

Complexes of 3,4-Dihydroxyphenyl Derivatives. VII*. Mixed Ligand Complexes of L-dopa and Related Compounds

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The stability constants of the mixed ligand complexes of manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) ions with L-dopa, dopamine, L-adrenaline and L-noradrenaline as ligand A and L-alanine, L-histidine, glycylglycine and ATP as ligand B were determined pH-metrically at 25 °C and an ionic strength of 0.2 mol/dm³ (KCl). From visible spectral studies, conclusions were drawn on the bonding properties of the ambidentate ligands in the complexes.

It was found that, even in its mixed ligand complexes, the ambidentate nature of dopa permits its coordination via the amino acid side-chain (N, O) at lower pH, and via the ortho phenolic hydroxy groups (O, O) at higher pH. At the same time, the complex-forming tendency of the ethanolamine side-chain in the catecholamines plays a minor role in the mixed ligand complexes.

The stabilities of the mixed ligand complexes were interpreted by consideration of the differences in the stepwise stability constants of the parent complexes, the back-coordination, the charge neutralization, and the electrostatic and hydrophobic interactions between the ligand pairs.

Introduction

The formation of mixed complexes may be important in the storage and transport of catecholamines in the organism [1]. Besides the biological significance, it is also essential from a coordination chemistry aspect to know how the presence of *B* ligands containing various donor groups influences the ambidentate nature of these compounds.

The transition metal–dopa and transition metal–catecholamine equilibrium systems are themselves fairly complex [2]. Therefore, few quantitative findings have yet been published on their mixed ligand systems. Rajan *et al.* [3–8] investigated the possibility of mixed ligand complex formation in various metal–dopa–*B* ligand and metal–catecholamine–*B* ligand systems. The formation of stable

mixed ligand complexes was considered highly likely in the mixed ligand systems of copper(II)–dopa and zinc(II)–dopa. It was concluded that, in the physiological pH range, dopa coordinates to the metal ion like an amino acid, but the bonding of one or both phenolic hydroxy groups cannot be excluded [7, 8]. It was similarly regarded as possible that 1:1:1 and in certain cases water-soluble polymeric complexes are formed at pH 4–6 in the metal–catecholamine–ligand *B* systems. Their findings relating to the formation of mixed ligand complexes in certain metal–adrenaline–ATP systems were also supported [9, 10]. According to Granot *et al.* [11, 12] two types of metal–catecholamine–ATP complex may be formed with nickel(II), cobalt(II) and manganese(II). In one of these the metal ion is bound only to the ATP, and there is a stacking interaction between the adenine ring and the benzene ring of the protonated catecholamine (this bonding mode does not differ essentially from that observed between catecholamines and nucleotides in the absence of metal ion [13, 14]). In the other type of complex, the catecholamine is also coordinated directly to the metal ion. Formation of this latter type of complex was considered to be negligible in the physiological pH range.

These divergent findings on the bonding modes in the mixed complexes, together with the small number of equilibrium studies, led us to investigate the possibility of mixed ligand complex formation in the systems of manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) ions with dopa, adrenaline, noradrenaline and dopamine as ligand *A*, with various *B* ligands. The compounds chosen as ligand *B* were alanine, histidine, glycylglycine and ATP, the donor groups of which (carboxylate, amino, imidazolyl-N, peptide-N and phosphate) frequently occupy metal ion binding sites in biological systems.

Experimental

Chemicals and Experimental Conditions

The L-alanine, L-histidine, glycylglycine and ATP were Reanal products of the highest analytical purity. The amino acids were purified by recrystall-

*Part VI, Ref. 22.

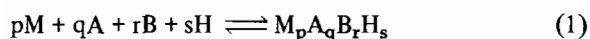
zation from an ethanol–water mixture. The purity of the ATP was checked by thin-layer chromatography on a Merck PEI-Cellulose F anion-exchange plate, with 0.4 mol/dm³ KH₂PO₄ as developing solvent. The catecholamines (dopamine, L-adrenaline and L-noradrenaline) and L-dopa*, Fluka products of *puriss.* quality, were used without further purification. Metal chloride stock solutions were prepared from compounds of the highest analytical purity; their concentrations were checked gravimetrically via the oxinates.

pH-metric measurements were made at 25 °C at an ionic strength of 0.2 mol/dm³ (KCl), with a Radiometer PHM 64 instrument, a G 202B glass electrode and a K 401 calomel electrode. The metal ion/ligand ratio in the samples was 1:1:1, 1:2:1, 1:1:2 or 1:2:2. Since the ligands have a tendency to undergo oxidation, efforts were made to exclude air completely during the measurements. Accordingly, titrations were carried out in a TTA 80 titration unit in an atmosphere of argon.

To establish the metal ion binding sites in the species formed, UV, visible and near IR spectra were recorded for the cobalt(II), nickel(II) and copper(II) complexes with a Beckman ACTA MIV double-beam recording spectrophotometer.

Calculations

The complexes formed can be characterized by the following general equilibrium process and stability constant:



$$\beta_{pqrs} = \frac{[M_pA_qB_rH_s]}{[M]^p[A]^q[B]^r[H]^s} \quad (2)$$

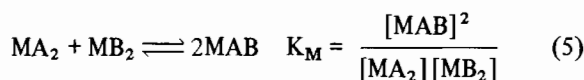
The stability constants defined by eqn. 2 were calculated from the pH-metric titration curves in the usual manner [15].

Mixed ligand complex formation is characterized by the stabilization constant, $\Delta \log \beta_{MAB}^{**}$, which is the difference between the measured stability constant and that calculated from statistical considerations [16]:

$$\Delta \log \beta_{MAB} = \log \beta_{MAB}^{\text{measured}} - \log \beta_{MAB}^{\text{stat.}} \quad (3)$$

$$\log \beta_{MAB}^{\text{stat.}} = 1/2(\log \beta_{MA_2} + \log \beta_{MB_2} + \log 4) \quad (4)$$

Use is also widely made of the exchange or distribution constant, K_M :

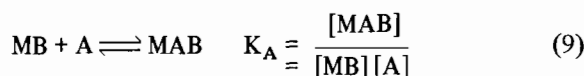
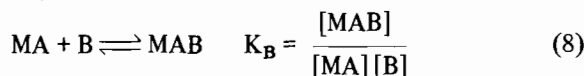


An important equilibrium characteristic for the parent complexes is $\log K_1/K_2$, which expresses the ratio of the stepwise stability constants. $-\Delta \log K^{**}$ [16], which is of analogous importance for the mixed ligand complexes, can be defined as follows:



$$-\Delta \log K = \log \beta_{MA} + \log \beta_{MB} - \log \beta_{MAB} \quad (7)$$

Useful information may also be derived from the equilibrium data K_A^{**} and K_B^{**} relating to the steps of mixed ligand complex formation:



Results and Discussion

The equilibrium conditions of the transition metal parent complexes of the *A* ligands are to be found in the preceding papers of this series [17–22], while the stability constants of the *B* ligands under similar experimental conditions have been reported elsewhere [23–27].

From the experience acquired in the study of the parent complexes [19, 20] it is to be expected that dopamine, adrenaline and noradrenaline will not display an ambidentate character in their mixed ligand complexes under the usual experimental conditions, and thus only pyrocatechol-like (O, O) coordination will result. At the same time, dopa is capable of both (N, O) coordination via the amino acid side-chain, and (O, O) coordination via the ortho phenolic hydroxy groups [17, 18, 21]. Complexes of the former type are formed in the initial pH range, and must contain at least two protons on the two phenolic hydroxy groups of the dopa. Accordingly, their stoichiometric composition is MAH₂B.

In the evaluation of the dopa titration curves, in some systems evidence was found for the formation of species MAH₂B. The results are given in Table I.

The data in Table I permit the following conclusions concerning the formation of mixed ligand complexes of dopa involving (N, O) coordination:

1. In accordance with earlier experience relating to the metal ion–amino acid–amino acid ternary systems [28], with alanine as ligand *B* the mixed ligand complex formation corresponds closely to the statistical case. Thus, the (N, O) coordination of both ligands does not particularly favour mixed ligand complex formation.

*Abbreviations used: dopm: dopamine; ad: L-adrenaline; and: L-noradrenaline; dopa: L-3,4-dihydroxyphenylalanine; ala: L-alanine; his: L-histidine; glygly: glycylglycine.

**Depending on the ligand, protonated mixed ligand complexes may also be formed. The constants (see Tables I–V) and the equations defining them are then modified accordingly.

TABLE I. Equilibrium Data on the Mixed Ligand Complexes of (N, O) Coordinated Dopa. $t = 25\text{ }^\circ\text{C}$, $I = 0.2\text{ mol/dm}^3\text{ KCl}$.

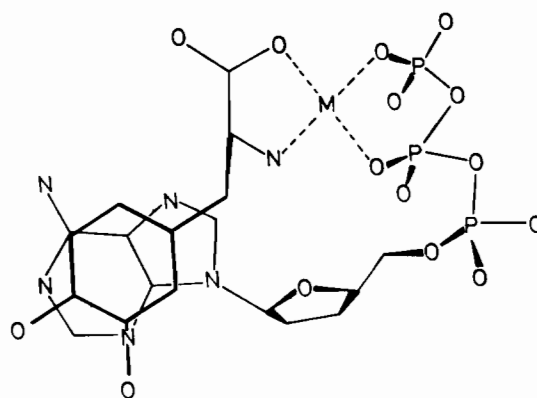
		$\log \beta_{\text{MAH}_2\text{B}}$	$\Delta \log \beta_{\text{MAH}_2\text{B}}$	$-\Delta \log K_{\text{MAH}_2\text{B}}$	$\log K_{\text{B}}$	$\log K_{\text{AH}_2}$	$\text{p}K_{\text{MAH}_2\text{B}}$
Co(II)	his	32.68	—	0.92	5.84	2.89	7.66
	ATP	31.11	—	0.18	4.27	3.63	—
Ni(II)	ala	32.74	0.04	0.71	4.61	4.39	8.12
	his	35.79	0.31	0.86	7.66	4.24	8.56
	glygly	31.61	0.03	0.56	3.48	4.14	8.17
	ATP	32.50	1.01	0.22	4.37	4.88	—
Cu(II)	ala	38.01	0.04	0.78	7.26	6.94	—
	his	40.45	0.94	0.34	9.70	7.38	7.40
	glygly	34.99	—	1.32	4.24	6.40	5.59
	ATP	30.32	1.22	0.43	5.41	7.29	—
Zn(II)	his	32.71	—	0.60	5.71	3.37	6.85
	ATP	27.23	—	-0.23	4.91	4.20	—

2. With histidine as ligand *B*, the $\Delta \log \beta_{\text{MAH}_2\text{B}}$ and $-\Delta \log K_{\text{MAH}_2\text{B}}$ values show an appreciable stabilization for copper(II), and a slight stabilization in the other cases. The $-\Delta \log K_{\text{MAH}_2\text{B}}$ values are smaller than the $\log K_1/K_2$ values [20] for the parent complexes of the *B* ligands. In agreement with this, it is also true that $\log K_{\text{B}} > \log K_{\text{MB}_2}$, *i.e.* there is a higher probability that the histidine is bonded to the MAH_2 complex of dopa than to its own parent complex MB. This suggests (N, N) coordination of the histidine, which conforms with the earlier observation [23] that histidine, in contrast with the simple amino acids, is mainly coordinated in a histamine-like way.

As a consequence of the ambidentate character of the histidine in the copper(II)–dopa–histidine system, it could be concluded that a species CuAH_2BH containing partially protonated histidine was also formed ($\log \beta_{\text{CuAH}_2\text{BH}} = 44.15$), in which the histidine too is coordinated like an amino acid. This is supported by the stabilization constant: $\Delta \log \beta_{\text{CuAH}_2\text{BH}} = -0.02$; *i.e.* the extent of formation is characteristic of an amino acid–amino acid mixed ligand complex.

3. In the mixed ligand complexes of copper(II) and nickel(II) with dopa and glycylglycine, the stoichiometric composition means that the peptide can be bound to the metal ions only via the amino group and the oxygen donor atom of the peptidamide. The stabilization data too correspond to those for amino acid mixed ligand complexes involving (N, O) coordination.

4. With ATP, the extent of mixed ligand complex formation is enhanced for all of the metal ions. This is reflected by the $\log K_{\text{AH}_2}$ data, which are in general 0.5–0.8 log unit higher than those calculated for the other *B* ligands. Mixed ligand complex formation is presumably favoured by the stacking interaction between the adenine ring of the ATP, bound via the phosphates, and the benzene ring of the dopa, coordinated via the (N, O) donor atoms. This



Scheme 1.

is illustrated in Scheme 1. A similar stability-increasing interaction has been demonstrated by Sigel *et al.* [30] in mixed ligand complexes of ATP, among others with the structurally very similar tryptophan, where the stacking arises between the adenine and the indole ring.

5. As the pH is raised deprotonation of the complexes MAH_2B and, at the same time, rearrangement of dopa coordination into the (O, O) bonding mode takes place. The deprotonation constants in the final column of Table I indicate that this rearrangement is practically complete, though from the pK values of ~ 8 found for the nickel(II) complexes it cannot be excluded that the deprotonation occurs in part on the phenolic hydroxy group not bound to the metal ion, the bonding mode remaining unchanged.

With the assumption of the (O, O) coordination of the catecholamines, we could characterize the metal ion–mixed ligand systems containing dopamine, adrenaline and noradrenaline in terms of the formation of the species MAHB and MAB. In certain cases, formation of the complex MABH_1 was also established. On the basis of the complete

structural rearrangement to the (O, O) bonding mode, the complexes MAHB and MAB of dopa may exhibit a considerable similarity to those for the catecholamine complexes with corresponding compositions. The stability constants found for the (O, O)-coordinated mixed ligand complexes of dopa and the catecholamines, and also the various derived equilibrium data, are listed in Tables II–V. Together with the results of visible spectral investigations on the cobalt(II), nickel(II) and copper(II) systems, the tabulated data allow the following conclusions.

1. The tendencies of the alanine complexes MAHB reveal the validity of the earlier finding [23, 29] that the greater the difference between the $\log K_1/K_2$ values for the parent complexes, the higher the stability of the mixed ligand complex. It is known that formation of the (O, O)-bonded 1:2 complexes of dopa and the catecholamines is hindered [17–21]; their $\log K_{MAH}/K_{MA_2H_2}$ values (2.5–3.1) are significantly larger than the $\log K_{MB}/K_{MB_2}$ values (0.6–1.35) for the (N, O)-coordinated amino acid complexes. Accordingly, the differing equilibrium conditions of the parent complexes may result in enhanced mixed ligand complex formation, without any specific interactions between the ligand pairs. This is supported by the stability data in Table II, for the $-\Delta \log K_{MAHB}$ values agree approximately with the $\log K_{MB}/K_{MB_2}$ values for the alanine parent complexes [17, 18, 21]. Hence, the probabilities that alanine is bound to its own parent complex

MB and to the species MAH are almost the same. Correspondingly, the $\log K_B$ values too agree well with the $\log K_{MB_2}$ values for the alanine parent complexes. At the same time, the $\log K_{AH}$ data characterizing the (O, O) coordination of dopa and the catecholamines in the mixed ligand complexes are substantially larger than the $\log K_{MA_2H_2}$ values for the catecholamines [19 20]. This can be interpreted in terms of the effect of the charge neutralization [19].

It is striking that for manganese(II) only the complex MnAB is formed with alanine. This is due to the low stability of the manganese(II) complexes; the mixed ligand complex is formed only at higher pH, where the ammonium group of the side-chain in ligand A is already deprotonated.

The deprotonation constants pK_{MAHB} of the complexes MAHB do not display an appreciable difference from the microconstants pK_{12} for the dissociation of the ammonium group of the ligands [18–20]. This is confirmation of the (O, O) coordination of the ligands, and also indicates that the B ligand has practically no influence on the deprotonation process of the chain-terminal donor group situated far from it (Table III).

2. The considerable increase in stability of the mixed ligand complexes with histidine as ligand B cannot be explained merely by the differences in the $\log K_1/K_2$ values of the parent complexes. The $-\Delta \log K_{MAHB}$ data are 0.5–0.7 log unit lower

TABLE II. Equilibrium Data on the Mixed Ligand Complexes Formed with Alanine and (O, O) Coordinated A Ligands. $t = 25^\circ\text{C}$, $I = 0.2 \text{ mol/dm}^3 \text{ KCl}$.

		$\log \beta_{MAHB}$	$\Delta \log \beta_{MAHB}$	$-\Delta \log K_{MAHB}$	$\log K_B$	$\log K_{AH}$	pK_{MAHB}
Mn(II)	dopa	—	—	—	—	—	10.14*
	dopm	—	—	—	—	—	10.12*
	nad	—	—	—	—	—	9.61*
	ad	—	—	—	—	—	9.63*
Co(II)	dopa	22.49	0.79	0.85	3.39	7.77	9.66
	dopm	22.70	0.48	0.64	3.60	8.10	—
	nad	21.44	0.31	0.78	3.46	7.78	9.62
	ad	22.04	0.38	0.80	3.44	7.99	10.06
Ni(II)	dopa	24.62	—	0.60	4.72	8.82	9.54
	dopm	23.68	0.66	1.01	4.31	8.00	10.36
	nad	22.84	—	0.74	4.58	8.10	9.32
	ad	23.31	0.80	0.85	4.47	8.18	9.75
Cu(II)	dopa	—	—	—	—	—	—
	dopm	31.06	0.48	1.20	6.84	12.66	10.44
	nad	29.90	0.54	1.14	6.90	12.44	9.34
	ad	30.24	0.58	1.37	6.67	12.39	9.65
Zn(II)	dopa	24.26	0.55	0.53	4.13	9.22	9.65
	dopm	24.39	0.37	0.38	4.18	9.47	10.43
	nad	23.10	0.38	0.58	3.98	9.12	9.37
	ad	23.87	0.32	0.44	4.12	9.50	—

* $\log \beta_{MAB}$ values.

than the $\log K_{MB}/K_{MB_2}$ values for histidine [23]; indeed, for copper(II) the difference is 1.5 log unit. The fact that the mixed ligand complex formation is preferred to such an extent presumably has the following explanation: because of the $d_{\pi}-p_{\pi}$ back-coordination due to the coordination of the imidazolyl-N in the metal-histidine systems, the charge on the metal ion increases and, just as in the copper(II)-histamine-ligand *B* systems [33], this favours (O, O) coordination associated with a ligand \rightarrow metal ion electron transfer.

Similarly, as in the case of alanine, a comparison with the microconstants of the free ligand shows that the deprotonation constants for the complexes MAHB can be ascribed to the deprotonation process of the side-chain ammonium group of the ligand *A*.

3. As shown by earlier results relating to the glycylglycine parent complexes and the copper(II) mixed ligand complexes [24, 25], it may be assumed that the characteristic coordination in the complexes MAHB is via the glycylglycine chain-terminal amino group and the peptidamide carbonyl group. This assumption is confirmed by the data in Table IV. All of the equilibrium data on the stability of the mixed ligand complexes ($\Delta \log \beta_{MAHB}$, $-\Delta \log K_{MAHB}$, $\log K_B$ and $\log K_{AH}$) indicate that the enhanced formation of the complex MAHB cannot be interpreted merely in terms of the differences between the $\log K_1/K_2$ values for the parent complexes. The minor extra stabilization may be explained by the role of the charge neutralization

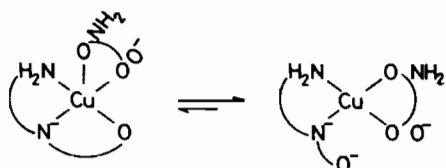
and presumably by the stabilizing effect of the electrostatic attraction between those donor groups of the two ligands which are not coordinated to the metal ion.

The bonding mode in the complexes MAB is not clear-cut. In the cases of cobalt(II), nickel(II) and copper(II), deprotonation of the peptide-NH would be expected [26, 31], with resulting coordination of the chain-terminal amino, the peptid-N and the carboxylate groups, so as to give the (N, N) or (N, N, O) bonding mode. The pK_{MAHB} values for these metal ions in Table IV are somewhat smaller than the values for deprotonation of the side-chain ammonium group in the *A* ligand, which may be indicative of the above-mentioned change in the bonding mode of the glycylglycine (the smaller pK_{MAHB} values obtained in the case of zinc(II) may be explained by the formation of hydroxo complexes [26]). Spectral studies, however, do not confirm a bonding rearrangement. The spectra of the complexes MAB for cobalt(II) and nickel(II) exhibit good agreement with the spectra of the (N, O) (O, O)-coordinated mixed ligand complexes formed with dopa and alanine. At the same time, there are considerable differences from the spectral properties of the glycylglycine parent complexes in which the peptide-N is coordinated. It may be assumed therefore that in the complexes CoAB and NiAB the glycylglycine remains coordinated via the chain-terminal amino and the peptidamide carbonyl groups, and that the slight decreases in their deprotonation constants may

TABLE III. Equilibrium Data on the Mixed Ligand Complexes Formed with Histidine and (O, O) Coordinated *A* Ligands. $t = 25^\circ\text{C}$, $I = 0.2 \text{ mol/dm}^3 \text{ KCl}$.

		$\log \beta_{MAHB}$	$\Delta \log \beta_{MAHB}$	$-\Delta \log K_{MAHB}$	$\log K_B$	$\log K_{AH}$	pK_{MAHB}
Mn(II)	dopa	20.47	—	0.67	2.71	6.81	9.66
	dopm	20.81	0.58	0.66	2.73	7.07	10.62
	nad	19.71	—	0.60	2.78	6.91	9.44
	ad	20.26	—	0.68	2.70	7.07	9.85
Co(II)	dopa	24.96	0.60	0.90	5.86	7.72	9.70
	dopm	25.01	0.53	0.85	5.91	7.89	10.62
	nad	23.87	0.48	0.87	5.89	7.69	9.66
	ad	24.46	0.55	0.90	5.86	7.89	10.02
Ni(II)	dopa	27.23	—	1.19	7.33	8.33	9.66
	dopm	26.80	1.02	1.09	7.43	7.92	10.49
	nad	26.39	—	1.07	7.45	7.77	9.56
	ad	25.71	1.21	0.97	7.55	8.06	9.97
Cu(II)	dopa	33.25	1.55	—	—	12.73	9.60
	dopm	33.57	1.45	0.69	9.35	13.17	10.33
	nad	32.39	1.52	0.64	9.40	12.94	9.40
	ad	32.99	1.50	0.63	9.41	13.13	9.76
Zn(II)	dopa	26.06	0.68	0.48	5.83	9.27	9.69
	dopm	26.34	0.65	0.18	6.13	9.67	10.36
	nad	25.13	0.52	0.30	6.01	9.40	9.56
	ad	25.82	0.61	0.24	6.07	9.70	9.92

be caused by the parallel processes of hydroxo complex formation. In the copper(II) mixed ligand systems, on the other hand, from the decrease in pK_{CuAHB} by 2–2.5 log unit it may be concluded that the peptide-N is coordinated. As a consequence of the structural rearrangement, the two donor group arrangements shown in Scheme 2 may arise.



Scheme 2.

It is conceivable that, besides the phenolate donor groups of the *A* ligand remaining in equatorial sites, the glycylglycine is coordinated merely via two donor atoms, the peptide-N and the amino-N, while the carboxylate group is displaced from the coordination sphere [16]. It may also be assumed that in the complex $CuBH_{-1}$ (in which the amino and peptide nitrogens and the carboxylate oxygen of the glycylglycine occupy three coordination sites in the equatorial plane), the phenolate groups of the *A* ligand are bound in axial–equatorial positions, in a similar way to the parent complex CuA_2H_{-1} of glycylglycine [24] and its mixed ligand complex with 2,2'-bipyridyl [32]. The first assumption is strongly suggested by the observation that, as may be seen in

Fig. 1, ligand *A* (e.g. dopamine) is even capable of displacing glycylglycine from its very stable complex $CuBH_{-1}$. Thus, it is hard to conceive that in its mixed ligand complex glycylglycine could be coordinated via three donor groups in the equatorial plane and that the phenolate groups occupy a much weaker equatorial–axial position. It may be presumed, therefore, that in the case of the (O, O)-coordinated ligand the equilibrium in Scheme 2 is shifted in the direction of the upper arrow.

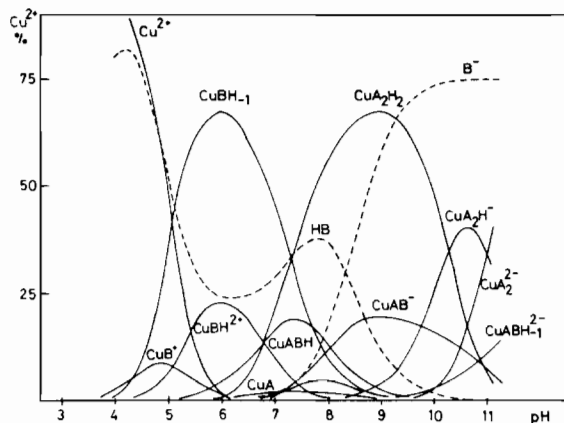


Fig. 1. Concentration-distribution of complexes formed in the copper(II)–dopamine (*A*)–glycylglycine (*B*) system as a function of pH at a metal ion: ligand *A*: ligand *B* ratio of 1:2:1.

TABLE IV. Equilibrium Data on the Mixed Ligand Complexes Formed with Glycylglycine and (O, O) Coordinated *A* Ligands. $t = 25^\circ\text{C}$, $I = 0.2 \text{ mol/dm}^3 \text{ KCl}$.

		$\log \beta_{MAHB}$	$\Delta \log \beta_{MAHB}$	$-\Delta \log K_{MAHB}$	$\log K_B$	$\log K_{AH}$	pK_{MAHB}
Mn(II)	dopa	20.08	–	0.18	2.32	7.46	9.33
	dopm	20.32	0.92	–0.09	2.23	7.82	10.51
	nad	19.62	0.76	–0.15	2.69	8.06	9.43
	ad	19.83	0.62	–0.13	2.67	7.87	9.94
Co(II)	dopa	22.27	1.56	–0.10	3.17	8.72	9.06
	dopm	22.01	0.78	0.16	2.91	8.58	10.05
	nad	21.16	1.02	0.11	3.18	8.67	9.26
	ad	21.51	0.84	0.16	2.91	8.63	10.02
Ni(II)	dopa	23.74	–	0.2	3.84	9.2	9.27
	dopm	22.88	1.12	0.53	3.51	8.48	9.63
	nad	21.84	–	0.46	3.58	8.38	8.98
	ad	22.54	1.38	0.34	3.70	8.69	9.35
Cu(II)	dopa	29.40	–	–	–	13.36	7.26
	dopm	29.04	–	0.74	4.82	13.12	7.96
	nad	28.04	–	0.52	5.04	13.06	7.80
	ad	28.45	–	0.68	4.88	13.08	8.13
Zn(II)	dopa	23.46	0.47	0.32	3.23	9.43	8.58
	dopm	23.56	0.77	0.20	3.35	9.65	8.94
	nad	22.29	0.57	0.38	3.17	9.32	8.45
	ad	22.95	0.62	0.35	3.20	9.59	9.06

TABLE V. Equilibrium Data on the Mixed Ligand Complexes Formed with ATP and (O, O) Coordinated *A* Ligands. $t = 25^\circ\text{C}$, $I = 0.2 \text{ mol/dm}^3 \text{ KCl}$.

		$\log \beta_{\text{MAHB}}$	$\Delta \log \beta_{\text{MAHB}}$	$\log \beta_{\text{MAB}}$	$\text{p}K_{\text{MAHB}}$
Co(II)	dopa	—	—	12.90	—
Ni(II)	dopa	22.8	-0.3	14.23	8.57
	dopm	21.8	0.0	—	—
	nad	20.5	-0.1	—	—
	ad	21.3	0.1	—	—
Cu(II)	dopa	—	—	—	—
	dopm	27.59	0.04	—	—
	nad	26.25	-0.05	—	—
	ad	26.96	0.06	—	—
Zn(II)	dopa	23.0	-0.3	14.35	8.65
	dopm	23.6	0.1	—	—
	nad	23.4	-0.05	—	—
	ad	23.2	0.0	—	—

In the copper(II) mixed ligand systems, as a result of a further deprotonation, the species CuABH_{-1} is also formed. Concerning their tendencies, the constants $\text{p}K_{\text{CuAB}}$ (dopa: 9.7; dopm: 10.7; nad: 9.9; ad: 10.1) agree well with the microconstants for the ammonium group of the *A* ligands [18, 20]; thus, the bonding mode is unchanged, and the $\text{p}K_{\text{CuAB}}$ values can be attributed to the deprotonation processes of the side-chain ammonium groups.

4. With ATP as ligand *B* the formation of mixed ligand complexes (primarily MAHB) was concluded only in the cases of copper(II), nickel(II) and zinc(II). In the metal–catecholamine–ATP systems, the formation of the mixed ligand complex practically corresponded to the statistical case. Because of the (O, O) coordination of both ligands, this is not in contradiction to expectation. The slight degree of destabilization observed in the metal–dopa–ATP systems can presumably be interpreted to indicate that the stacking interaction with the adenine ring of the ATP ceases with the rearrangement of the dopa to (O, O) coordination. The steric hindrance due to the size of the molecules increases, which does not favour formation of this species.

With manganese(II) and cobalt(II) the pH-metric method did not prove sufficiently sensitive to demonstrate mixed ligand complex formation: in these systems there is considerable separation in the pH interval of formation of the parent complexes of ATP and of dopa and the catecholamines. Accordingly, in the pH range in which mixed ligand complexes are formed, the ATP is present in fully deprotonated form, and its coordination is therefore not accompanied by a direct pH effect. At the same time, spectral measurements at higher pH point to an interaction between the ligands bonded to the metal ion in the cobalt(II)–dopa–ATP system. As may be seen in Fig. 2, the spectrum of the cobalt(II)–dopa–

ATP system differs considerably from those of the parent complexes, which cannot be explained without the assumption of mixed ligand complex formation. This assumption is supported by the NMR studies by Granot and Fiat [11] in cobalt(II)–dopamine–ATP systems.

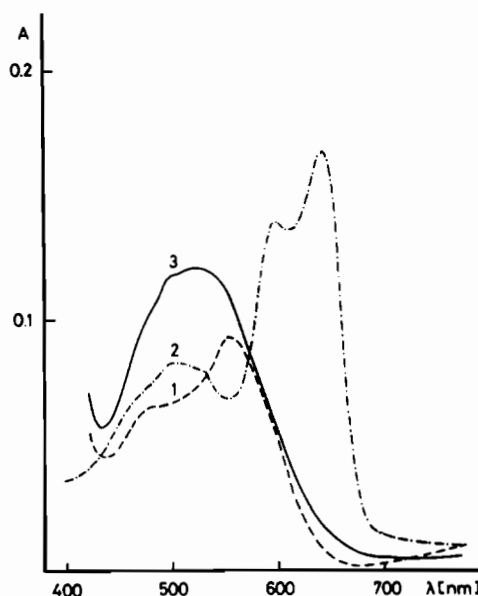


Fig. 2. Spectral behaviour of cobalt(II) complexes. 1. (---): Co(II)–dopa 1:2, pH 10.8; 2. (- · -): Co(II)–ATP 1:2, pH 10.9; 3. (—): Co(II)–dopa–ATP 1:1:1, pH 10.9.

5. As was mentioned above, the stability of the mixed complexes tends to be higher the greater the difference between the $\log K_1/K_2$ values of the parent complexes. Correspondingly, if the distribution constant $\log K_M$ of the mixed ligand complexes is plotted as a function of the difference between the

$\log K_1/K_2$ values of the parent complexes ($\delta \log K_1/K_2$), the result is that shown in Fig. 3.

It is clear from the Figure that, with a few exceptions, $\log K_M$ generally rises with increase of $\delta \log K_1/K_2$. Appreciable differences from this tendency appear only in the copper(II)–(O, O)-coordinated ligand *A*–histidine (A), the nickel(II)–(O, O)-coordinated ligand *A*–histidine (B), the metal ion–(N, O)-coordinated dopa–ATP (C) and the metal ion–(O, O)-coordinated dopa–ATP (D) systems. This is a result of special effects arising in the stability of the mixed ligand complex, due to the nature of the ligand pairs. Thus, in cases A and B back-coordination occurs through coordination of the aromatic nitrogen; in case C there is a stabilizing effect from the stacking interaction between the ligands; and in case D there is destabilization because of the cessation of this interaction.

6. The data in Tables II–V reveal that (O, O)-coordinated mixed ligand complexes are not formed between copper(II)–dopa and alanine or ATP as ligand *B*. Evidence of such species was found only with histidine and glycylglycine. The equilibrium results are supported by the spectral studies. Figure 4 depicts spectra of the parent and mixed ligand systems of copper(II)–dopa at pH ~ 11.0 .

This particular behaviour stems from the marked ambidentate character of dopa, and its tendency to form the dimeric species Cu_2A_2 with the copper(II) ion [17]. The *B* ligands alanine and ATP, which can bind only as strongly as the dopa in the (N, O) bonding mode, are not able to displace one of the (N, O)-coordinated dopa molecules from the dimeric complex, and consequently their spectra practically coincide with the spectrum of the copper(II)–dopa system. At the same time, the coordination of the peptide nitrogen of glycylglycine, or the aromatic nitrogen of histidine, is favoured in comparison with the (O, O) donor atoms; this results in the partial or practically complete decomposition of the dimer Cu_2A_2 , and the formation of the mixed ligand complex CuAB .

An essentially similar situation arises in the copper(II)–adrenaline–ligand *B* and the copper(II)–noradrenaline–ligand *B* systems too. In an earlier publication [20] it was mentioned that the ambidentate character of adrenaline and noradrenaline may be manifested in the case of the copper(II) ion at high pH; this shows up in the formation of a polymeric species involving participation of the donor atoms of the side-chain. As shown by Fig. 5, even in the presence of alanine and ATP as the *B* ligand, which are capable of forming bonds of similar strength, it is probable that only the polynuclear species involving the side-chain donor groups of the *A* ligand is formed in the mixed ligand systems.

At the same time, because of the preferred nature of mixed ligand complex formation in the presence of

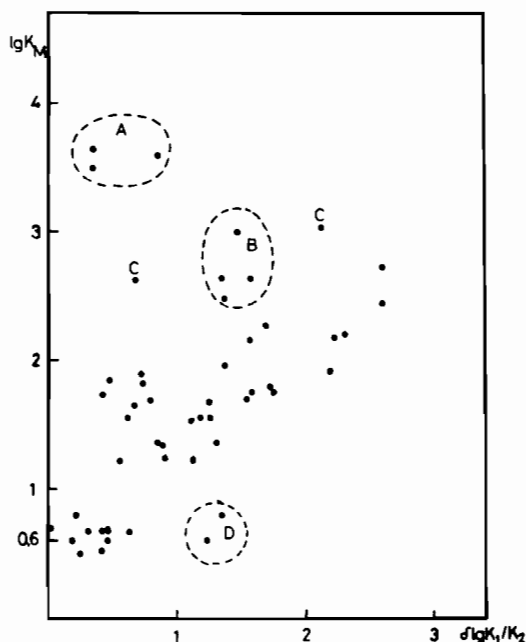


Fig. 3. Variation of $\log K_M$ values of mixed ligand complexes as a function of the difference between the $\log K_1/K_2$ values for the parent complexes.

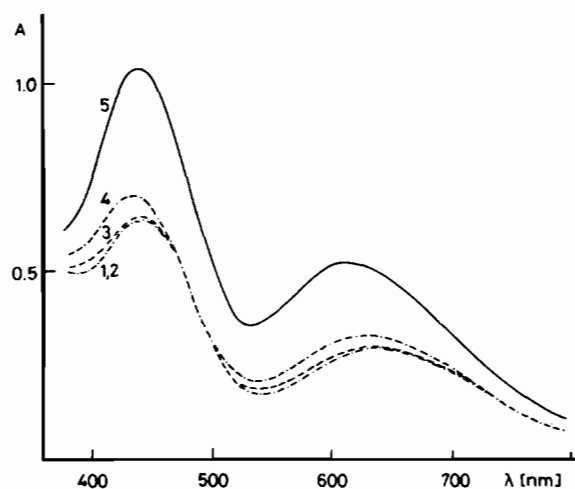


Fig. 4. Spectra of the copper(II)–dopa–ligand *B* systems at a metal ion:ligand:ligand ratio of 1:1:1, at pH 11.0. 1. (· · ·): Cu(II)–dopa 1:1; 2. (- · -): Cu(II)–dopa–ala; 3. (- - -): Cu(II)–dopa–ATP; 4. (- · · ·): Cu(II)–dopa–glygly; 5. (—): Cu(II)–dopa–his.

histidine and glycylglycine, the ambidentate character of adrenaline and noradrenaline can no longer be displayed. Accordingly, a considerable spectral change may be observed relative to the parent complex.

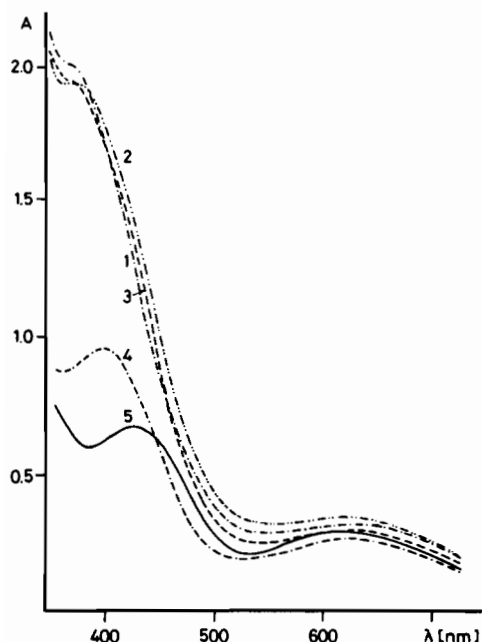


Fig. 5. Spectra of the copper(II)-adrenaline-ligand *B* systems. 1. (· · ·): Cu(II)-ad 1:1; 2. (- · - ·): Cu(II)-ad-ala; 3. (- - -): Cu(II)-ad-ATP; 4. (- - - -): Cu(II)-ad-glygly; 5. (—): Cu(II)-ad-his.

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