Metal Complexes of Sulphur-containing Ligands. V. Interactions of Cobalt(II) Ion with L-cysteine and its Derivatives

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Potentiometry and spectrophotometry were used to study the cobalt(II) complexes of L-cysteine, D-penicillamine, DL-mercaptosuccinic acid, N-acetyl-L-cysteine, N-acetyl-D-penicillamine, α-mercaptopropionylglycine and glutathione. It was found that L-cysteine and D-penicillamine form octahedral bis complexes of composition CoA_2 with the cobalt(II) ion. At intermediate pH values polynuclear complexes too are formed; this is the case primarily for L-cysteine. Pentacoordinated complexes CoA_2 are produced with mercaptosuccinic acid, and the concentration of the polynuclear species is negligible. N-acetyl-D-penicillamine forms a distorted tetrahedral bis complex by (S,O) coordination. Besides an analogous species, polynuclear and mixed hydroxo complexes too may be present in the cobalt(II)–N-acetyl-L-cysteine system. In a weakly basic medium the cobalt(II) ion does not induce deprotonation of the peptide NH group for either mercaptopropionylglycine or glutathione. In the case of mercaptopropionylglycine the formation of the parent complexes is accompanied by their hydrolysis, while for glutathione there is the possibility of both cysteine-type and glutamic acid side-chain coordination.

Introduction

The importance of cobalt(II) complexes containing a Co–S bond has recently been enhanced considerably by the recognition that these compounds may be suitable models for acquiring a deeper understanding of the properties of 'blue copper proteins' [1]. The complexity of the interactions of L-cysteine and D-penicillamine with metal ions has been reviewed previously [2–4]. Concerning the cobalt(II)–L-cysteine (or D-penicillamine) systems, however, it appears that relatively few investigations have been performed in comparison with the other 3d transition metals, and the available findings relating to the structures and compositions of the complexes are contradictory. The possibility of the formation of polynuclear complexes in the case of L-cysteine was pointed out by Williams *et al.* [5], who suggested that these contained tetrahedral and square-planar cobalt(II) ions. Their investigations indicated that parent complexes of the types CoA_2 and CoA_3 are also formed, containing tetrahedral and octahedral cobalt(II) ions, respectively. Boggess and Martin [6] studied the cobalt(II)–L-cysteine and D-penicillamine systems by means of pH-metry, spectrophotometry and CD. They concluded that neither ligand yields tris complexes, and that the species CoA_2 contain cobalt(II) ions in octahedral symmetry.

The main results on the metal complexes of glutathione and other SH-containing peptides are to be found in the reviews by Rabenstein *et al.* [7] and ourselves [4]. As concerns the cobalt(II)–glutathione interaction, it was first pointed out by Martin and Edsall [8] that this ligand may coordinate to the metal ion both as an aminoacid, via the γ-glutamyl side-chain, and via the sulphur atom. Jeżowska-Trzebiatowska *et al.* [9] subsequently assumed deprotonation of the peptide NH group at pH ~ 12, and the formation of tetrahedral complexes involving (S, N) coordination. More recently, Abello *et al.* [10] used a potentiometric method to study the cobalt(II)–glutathione system, and concluded that a complex of type CoA is produced in a predominant amount. Sugiura *et al.* [11] have carried out studies on the cobalt(II) complexes of α-mercaptopropionylglycine. They established that only 1:1 and 1:2 parent complexes are formed, and that the amide hydrogen does not undergo deprotonation in the pH interval of the investigation. As regards the results on the cobalt(II) complexes of other SH-containing peptides, it appears that the cobalt(II) ion in these compounds is generally tetrahedral [1, 12]. At the same time, the presence of a pentacoordinated cobalt(II) ion is probable in the monomeric complexes of thioglycollic acid, which is likewise capable of (S, O) coordination [5]. This ligand yields polynuclear complexes too, a feature

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TABLE I. Protonation Constants of the Ligands. T = 298 K; I = 0.2 mol/dm^3 KCl.

$$\beta_{pqr} = \frac{[M_pA_qH_r]}{[M]^p[A]^q[H]^r}$$

	$\log \beta_{011}$	$\log \beta_{012}$	$\log \beta_{013}$
L-cysteine	10.16	18.26	20.12
D-penicillamine	10.63	18.56	20.46
N-acetyl-L-cysteine	9.52	12.56	–
N-acetyl-D-penicillamine	10.05	13.25	–
DL-mercaptosuccinic acid	10.33	14.84	17.00
α-mercaptopropionylglycine	8.33	11.75	–
glutathione	9.46	18.10	21.58

which is also characteristic of the (S,N)-coordinated β-mercaptoethylamine [13].

From the foregoing it is seen that the cobalt(II) ion may take part with variable geometry in its complexes with sulphur-containing ligands. However, the available data do not allow a general picture of the effects of the nature and steric situation of the donor atoms on the geometry and composition of the complexes.

The aim of the present work is to obtain further data on the interactions between the cobalt(II) ion and sulphur-containing ligands, primarily with consideration as to how the complex formation is influenced by the donor atoms and substituents in the vicinity of the SH group. Accordingly, we have used potentiometry and spectrophotometry to study the systems of cobalt(II) with L-cysteine, D-penicillamine, DL-mercaptosuccinic acid, N-acetyl-L-cysteine, N-acetyl-D-penicillamine, α-mercaptopropionylglycine and glutathione over wide concentration ranges.

Experimental

L-cysteine hydrochloride monohydrate (Merck), DL-mercaptosuccinic acid (Serva), D-penicillamine, α-mercaptopropionylglycine, N-acetyl-L-cysteine, N-acetyl-D-penicillamine and glutathione (Fluka) of analytically pure quality were used without further purification. The other chemicals were Reanal products of the highest analytical purity.

The pH-metric titrations were performed in the concentration interval 10^{-3}–2×10^{-2} mol/dm^3, and at metal ion–ligand ratios between 1:1 and 1:8. 200 to 300 experimental points were used for the computer analysis of every system. The practical details of the pH-metric measurements and the computer evaluation of the data were described earlier [14]. The differences between the experimental and calculated titration curves do not exceed 0.01 cm^3 which is less than 1% of the titrant. A Radiometer pH M 64 pH-meter, G 202 B glass and K 401 calomel electrodes, and an ABU 13 automatic burette were used for the experiments. All titrations were carried out at 298 K, at an ionic strength of 0.2 mol/dm^3 KCl. The measurements were made in an argon atmosphere in a closed system, because of the readiness of cobalt(II) complexes to undergo oxidation.

A Beckman Acta MIV double-beam recording spectrophotometer was used for the spectrophotometric examinations. The application of 1 or 10 mm flow cells meant that the cells, the titration vessel and the home-made pump effecting the circulation could be built into a closed system which ensures the absence of O_2 and the simultaneous measurement of the pH and the spectrum. Magnetic moments were determined on a JEOL MH-100 NMR spectrometer as described by Löliger and Scheffold [15].

Results and Discussion

The pH-metric measurements were made in a wide concentration range at metal ion–ligand ratios between 1:1 and 1:8. As the relevant Figures show, there are considerable differences between the titration curves for the individual systems, and hence between the complex-forming properties of the ligands. Below, therefore, the results will be discussed grouped according to the ligands. The protonation constants determined from the experimental pH-metric data on the free ligands are listed in Table I.

L-cysteine and D-penicillamine

Figure 1 presents the relevant titration curves at metal ion–ligand ratios of 2:10, 3.33:10, 5:10, 7.5:10 and 10:10 ($\times 10^{-3}$ mol/dm^3).

Figure 1 indicates that at pH $\geqslant$ 9 both cysteine and penicillamine form 1:2 complexes; this is shown by titration of the free ligand in the systems with

TABLE II. Stability Constants of Cobalt(II) Complexes with Various Thiol Containing Ligands. T = 298 K; I = 0.2 mol/dm^3 KCl.
$\beta_{pqr} = \frac{[M_pA_qH_r]}{[M]^p[A]^q[H]^r}$

	Ligands					
	L-cysteine	D-penicillamine	MSA	NAP	MPG	glutathione
$\log \beta_{110}$	8.00	8.98	6.71	4.63	2.93	–
$\log \beta_{120}$	14.20	16.88	11.15	10.10	7.03	9.55
$\log \beta_{130}$	–	–	–	–	9.47	–
$\log \beta_{111}$	–	–	–	–	–	13.20
$\log \beta_{11-1}$	–	–	–	–	−5.3	–
$\log \beta_{121}$	–	23.32	–	–	–	18.52
$\log \beta_{12-1}$	–	–	–	–	−2.11	–
$\log \beta_{230}$	26.34	28.35	(19.61)	–	–	18.93
$\log \beta_{231}$	–	–	–	–	–	26.97
$\log \beta_{340}$	37.98	–	(28.91)	–	–	–
$\log \beta_{341}$	43.74	–	–	–	–	–
$\log \beta_{220}$	–	–	–	–	–	14.05
$\log K_1/K_2$	1.80	1.08	2.37	−0.84	−1.17	–

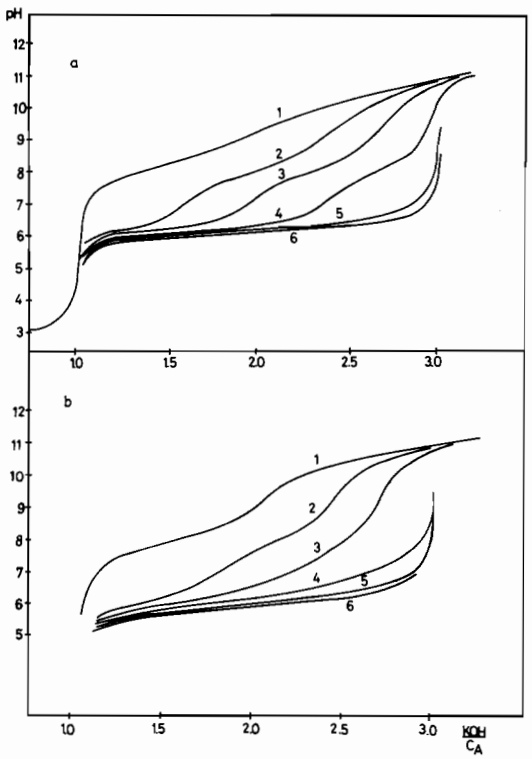

Fig. 1. pH-metric titration curves for Co(II)–L-cysteine (a) and Co(II)–D-penicillamine (b) systems. C_{A_0} = 0.01 mol/dm^3; C_{Co} = 0 (1); 2 × 10^{-3} (2); 3.33 × 10^{-3} (3); 5 × 10^{-3} (4); 7.5 × 10^{-3} (5); 10^{-2} mol/dm^3 (6).

ratios of 1:3 or more. However, there is a significant difference between the complex-formation processes of the two ligands in the interval $5 \leqslant pH \leqslant 9$. The titration curves for the systems containing L-cysteine reveal the appearance of two inflection points even at a ratio of 5:10. The position of the first inflection point corresponds to the ratio Co/A = 3/4, which is indicative of the formation of the previously assumed polynuclear complex Co_3A_4 [5]. The presence of polynuclear complexes is confirmed by computer evaluation of the titration data. The stability constants obtained in the case of the best approximation, together with the data on the other ligands, are given in Table II.

The relevant concentration distribution curves demonstrate that the formation of polynuclear complexes is primarily characteristic of cysteine, in accordance with what has been observed in the case of other metal ions (Ni(II), Zn(II)) [3]. Corresponding to the information obtained from the titration curves, the polynuclear complex of cysteine, Co_3A_4, is formed virtually exclusively at pH ~ 7. However, the donor groups becoming free on titration of the excess of ligand break down the polynuclear structure, and at pH > 9 the bis complex CoA_2 predominates. In agreement with the observations of Boggess and Martin [6], the formation of 1:3 complexes is negligible for both ligands, and the polynuclear complex is also produced in the case of D-penicillamine. Nevertheless, its quantity and stoichiometric composition are different from those for L-cysteine, which can be attributed to the steric inhibitory effect of the β-methyl substituents.

We carried out spectrophotometric and magnetic susceptibility measurements also to confirm the above results. These studies gave the possibility at the same time to establish the symmetry of the com-

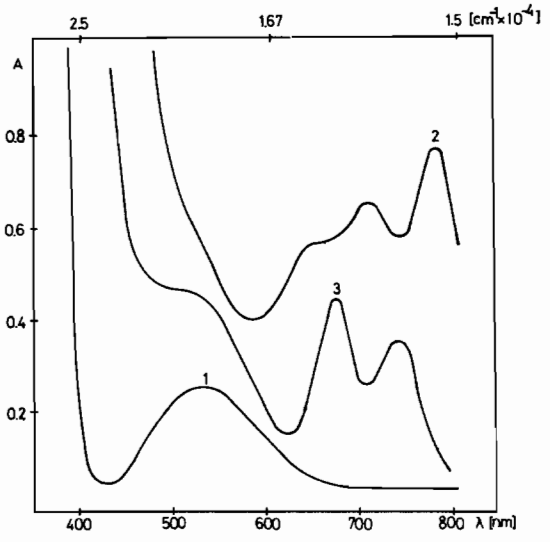

Fig. 2. Absorption spectra of cobalt(II) complexes of L-cysteine (1 and 2) and D-penicillamine (3); (1 = 10 mm). C_{Co} = 5×10^{-3}; C_{ligand} = 10^{-2} mol/dm^3 (1) pH = 9.5; (2–3) pH = 7.

plexes. Some typical spectra for the Co(II)–L-cysteine and Co(II)–D-penicillamine systems are shown in Fig. 2.

Spectrum 1 relates to the cysteine complex of type CoA_2 (the analogous spectrum for D-penicillamine is completely similar); the position and intensity of the band are indicative of octahedral symmetry for the cobalt(II) ion. In contrast, spectra 2 and 3 (and the intense green colour of the solutions) suggest the presence of a tetrahedral central ion. Like the concentration of the polynuclear complex, the intensities of these absorbance bands vary according to maximum curves. Thus, the tetrahedral bands presumably originate from the polynuclear complex, which is formed in the following process:

$$2CoA_2^{2-} + Co^{2+} \rightleftharpoons$$

$$[Co(\text{tetrahedral})(CoA_2(\text{octahedral}))_2]$$

i.e. the Co_3A_4 unit contains 1 tetrahedral and 2 octahedral cobalt(II) ions.

However, the magnetic susceptibility measurements appear to contradict this assumption. The magnetic moment of the Co(II)–L-cysteine solutions displays a minimum curve as a function of pH (μ_{min} = 3.4 BM). The position of the minimum coincides with the formation maximum for the polynuclear complex. This would indicate that, in agreement with findings relating to cysteine and β-mercaptoethylamine [5, 13], the central cobalt(II) ion has square-planar geometry. Since the spectra do not support this assumption, it is more probable that the decrease in the magnetic moment is caused by the spin-exchange interaction between tetrahedral and octahedral cobalt(II) ions in the polynuclear complex.

DL-mercaptosuccinic acid (MSA)

The complexes formed in the range $5 \leqslant pH \leqslant 10$ are to be found in Table II, together with their stability constants. Both these tabulated data and the relevant titration curves show that 1:3 complexes are not formed in this system either. The results demonstrate that species of the types CoA and CoA_2 are present in predominant amounts over the entire pH interval. However, the fit of the experimental data can be improved by the assumption of polynuclear complexes. The overall quantity of these does not exceed 10% of the total metal ion concentration, and hence the presence of Co_3A_4 and Co_2A_3, alone or together, cannot be decided from the pH-metric data.

The spectrophotometric measurements reveal a very complicated band system at $pH < 6.5$, with λ_{max} = 580, 670 and 740 nm. As the pH is increased, the intensity of the band at 580 nm increases, and then becomes constant (ϵ = 70 mol^{-1} dm^3 cm^{-1}) after base consumption corresponding to the composition CoA_2; at the same time the other two bands disappear. On this basis it may be assumed that the absorbance at the higher wavelengths is caused by the polynuclear complexes. The 670 and 740 nm bands coincide with the bands for the D-penicillamine complex of type Co_2A_3, and thus the spectrophotometric results suggest that a polynuclear complex of composition Co_2A_3 also is formed in the case of MSA.

The spectral data on the blue complex formed in the higher pH range are very similar to the parameters determined by Williams *et al.* [5] for the Co(II)–thioglycollic acid CoA_2 complex, in which a pentacoordinated cobalt(II) ion was postulated. The pH-metric titration curves clearly show that a hydroxo complex is not formed in the interval in question, *i.e.* the fifth coordination site is occupied by a water molecule or a carboxyl group. The large positive value in this case compared to the values of $\log K_1/K_2$ for the other ligands is indicative of coordination of the carboxyl group. In the complex of type CoA the MSA behaves as a tridentate ligand, while for steric reasons coordination of the second MSA is less favoured and it behaves as a bidentate ligand.

N-acetyl-L-cysteine (NAC) and N-acetyl-D-penicillamine (NAP)

It is well known that the presence of N-acetyl substituents leads to a very considerable decrease in the coordinating ability of the nitrogen atoms

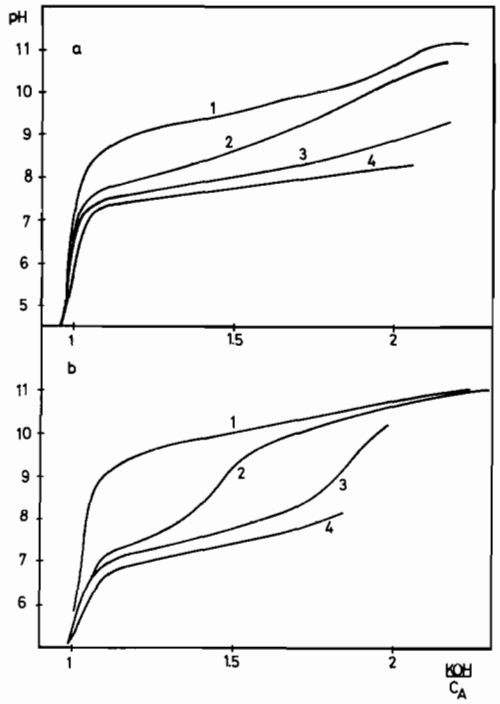

Fig. 3. pH-metric titration curves for Co(II)–NAC (a) and Co(II)–NAP (b) systems. a: C_{NAC} = 0.01 mol/dm^3; C_{Co} = 0 (1); 2×10^{-3} (2); 5×10^{-3} (3); 10^{-2} mol/dm^3 (4). b: C_{NAP} = 0.005 mol/dm^3; C_{Co} = 0(1); 10^{-3} (2); 2×10^{-3} (3); 5×10^{-3} mol/dm^3 (4).

of aminoacids. In principle, however, the presence of the NH–CO–peptide group permits coordination like that in peptides in these compounds. Nevertheless, the studies by Sóvágó and Martin [16] show that deprotonation and coordination of the amide does not occur at pH < 12 even in the NAC complexes of Pd(II), which is a metal ion exhibiting one of the highest inductive effects.

Our results for the Co(II)–NAC and Co(II)–NAP systems can presumably be interpreted in a similar way. The relevant titration curves are presented in Fig. 3.

This figure shows that the differences between the complex-forming properties of L-cysteine and D-penicillamine are manifested even more markedly in the case of the N-acetyl derivatives. The complex-formation processes occurring in the cobalt(II)–NAP system can be described unambiguously by the assumption of the parent complexes CoA and CoA_2, and the amide hydrogen cannot undergo deprotonation at pH < 11. The relevant stability constants are to be found in Table II. The appreciably negative value for log K_1/K_2 points to the marked stability of tetracoordination and to the virtually exclusive formation of the bis complex CoA_2.

In spite of the lower pK values of the ligand, the complexes of NAC are formed at somewhat higher pH, *i.e.* the corresponding species are less stable than in the case of Co(II)–NAP. It is striking, however, that even in the presence of a considerable ligand excess more than 2 equivalents of proton can be titrated at pH < 10.5. This base-consuming process can be interpreted in terms of deprotonation of the amide group, or the formation of mixed hydroxo complexes. Our spectrophotometric examinations are clearly in support of the base consumption being caused by hydrolysis. A spectrum with one absorbance band is obtained for each of the cobalt(II)–NAP and the cobalt(II)–NAC systems, with λ_{max} = 625 and 670 nm, respectively. The complex nature of these bands (shoulders at 580 and 640 nm in the case of NAP, and at 610 and 720 nm in the case of NAC) points to the distorted tetrahedral geometry of the cobalt(II) ion [1b, 17]. In solutions containing a cobalt(II)–NAP ratio of 1:3 or more ligand, the intensity of the absorption maximum at 625 nm varies according to a saturation curve (ϵ_{max} = 150 mol^{-1} dm^3 cm^{-1}) as a function of pH, which is indicative of the formation of a 1:2 complex. The intensity of the 670 nm band for the cobalt(II)–NAC system is substantially higher, and depends considerably on the metal ion–ligand ratio. The high molar absorbance ($\epsilon_{max} \leqslant 500$ mol^{-1} dm^3 cm^{-1}) strongly suggests that polynuclear species also may be formed besides the parent complexes. In solutions containing a large ligand excess (ratios between 1:4 and 1:8), the intensity (before precipitate formation) varies according to a maximum curve. The intensity decrease becomes determining in the interval of the excess base-consuming process (after 2 equivalents), but it is not accompanied by a change in form or a shift in the spectrum; this points to hydrolysis of the metal ion rather than amide deprotonation. These findings show that formation of the parent complex in the cobalt(II)–NAC system is not complete even in the case of a ligand excess, and formation of the bis complex CoA_2 is accompanied by the appearance of polynuclear and mixed hydroxo complexes. It follows from this that the computer evaluation of the pH-metric titration data could not be performed since, because of the low stability, the free ligand concentration is very high in the range of complex formation, and very many species of various compositions may be formed.

α-Mercaptopropionylglycine (MPG) and glutathione

As observed with other metal ions [16], the cobalt(II) complexes of the two peptide molecules differ considerably, as shown by the titration curves in Fig. 4.

Figure 4 reveals that even in the case of a ligand excess in cobalt(II)–MPG solutions another base-consuming process occurs besides that involving the SH group. By comparison with NAC, MPG is very prone to coordination via the peptide-N [11, 16],

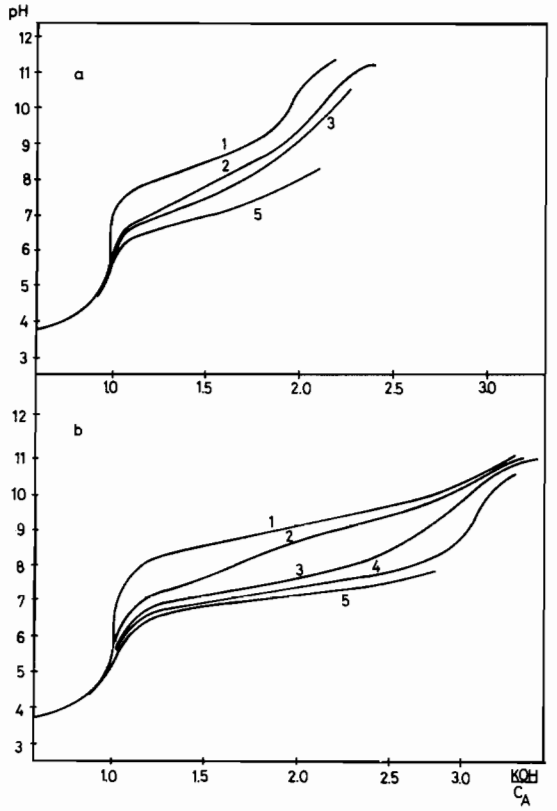

Fig. 4. pH-metric titration curves for the Co(II)–MPG (a) and Co(II)–glutathione (b) systems. C_A = 0.01 mol/dm^3; C_{Co} = 0 (1); 2 × 10^{-3} (2); 5 × 10^{-3} (3); 7 × 10^{-3} (4 – for glutathione); 10^{-2} mol/dm^3 (5).

and hence this process is also likely in the cobalt(II) complexes. Spectrophotometry seemed to be a suitable method to decide this question. However, the evaluation was severely hampered by the extremely ready oxidation of the system and by the complexity of the spectra recorded in the argon atmosphere. Figure 5 illustrates spectra at two different pH values for a solution with a cobalt(II)–MPG ratio of 1:4.

The cobalt(II)–MPG system is seen to be characterized by two absorbance bands, with λ_{max} = 675 and 600 nm, respectively. However, as a function of pH the 675 nm band varies according to a maximum curve, and the 600 nm band according to a saturation curve. At the same time, the intensiy of the absorbance at the greater wavelength is enhanced significantly by an increase in the ligand–metal ion ratio. Since these spectral changes are not accompanied by the appearance of a new band in the range of base consumption above 2 equivalents, it is probable that, just as for NAC, deprotonation of the amide hydrogen does not occur for MPG either. Accordingly, our data can best be interpreted if it is assumed that the process of formation of the parent complex is accompanied by its hydrolysis. This assumption is supported by a computer analysis of the titration curves. The relevant stability constants are given in Table II, and the corresponding concentration distribution is depicted in Fig. 6.

Figure 6 shows that the bis complex CoA_2 is the main species in the cobalt(II)–MPG system; the 600 nm band can be ascribed to this species (ϵ_{600} < 160

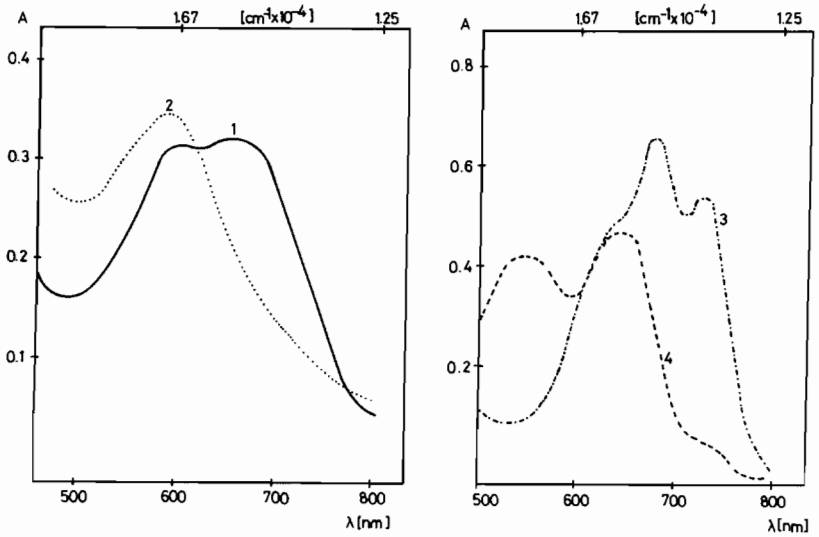

Fig. 5. Absorption spectra of cobalt(II) complexes of MPG (1 and 2) and glutathione (3 and 4); (1 = 10 mm). C_{Co} = 0.0025; C_{ligand} = 10^{-2} mol/dm^3. (1) pH = 8.6; (2) pH = 10.3; (3) pH = 9.5; (4) pH = 10.2.

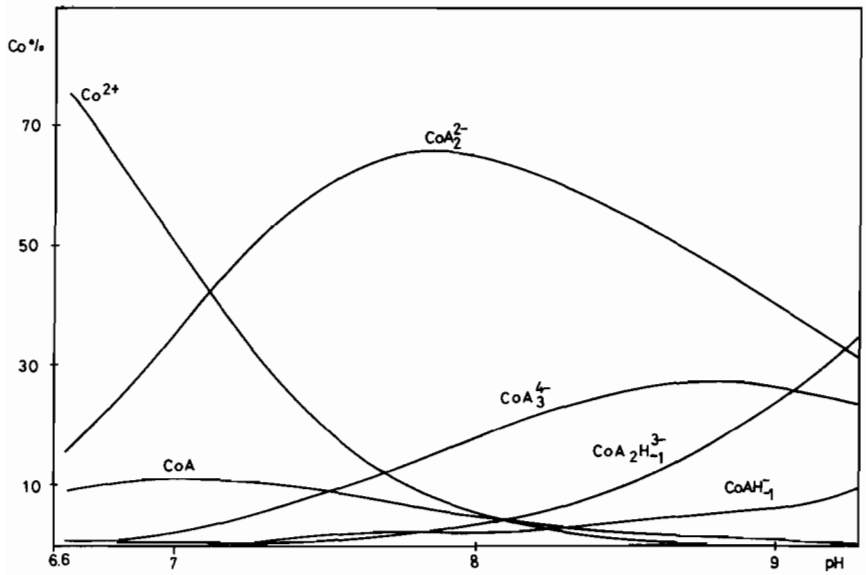

Fig. 6. Concentration distribution of complexes formed in the Co(II)–MPG system as a function of pH. $C_{MPG} = 0.01$; $C_{Co} = 3 \times 10^{-3}$ mol/dm^3.

mol^{-1} dm^3 cm^{-1}). The formation of CoA_3 ($\lambda_{max} \sim$ 675 nm) is promoted by an excess of ligand, but is suppressed by the hydrolytic processes at higher pH.

Figure 4 indicates that no additional base-consuming process occurs at pH < 10 in the cobalt(II)–glutathione system, so that neither deprotonation of the amide groups nor hydrolysis of the complexes need be reckoned with. Nevertheless, the complex formation is very involved, for the processes of type $MA \rightarrow MA_2$ are not suitable for the description of the titration data.

However, the variations in the spectral parameters of the cobalt(II)–glutathione systems of various compositions are surprisingly simple. Figure 5 shows the spectra for 1:3 solutions of cobalt(II)–glutathione in weakly and in strongly basic medium. Spectrum 3 in this Figure exhibits a characteristic tetrahedral band structure, with a shoulder at 630 nm and peaks at 680 and 735 nm. At 6 < pH < 10 the positions of these bands do not change as functions of the pH or the ligand–metal ion ratio; as shown in Fig. 7, only their intensities vary.

It emerges from Fig. 7 that the tetrahedral band system cannot yet be observed in the initial range of complex formation, *i.e.* the coordination begins on the aminoacid side-chain. The subsequent changes are suggestive of the formation of a tetrahedral bis complex, accompanied at pH > 10 by a further decrease in the intensity values. On the above basis, in the interval 6 < pH < 10 the titration curves should be describable by the assumption of MAH and MA_2. The best approximation, however, was

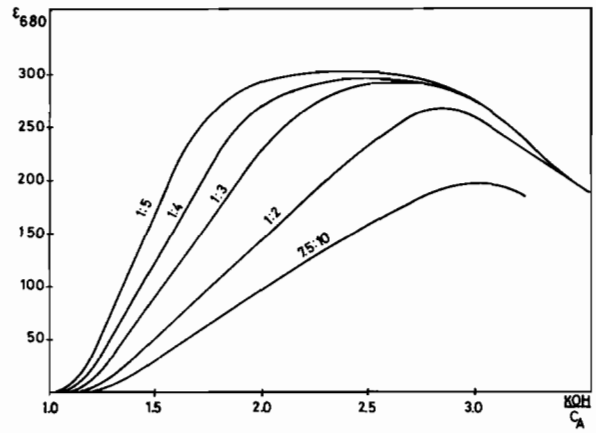

Fig. 7. Variation of the 680 nm absorbance band in the Co(II)–glutathione system as a function of the number of equivalents of base at different metal ion–ligand ratios. $C_A = 0.01$ mol/dm^3.

obtained from the data in Table II, which points to the formation of additional protonated and polynuclear complexes. The relevant concentration distribution is depicted in Fig. 8.

The complex CoAH presumably corresponds to the aminoacid-like coordination of the ligand via the -glutamyl side-chain, the SH group remaining protonated. The bis complex CoA_2 is formed only at higher pH, but the various protonated and polynuclear species are present in appreciable amounts throughout. On the basis of the spectrophotometric data, this can be interpreted only if it is assumed that

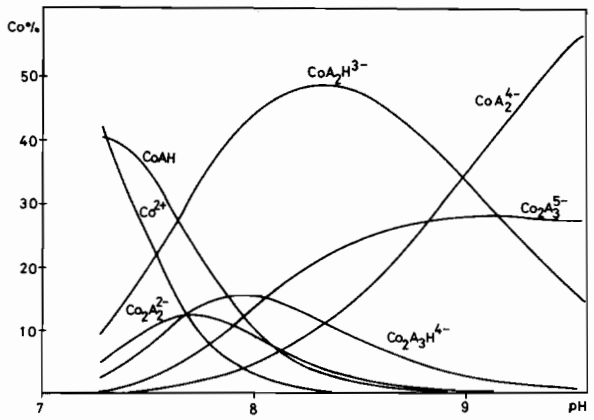

Fig. 8. Concentration distribution of complexes formed in the Co(II)–glutathione system as a function of pH. C_A = 0.01; $C_{Co} = 2 \times 10^{-3}$ mol/dm^3.

TABLE III. Stability Constants of Mixed Ligand Complexes of D-penicillamine, T = 298 K; I = 0.2 mol/dm^3 KCl.

$$\beta_{pqrs} = \frac{[M_pA_qB_rH_s]}{[M]^p[A]^q[B]^r[H]^s}$$

$\Delta \log \beta_{1110} = \log \beta_{1110} - \frac{1}{2}(\log \beta_{1200} + \log \beta_{1020} + 0.6)$

	$\log \beta_{1110}$	$\Delta \log \beta_{1110}$
Glycine	12.84	0.11
Ethylenediamine	14.09	0.15
L-histidine	15.05	0.22

Stability constants of B-ligands:

glycine:	$\log \beta_{1010} = 4.56$	$\log \beta_{1020} = 8.30$
en:	$\log \beta_{1010} = 5.60$	$\log \beta_{1020} = 10.24$
L-histidine:	$\log \beta_{1010} = 6.76$	$\log \beta_{1020} = 12.18$
	$\log \beta_{1011} = 10.98$	$\log \beta_{1021} = 17.36$

all of the complex species contain the same chromophoric group. This means that the tetrahedral coordination characteristic for CoA_2, via the sulphur atom and the carbonyl oxygen is present in the protonated and polynuclear complexes as well. That is, the free amino group of the species CoA_2 can bind either to a proton (CoA_2H) or to a cobalt(II) ion (octahedrally coordinated Co_2A_2), and the spectral effects of these species can be taken as the same as those of CoA_2. The appearance of the species Co_2A_3 and Co_2A_3H points to the formation of a chain-like polymer, in which one of the cobalt(II) ions has tetrahedral symmetry involving (S, O) coordination, while the other cobalt(II) ion has octahedral symmetry involving (N, O) coordination.

In order to test these assumptions we also performed pH-metric and spectrophotometric studies on the cobalt(II)–NAP–glycine system. The results demonstrated that the mixed ligand complex CoAB is not formed, but only the bis complexes CoA_2 and CoB_2 are present, *i.e.* the mixed coordination mode (S, O)–(N, O) is not favoured in the case of cobalt(II). As concerns the parent complexes of glutathione, this means that the complex Co_2A_2 binds the third ligand in an aminoacid-like manner This is supported by the relevant pK values, since $pK_{Co_2A_3}^{Co_2A_3H} = 8.04$, which corresponds closely to the value for deprotonation of the SH group. At the same time, $pK_{CoA_2}^{CoA_2H} = 8.97$, which can be ascribed to the amino group.

Spectrum 4 in Fig. 5 shows the absorbance measured at pH ~ 12.0. As a consequence of the considerable spectral change it may be assumed that, in the case of glutathione in strongly basic medium, the complexes do not undergo hydrolysis but the amide nitrogen is coordinated [9].

Mixed Ligand Complexes

From the data for the cobalt(II)–NAP–glycine system it may be assumed that mixed ligand complex formation is not favoured in the case of the tetrahedral and octahedral bis complexes. D-penicillamine does form an octahedral complex CoA_2, however, and our investigations were therefore extended to the corresponding mixed complexes with glycine, ethylenediamine or histidine as ligand B. The stability constants obtained from the pH-metric measurements are listed in Table III.

The data in Table III demonstrate that in all three cases the observed stability is only slightly in excess of the statistical value, *i.e.* the aminoacids containing the SH group display a similarity to the simple aminoacids in the course of mixed complex formation with the cobalt(II) ion [18]. At the same time, it is noteworthy that the value of $\Delta \log \beta_{1110}$ is largest for the cobalt(II)–penicillamine–histidine system. The participation of the sulphur atom in the coordination therefore favours primarily the coordination of the imidazole nitrogen, as in the results obtained earlier for the nickel(II) and zinc(II) complexes [19].

Conclusions

It was found experimentally that the symmetry of the central cobalt(II) ion and the formation of polynuclear complexes depend to a large extent on the number and nature of other donor atoms situated in the vicinity of the SH group and on the presence of alkyl substituents. L-cysteine and D-penicillamine, which are capable of (S, N, O) coordination, form octahedral bis complexes with the cobalt(II) ion, *i.e.* all three donor atoms participate in the coordi-

nation. At intermediate pH values, the polynuclear complexes Co_2A_3 and Co_3A_4 are also formed, which in part contain tetrahedral cobalt(II) ions. Formation of the polynuclear complexes is primarily favoured in the case of L-cysteine, since the β-methyl substitutents in D-penicillamine have a considerable steric hindrance effect.

Like the preceding ligands, MSA contains three potential donor groups, but its complex-formation processes differ appreciably from those of the SH-containing aminoacids. The spectral parameters of the bis complex CoA_2 and the large positive value found for log K_1/K_2 can best be interpreted by the presence of pentacoordinated cobalt(II), which strongly suggests the coordination of both carboxyls of one of the ligands. The quantity of polynuclear complexes formed in the intermediate pH range is not considerable, presumably because of the bulk of the chain-terminal carboxyl group.

Deprotonation of the amide hydrogen does not occur during the interactions of NAP and NAC with cobalt(II), and thus the coordination can proceed only via the sulphur and the carboxyl oxygen donor atoms, with the formation of a six-membered chelate ring. As shown by the negative log K_1/K_2 value, NAP yields a very stable bis complex CoA_2. Formation of the polynuclear complexes is negligible, as in the cobalt(II) complex of β-mercapto-β′,β″-dimethylethylamine [20], and in both cases the central ion has distorted tetrahedral symmetry. In the case of NAC the absence of the β-methyl substituents means that the coordinating ability of the sulphur atom is less, and thus the stabilities of the corresponding complexes are lower. As for L-cysteine and β-mercaptoethylamine [13], however, there is a possibility for sulphur-bridged coordination and also, with regard to the complex-formation process occurring at high pH, for the formation of mixed hydroxo complexes.

At pH $<$ 10 the amide hydrogen is not deprotonated for MPG or glutathione. This process does take place to an appreciable extent at pH $\sim$ 12 in the cobalt(II)–glutathione system. The complex-formation processes in the weakly alkaline pH interval also differ considerably from the foregoing ones, since only thiol and carbonyl coordinations are possible. As for NAP, in the case of MPG this results in distorted tetrahedral coordination, which is reflected in the negative value of log K_1/K_2. However, the lower strength of the bond between the metal ion and the carbonyl oxygen does not inhibit the hydrolysis of the complexes, or the formation of CoA_3 in the presence of a ligand excess, *i.e.* the monofunctional coordination of the MPG.

As in the corresponding nickel(II) complexes, the cobalt(II)–glutathione interaction begins on the aminoacid side-chain [8]. Coordination of the sulphur atom is accompanied by the appearance of the characteristic tetrahedral band structure, which is indicative of the formation of (S, O)-coordinated bis complexes, just as for MPG. In contrast with earlier results [10], the formation of 1:1 complexes is not probable. However, a difference from the results found for MPG is that the presence of the aminoacid side-chain inhibits hydrolysis of the complexes. On the other hand, the glutamyl group allows the coordination of two metal ions to one glutathione molecule and hence chain-like polynuclear complexes may be formed.

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