include a large range of protonated complexes with the ligand behaving as tri- or quadri-dentate chelate depending on the pH. The determination of the formation constants of complexes formed by dtba with divalent metals has shown that there are the following species in solution: $[Mn(HL)]^+$, $[MnL_2]^{2-}$; $[Co(HL)]^+$, $[CoL]$, $[CoL_2]^{2-}$; $[Ni(HL)]^+$, $[NiL]$, $[NiL_2]^{2-}$, where the ligand $L^{2-} = [^-O_2CCH(NH_2)CH_2CH_2S-]_2$. These complexes are very likely to be *N,O*-chelates of a glycine type. The proton of the hydrogen complexes is probably bound to the amine group which is not a part of the chelate ring. The cumulative and stepwise formation constants follow the Irving–Williams series. Probable structures for the various complexes are discussed by comparing them with the crystal structure of the solid ligand and by considering the values of log (K_n/K_{n+1}).

FOLLOWING the research performed on protonation equilibria [1] and as part of a research programme on the properties of the sulphur-containing amino-acids,[2] a study of the properties of DL-4,4′-dithiobis(2-aminobutyric acid), $[-S-(CH_2)_2-CH(NH_2)-COOH]_2$ or homocystine (dtba), solutions with divalent metals has been undertaken. Many amino-acids are potentially tridentate ligands. When the third donor site is an oxygen atom ($-OH$) the tendency to form tridentate chelates is limited,[3] but when this third site is a nitrogen atom ($-NH_2$) marked tridentate behaviour is frequently found particularly when the additional chelate ring is five- or six-membered, as in the case of the histidine,[4] ornithine,[5] and other α-amino-acids.[5] In the present work this has not been realised, because the amine group ($-NH_2$) as a third donor site is very far away from the chelate ring; therefore the introduction of the nitrogen atom ($-NH_2$) in the co-ordination polyhedron to a certain extent implies a rearrangement of the ligand, which is not expected from structural information.[2] The nitrogen donor atom, not involved in the chelate ring, is generally able to co-ordinate with hydrogen ions at intermediate pH values (*i.e.* those found in biological systems). We report here the results of a detailed study of the manganese(II), cobalt(II), and nickel(II) complexes with DL-4,4′-dithiobis(2-aminobutyric acid) in aqueous solution. Complexes formed with Cu^{2+} are not included in the present work since a large number of equilibria is present simultaneously and a complicated range of protonated complexes is produced in acidic media, because of this an analysis of the experimental data brings to the surface major problems in the species selection.

EXPERIMENTAL

The amino-acid was employed without further purification (Found: C, 29.7; H, 4.85; N, 11.65; S, 23.35. Calc. for $C_8H_{16}N_2O_4S_2$: C, 29.95; H, 5.00; N, 11.7; S, 23.9%). All the solutions for potentiometric measurements were prepared with doubly distilled water. Hydrochloric acid solutions were prepared and their concentrations (0.100 26 mol dm^{-3}) determined against tris(hydroxymethyl)methylamine (tham); potassium hydroxide solutions were prepared by diluting concentrated K[OH] (BDH) with CO_2-free water and their titres checked against potassium hydrogenphthalate. Concentrations of stock solutions of divalent metal chlorides were determined by conventional analytical methods. The starting solutions for each potentiometric titration were prepared by adding successively to the titration vessel a weighed amount of amino-acid in a water bath, a known volume of hydrochloric acid, and an exact volume of metal chloride; then the required quantities of potassium chloride and a sufficient amount of doubly distilled water were added to reach the total volume V_0, which was 99.695 ± 0.010 cm^3. The ionic medium was 0.1 mol dm^{-3} KCl at the beginning of each potentiometric titration. The volume of the titrant K[OH] solution was added by a Metrohm Multidosimat E 414 piston burette, with a precision of 0.005 cm^3. The total volumes added (*ca.* 5—7 cm^3) were subdivided into about 39—48 steps for each titration.

The compositions of the starting solutions and the pH ranges for each titration are quoted in Table 1. The potentiometric measurements have been performed by a Radiometer PHM52 digital potentiometer, equipped with a

TABLE 1

Complex formation constant determinations. Initial solution compositions (T_M, T_H, T_L/mmol) and pH ranges for the titrations of homocystine with divalent metals at 25 °C and $I = 0.1$ mol dm^{-3} KCl

Expt. no.	Ion	T_M	T_H	T_L	pH
1	Mn^{2+}	0.029 685	0.591 510	0.090 620	2.46—9.74
2		0.036 362	0.708 560	0.144 130	2.48—9.65
3		0.032 650	0.814 120	0.166 940	2.43—9.65
4	Co^{2+}	0.046 350	0.618 220	0.108 990	2.48—9.49
5		0.056 908	0.624 190	0.111 970	2.48—9.49
6		0.051 550	0.756 270	0.153 000	2.43—9.75
7		0.041 237	0.847 810	0.173 757	2.43—9.79
8	Ni^{2+}	0.055 480	0.452 060	0.110 930	2.74—9.67
9		0.019 820	0.328 430	0.079 150	2.85—9.77
10		0.207 106	0.795 407	0.203 886	2.56—8.40
11		0.049 940	0.794 780	0.197 270	2.54—9.92

Radiometer G2025B glass electrode and a saturated calomel electrode (s.c.e.) as reference. The titration vessel was maintained at 25.0 ± 0.1 °C by the circulation of thermostatted water. A nitrogen stream, presaturated by bubbling it through an appropriate KCl solution (0.1 mol dm^{-3}), was maintained in the cell by a continuous slow gas atmosphere. The glass electrode was standardized by titrating an HCl solution of known concentration with K[OH] solution at the chosen ionic strength. About 25 equally spaced points both in acidic and alkaline media were measured. The equivalent point and the standard electrode potential, $E^{\ominus}$, were determined following the Gran [6] method by a least-squares procedure. The NBAR computer program [7] was used. The liquid junction potential was assumed to be constant, since $E^{\ominus}$ included both the effects of the ionic strength and the junction potential. The ionic product K_w at 25 °C and at 0.1 mol dm^{-3} KCl ionic strength was obtained by varying its value until the average $E^{\ominus}$ in alkaline media equalled that in acidic media. Values of K_w were found to be in good agreement with those reported in the literature.[8] Further details have been presented in previous publications.[9,10]

RESULTS

Protonation constants of the ligand [1] have already been determined. The results obtained by using homocystine, H_2L, with different metal ions are summarized in Table 2. The constants quoted are generally overall formation constants $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$. When, in Table 2, values for a particular species are not included this means that the species was apparently absent or contributed little to the equilibrium (and hence had an insignificant effect on the statistical fit of the data). The calculated titration curves of the protonated ligand, H_4L^{2+}, in the presence or in the absence of Mn^{2+}, Co^{2+}, and Ni^{2+}, are shown in Figure 1. In the presence of metal ions these curves did not reveal an appreciable complexing capacity in the acidic media, but showed, after neutralization of the two –COOH groups, one or two distinct buffer zones, at pH *ca.* 5.0—7.5 and 8.0—9.5, respectively.

TABLE 2

Cumulative and stepwise formation constants [a] of DL-4,4′-dithiobis(2-aminobutyric acid) with Mn^{II}, Co^{II}, and Ni^{II} at 25 °C and $I = 0.1$ mol dm^{-3} KCl. Standard deviations (σ values) are given in parentheses

	Mn^{II}	Co^{II}	Ni^{II}
log β_{111}	12.551(37)	13.543(52)	14.327(10)
log β_{101}		5.759(25)	7.653(4)
log β_{102}	7.002(21)	9.308(41)	11.489(18)
log K_2		3.549(48)	3.836(18)
log K_{111}^{101} [b]		7.784(58)	6.674(11)
ΔpK [c]		1.629	2.739
$\Sigma\Delta^2$ [d]	0.178 871 × 10^{-7}	0.916 637 × 10^{-7}	0.431 593 × 10^{-7}
R [e]	0.38%	0.70%	0.40%
N [f]	133	188	243

[a] $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$. [b] $\log K_{111}^{101} = \log \beta_{111} - \log \beta_{101}$. [c] $\Delta pK = \log K_1^H$ (9.413) $- \log K_{111}^{101}$. [d] $\Sigma\Delta^2 = \Sigma(\Delta C_M^2 + \Delta C_H^2 + \Delta C_L^2)$. [e] R factor $= \left[\frac{\Sigma_i(X_{o,i} - X_{c,i})^2}{\Sigma_i(X_{o,i})^2}\right]^{\frac{1}{2}}$, where $X_{o,i}$ and $X_{c,i}$ are observed and calculated quantities at point *i*. [f] N = Total number of data points used in the refinement.

The formation functions, $\bar{n}$, calculated on the assumption that the protonated complexes do not exist, roughly suggest the existence of complexes with metal to ligand ratios of 1 : 1 (ML) and 1 : 2 (ML_2), but their abnormal features indicate the presence of protonated complexes. Calculations were carried out with the aid of the MINIQUAD computer program; [11] thus the sum of the squares of the residuals, $\Sigma\Delta^2 = \Sigma(\Delta C_M^2 + \Delta C_H^2 + \Delta C_L^2)$, where C_M, C_H, and C_L are the total molar metal, acid, and ligand concentrations respectively, is minimised. A number of metal to ligand ratios was used and various models were fitted to the data. We selected a model which gave the best statistical fit for the range of titration experimental data and one which did not contain any systematic drifts in the magnitudes of the residuals ΔC_M [*i.e.* C_M(calc.) − C_M(obs.)], ΔC_H, or ΔC_L. The best set of complexes was $[M(HL)]^+$ and $[ML_2]^{2-}$ for Mn^{2+}, $[M(HL)]^+$, [ML], and $[ML_2]^{2-}$ for Co^{2+}, and $[M(HL)]^+$, [ML], and $[ML_2]^{2-}$ for Ni^{2+}. In addition to this the MINIQUAD computer program was also applied to each titration, and then the results were averaged and analysed. This procedure of refinement makes it possible to reveal the experimental errors, which are inherent in each set of titration data. Values of the overall formation constants (Table 3) were obtained as weighted means of the values in a single titration. At any rate, the comparison of the data in Tables 2 and 3 shows that the mean values are practically the same; nevertheless the

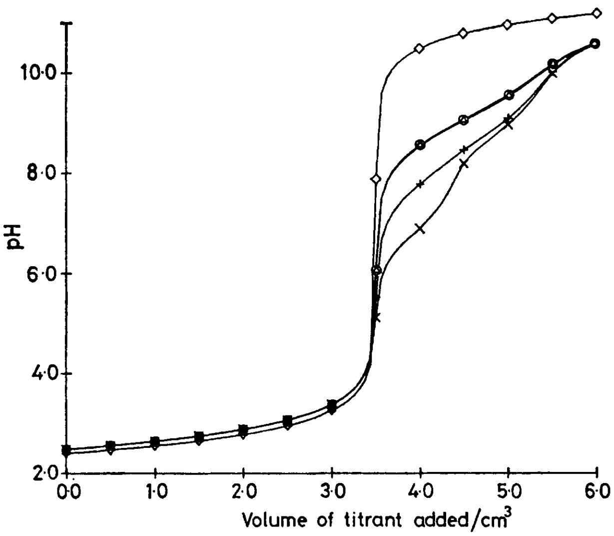

FIGURE 1 Titration curves of pH as function of the volume of K[OH] added, calculated by HALTAFALL program (N. Ingri, W. Kakalowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261), equipped with PLOTTER Calcomp 936. Homocystine (○), with Mn(△), with Co (+), with Ni (×), and HCl–K[OH] (◇). $C_L = 1.093 \times 10^{-3}$, $C_H = 6.201 \times 10^{-3}$, $C_{Mn^{II}} = 6.698 \times 10^{-5}$, $C_{Co^{II}} = 4.649 \times 10^{-4}$, $C_{Ni^{II}} = 4.766 \times 10^{-4}$, $C_{K[OH]} = 0.1144$ mol dm^{-3}; total initial volume of solutions, $V_0 = 99.695$ cm^3

TABLE 3

Overall formation constants of DL-4,4′-dithiobis(2-aminobutyric acid) with Mn^{II}, Co^{II}, and Ni^{II} obtained as weighted means of the values refined for each set of experimental data. Standard deviations (σ values) are given in parentheses

	Mn^{II}	Co^{II}	Ni^{II}
log β_{111}	12.539(35)	13.530(42)	14.324(4)
log β_{101}		5.770(19)	7.653(1)
log β_{102}	6.989(19)	9.412(32)	11.745(22)

refinement of each titration separately is recommended because it gives a more reasonable picture of the experimental errors distribution. The mean values of the constants were calculated by using the WGMEAN computer program, based on the analysis of observation errors.[12] All the calculations were carried out on the CDC CYBER computer 70/76 of the 'Centro di Calcolo Interuniversitario dell'Italia Nord-Orientale (Casalecchio, Bologna)' with the financial support of the University of Parma. A complete list of the experimental data is available as Supplementary Publication No. SUP 23047 (11 pp.).*

DISCUSSION

The analysis and refinement of the formation constants of the complexes formed by homocystine with Mn^{2+}, Co^{2+}, and Ni^{2+} show that the following species are formed: $[Mn(HL)]^+$, $[MnL_2]^{2-}$, $[Co(HL)]^+$, [CoL], $[CoL_2]^{2-}$, $[Ni(HL)]^+$, [NiL], $[NiL_2]^{2-}$. Using the stability constants given in Table 2 and the protonation constants of the ligand under the same conditions, the concentration of each complex involving H^+, metal ions, and ligand has been calculated. Representative distribution functions are presented for Mn^{2+} (Figure 2), Co^{2+} (Figure 3), and Ni^{2+} (Figure 4). Some general observations about the behaviour of the various systems are: (*a*) for the homocystine–Mn^{2+} system (M : L = 1 : 3) the $[Mn(HL)]^+$ complex reaches a maximum concentration of 32.9% total metal at pH 8.8, and $[MnL_2]^{2-}$ reaches a maximum of 69.5% at pH 11.0; (*b*) for the homocystine–Co^{2+} system (M : L = 1 : 3) $[Co(HL)]^+$ reaches 40.1% total metal at pH 7.5, [CoL] reaches a peak of 71.9% at pH 8.5, and $[CoL_2]^{2-}$ reaches a maximum of 69.4% at pH 11.0; (*c*) for homocystine–Ni^{2+} system (M : L = 1 : 2) $[Ni(HL)]^+$ has a maximum of 28.2% total metal at pH 6.8, [NiL] a maximum of 92.7% at pH 8.0, and $[NiL_2]^{2-}$ of 59.8% at pH 11.0. The fully deprotonated complexes [CoL], $[CoL_2]^{2-}$, [NiL], and $[NiL_2]^{2-}$ become important only above *ca.* pH 8.0. These observations correlate well with the values for ΔpK (*i.e.* $\log K_1^H - \log K_{111}^{101}$ for Ni^{2+} and Co^{2+} are 2.739 and 1.629 respectively) when compared with other α-amino-acids[5] in as far as the decrease in basicity of the ω-amino-group on complex formation is concerned. The order of decreasing ΔpK is 2,3-diaminopropionic acid >2,4-diaminobutanoic acid > homocystine > ornithine > lysine with various cations.

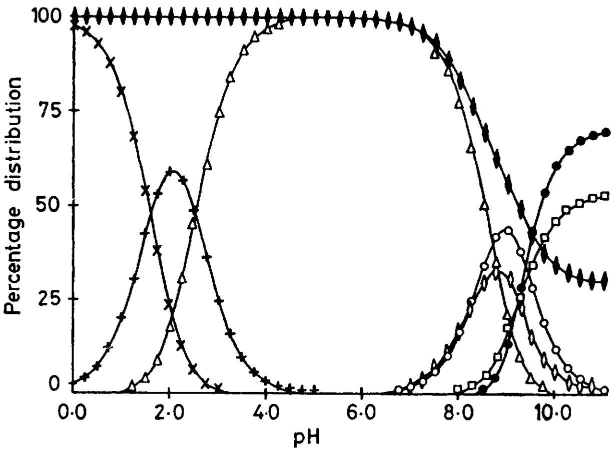

FIGURE 2 Typical distribution diagram for the system homocystine–Mn^{2+}; HL^- (○), H_2L (△), H_3L^+ (+), H_4L^{2+} (×), $[M(HL)]^+$ (◇), $[ML_2]^{2-}$ (●), L^{2-} (□), and M^{2+} (◆). The percentage of each species has been calculated for a hypothetical solution of manganese ions (0.000 297 8 mol dm^{-3}) and homocystine (0.000 909 0 mol dm^{-3}) by HALTAFALL program equipped with PLOTTER Calcomp 936. Concentrations of the species not containing metal have been calculated as percentages of total ligand, those containing metal as percentages of total metal

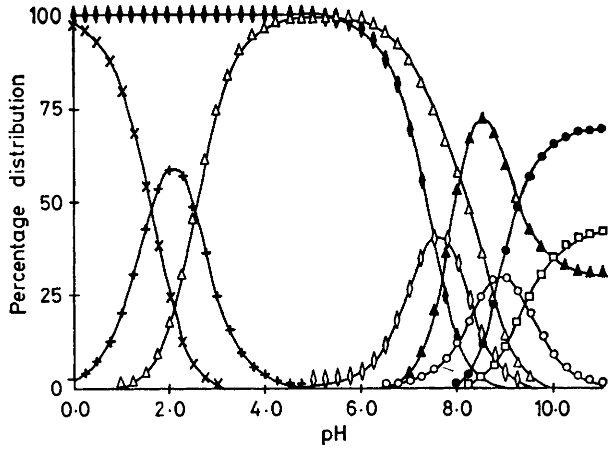

FIGURE 3 Typical distribution diagram for the system homocystine–Co^{2+}; see key to Figure 2, [ML] (▲). The percentage of each species has been calculated for a hypothetical solution of cobalt ions (0.000 517 1 mol dm^{-3}) and homocystine (0.001 534 7 mol dm^{-3}) by HALTAFALL program equipped with PLOTTER Calcomp 936. Concentrations of the species not containing metal have been calculated as percentages of total ligand, those containing metal as percentages of total metal

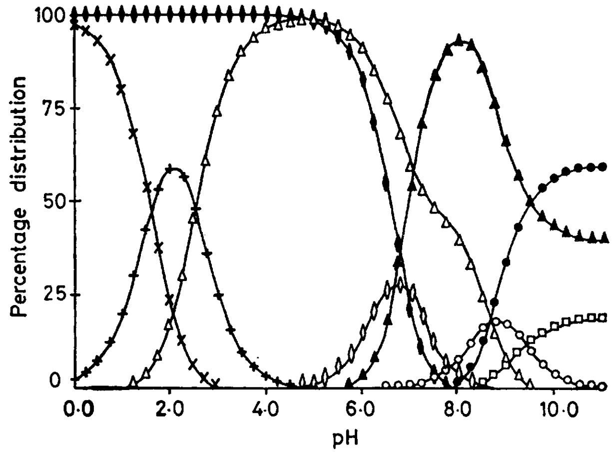

FIGURE 4 Typical distribution diagram for the system homocystine–Ni^{2+}; see key to Figure 2, [ML] (▲). The percentage of each species has been calculated for a hypothetical solution of nickel ions (0.000 556 5 mol dm^{-3}) and homocystine (0.001 112 7 mol dm^{-3}) by HALTAFALL program equipped with PLOTTER Calcomp 936. Concentrations of the species not containing metal have been calculated as percentages of total ligand, those containing metal as percentages of total metal

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* For details see Notices to Authors No. 7, *J. Chem. Soc., Dalton Trans.*, 1979, Index issue.

TABLE 4

Comparison of equilibrium constants for equation (1) of various metal ions with different α-amino-acids at 25 °C. Standard deviations (σ values) are given in parentheses

Ligand	Equilibrium constant	Co^{II}	Ni^{II}	Cu^{II}
DL-2,3-Diaminopropionic acid [a]	log β_{111}	12.30(2)	13.428(6)	15.551(2)
	log β_{101}	6.28(1)	8.159(3)	10.514(6)
	log K [b]	6.02(2)	5.269(7)	5.037(6)
L-Ornithine [c]	log β_{111}	14.168(5)	15.255(8)	17.812(1)
	log β_{101}	5.01(1)	7.113(9)	11.30 [d]
	log K [b]	9.158(11)	8.142(12)	6.512
L-Lysine [e]	log β_{111}	14.505(4)	15.600(8)	18.29(2)
	log β_{101}		5.75(7)	11.50
	log K [b]		9.85(7)	6.79
Homocystine [f]	log β_{111}	13.543(52)	14.327(10)	
	log β_{101}	5.759(25)	7.653(4)	
	log K [b]	7.784(58)	6.674(11)	
DL-2,4-Diaminobutyric acid [a]	log β_{111}	13.60(1)	14.738(2)	17.137(1)
	log β_{101}	6.748(4)	8.909(1)	10.500(2)
	log K [b]	6.852(11)	5.829(2)	6.637(2)

[a] Ref. 5. [b] $\log K = \log \beta_{111} - \log \beta_{101}$. [c] L-2,5-Diaminopentanoic acid, ref. 5. [d] E. R. Clarke and A. E. Martell, *J. Inorg. Nucl. Chem.*, 1970, **32**, 911. [e] L-2,6-Diaminohexanoic acid, ref. 5. [f] DL-4,4′-Dithiobis(2-aminobutyric acid), present work.

The cumulative (log β_{101} and log β_{102}) and stepwise (log K_2) formation constants are in accordance with the Irving–Williams series. We think the complexes formed by homocystine with metal ions are five-membered ring chelates made up of only one α-amino-acid extremity; the mode of bonding in the $[M(HL)]^+$ complexes of homocystine is almost certainly glycine-like with the proton residing on the amino-nitrogen atom not involved in the chelation. The glycine-type chelate with *N,O* donor atoms is confirmed by a comparison of the constants in Table 2 with $\log \beta_{101} = 6.18$ and $\log K_2 = 4.95$ for Ni^{2+}–glycine,[13] and $\log \beta_{101} = 5.07$ and $\log K_2 = 3.97$ for Co^{2+}–glycine systems respectively.[13] Equilibrium constants for reaction (1) may be compared with those for the analogous reaction with some α-amino-acids, and results are quoted in Table 4.

$$[ML] + H^+ \rightleftharpoons [M(HL)]^+ \qquad (1)$$

A first glance at these results would suggest that the order of stability constants (log K) for different ligands is inverted with respect to the Irving–Williams series. It is reasonable to suppose that this enhancement in the [ML] metal complex stability ($\beta_{CoL} < \beta_{NiL} < \beta_{CuL}$) causes a decrease in the basicity of the amino-group not chelated ($K_{Co^{II}} > K_{Ni^{II}} > K_{Cu^{II}}$). The higher value of log (β_{101}/K_2) for Co^{2+} [log $(\beta_{101}/K_2) = 2.21$] and Ni^{2+} [log $(\beta_{101}/K_2) = 3.82$] could be explained either by the Jahn–Teller effect or even by a different rearrangement of the ligand. The ligand is in a nearly extended form in its solid state according to *X*-ray conformational analysis. It is thus reasonable to assume that the chelation occurs only through the *O,N* bite on one half of the amino-acid for the different species found in solution.

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