Structure Dependence of ESR Spectra of Cu(II) Complexes of Aliphatic and Tryptophan Containing Dipeptides and ATP§

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Abstract

Spectra and stability constants of binary and ternary complexes were derived from ESR† spectra of aqueous solutions of Cu(2+), ATP† and the dipeptides gly-L-trp and L-trp-gly. The stability of binary as well as ternary complexes is only slightly influenced by the tryptophan side chain.

Parameters affecting the shape of an ESR spectum of a Cu(II) complex in solution are discussed in detail. A comparison of the ESR spectra of the several complex species reveals the different influence of electronic and mobility contributions of the tryptophan side chain depending on the position of tryptophan in the dipeptide. In ternary complexes, the spectral shape is determined by the stronger binding ligand.

Introduction

The investigation of the interaction of peptides with metal ions and ATP is stimulated for several reasons. One is the use of the peptide as a model compound for proteins to get information about the parameters influencing protein–metal–ATP interactions which are very important in nature. Although the results derived from such model systems yield only limited information about the system itself [1] it is useful to know the behaviour of the basic functional groups in order to classify an observed complex formation as a 'specific' or 'unspecific' interaction of the particular molecule. Further, investigations using such model compounds allow the study of effects in a well characterizable system. Such an effect concerns the parameters affecting the shape of solution ESR spectra of copper complexes of biologically important molecules. This knowledge should be useful for further work using more complicated systems.

Copper was chosen as metal ion due to its excellent properties for ESR measurements, and because it is one of the most important metal ions in biological systems, forming the active center of many important oxidases [2]. It is no surprise, therefore, that copper complexes involved in the controlled transport of this metal ion into the cell also act as growth factors [3, 4]. Apart from its role in oxidases, copper is linked to the hormone system by mechanisms which are as yet poorly understood [2, 5]. One report related to this problem is that of the specific release of the luteinizing hormone releasing hormone (LHRH) from isolated hypothalamic granules by the combination of ATP and Cu(II) [5]. The question of how such an effect can be understood on a molecular basis gives another motivation to study the complex formation of ATP and Cu(II) with other ligands.

In a previous work we reported the complex formation of Cu(II) with ATP and aliphatic dipeptides [6]. Tryptophan containing dipeptides were chosen for the present study in order to determine the influence of a possible 'stacking' interaction of the aromatic moieties of ATP and tryptophan on the stability of the complexes, especially since such an interaction is believed to be relevant in ternary complexes of ATP [7–10].

As shown in earlier papers [6, 11–13], the quantitative evaluation of solution ESR spectra is especially useful to investigate the complex formation of Cu(II) in aqueous solution. The outstanding features of this method are: (i) The observation of the metal in the complex formation in contrast to the study of the influence of the metal ion on ligand properties as in most other methods. (ii) Several independent parameters influence the shape of the ESR spectra of the complexes in solution giving spectra which are distinct and characteristic for almost every copper complex occurring in solution.

As the quantitative evaluation of spectra is necessary to obtain ESR spectra of copper species in solution, and as few works using this method have been published up to now [6, 11–14], very few ESR spectra of copper species in solution are known. In this paper, parameters influencing the spectral shape are discussed on the basis of this limited knowledge.

§ Adenosine-5'-triphosphate.

†Abbreviations: ESR: electron spin resonance; ATP: adenosine-5'triphosphate; gly-L-trp: N-glycyl-L-tryptophan; L-trp-gly: N-L-tryptophyl-L-glycine; gly-L-pro: 1-glycyl-L-proline; gly-gly: N-glycyl-glycine.

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Experimental

Materials

Copper was used as $CuCl_2 \cdot 2H_2O$ analytical grade (Mallinckrodt), the dipeptides and ATP (as Na_2H_2-ATP$\cdot 3H_2O$ 'puriss.') were obtained from Serva. NaOH and HCl used for titrations were 'Titrisol' products (Merck).

Potentiometric Titrations and ESR Experiments

Stock solutions of ATP and the dipeptides were freshly prepared daily. The dissolved $Na_2H_2ATP \cdot 3H_2O$ was titrated rapidly to the equivalence point to form an ATP(4–) solution. The content of the stock solutions was determined by potentiometric titration to check the water content of the peptides and the ATP salt. After mixing of the ligand and metal stock solutions with an appropriate amount of HCl the titration was carried out immediately to prevent errors due to dephosphorylation [15]. 3 cm^3 solution were titrated at 20 ± 0.2 °C using a 0.2 cm^3 burette (Gilmont). The copper concentration was always 0.00490 *M*, the ATP and/or peptide concentrations were varied independently from a ratio of 1:1 to 3:1 to Cu(2+). For the ESR measurements 8 to 15 samples per titration were taken from the titration vessel by a micropipette, immediately frozen in liquid nitrogen and rapidly defrosted just before the ESR measurement. The spectra were recorded at 20 °C within four minutes.

Apparatus

For the pH measurements a Schott pH-meter CG 803 and an Ingold electrode 104051393 calibrated with standard buffer solutions (Merck) were used. The ESR spectra were recorded on a Varian E 104 spectrometer (calibrated microwave frequency = 9.907 GHz) in tubes with a much smaller diameter than standard ESR tubes to reduce the dielectric losses caused by water.

Calculations

All calculations were carried out using the CDC Cyber 74 computer of the University of Innsbruck. 24 to 35 points per potentiometric titration curve and 400 points per ESR-spectrum (digitized with a Summagraphics ID 2000, resolution 0.1 mm) were included. Details of the computational procedure are given in ref. 12.

Method

The ESR titration method was used as described in ref. 11. The main differences from most other ESR investigations in this field are the recording of spectra at room temperature and the evaluation focused on quantitative data (peak heights, stability constants calculated therefrom). The basic assumption used in the interpretation is that the amplitude of the ESR signal is proportional to the concentration of the complex in solution. The spectrum recorded for a metal/ligand solution is assumed to be the direct sum of the spectra of all the species present. Although it is just this sum of the spectra which is recorded and although the stoichiometry of the occurring complex species as well as their stability constants and their ESR spectra are not known, all these quantities can be evaluated from a series of spectra because the species concentration as a function of pH and metal to ligand ratio must obey the law of mass action (see ref. 12 for details).

Such a calculation, which is in principle independent of the type of spectra used, can successfully be performed with ESR spectra because of their high information content, *i.e.* because the spectra of the different complex species show a distinct, characteristic shape.

For non-macromolecular complexes containing two copper atoms a zero ESR spectrum is generally observed in aqueous solution at room temperature [16 17], known exceptions being copper complexes of N-β-alanyl-L-histidine ('carnosine', [18]) and 2,2′-bisaminomethyl-1,3-propane-diamine [19].

Results and Discussion

Complex Formation of Tryptophan Containing Dipeptides with Cu(II) and ATP

Gly-l-trp and L-trp-gly show the same copper species as gly-gly [12] in solution, indicating that the tryptophan side chain does not participate in complex formation. This result is consistent with X-ray studies of gly-L-trp-aquocomplexes [20, 21]. Table I shows a comparison of the stability constants of binary and ternary complexes of tryptophan containing dipeptides with gly-gly (for pK-values and equilibria of ATP–Cu(II) species see ref. 6). It is seen from the table that the influence of the tryptophan side chain on the stability of the complexes is low and within the limits of accuracy for the stability constants. In particular, the contribution of the tryptophan side chain to the stability of the ternary complexes with ATP is not significantly higher than the contribution to the stability of the dipeptide–Cu(II) complexes. This agrees with results for ATP–Cu–tryptophan complexes, where the contribution of the stacking interaction to the stability of the ternary complex is also found to be small [22, 23].

Parameters Affecting the Shape of X-band ESR Spectra of Copper Complexes in Solution at Room Temperature

Figure 1 shows three spectra which demonstrate the influence of some parameters on the shape of the spectra of the complexes. The solvated copper-(2+)–ion shows no hyperfine structure, because the

TABLE I. Comparison of the Stability of Binary and Ternary Complexes of Tryptophan Containing Dipeptides with the Corresponding glygly Complexes.

No.	Equilibrium	gly-gly	L-trp-gly	gly-L-trp
1	PE(O) + H(+) → PEH(+)	−3.18 ±0.03	−3.27 ±0.07	−3.21 ±0.05
2	PE(O) → PE(−) + H(+)	8.25 ±0.03	7.95 ±0.05	8.29 ±0.03
3	PE(−) + Cu(2+) → (PE)Cu(+)	−5.91 ±0.25	−5.29 ±0.45	−5.76 ±0.75
4	PE(−) + Cu(2+) → (PE)Cu(O) + H(+)	−1.57 ±0.14	−1.49 ±0.15	−1.80 ±0.19
5	PE(−) + Cu(2+) + H_2O → (PE)Cu(OH)(−) + 2H(+)	8.24 ±0.23	8.04 ±0.13	7.80 ±0.15
6	2PE(−) + 2Cu(2+) + H_2O → $(PE)_2Cu_2(OH)(-)$ + 3H(+)	4.08 ±0.25	4.01 ±0.20	3.41 ±0.30
7	2PE(−) + Cu(2+) → $(PE)_2Cu(-)$ + H(+)	−4.40 ±0.30	−4.85 ±0.25	−5.10 ±0.35
8	PE(−) + ATP(4−) + Cu(2+) → (PE)Cu(ATP)(3−)	−10.57 ±0.39	−10.56 ±0.29	−10.93 ±0.33
9	PE(−) + ATP(4−) + Cu(2+) → (PE)Cu(ATP)(4−) + H(+)	−3.67 ±0.45	−4.12 ±0.27	−4.19 ±0.16

The deviations denote the values by which the pK-value has to be changed to yield a doubled sum of squared errors in the comparison of calculated to measured spectra.

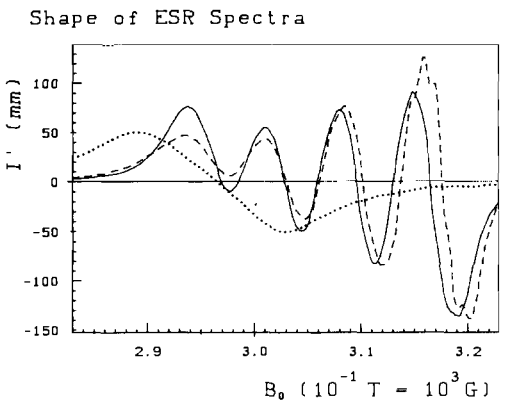

Fig. 1. The concentration of all species is 0.00490 *M*, ··· hydrated Cu(2+), —— (gly-gly)Cu(0), − − − (gly-L-trp)-Cu(O).

distorted octahedral coordination sphere is formed by six water molecules, enabling a fast reorientation of the distortion axis (dynamic Jahn–Teller-effect [14]). A comparison of the spectra of the complexes (gly-gly)Cu(O) and (gly-L-trp)Cu(O) reveals three characteristic differences (Fig. 1).

a) The spectrum of the gly-L-trp complex is more asymmetric than the corresponding gly-gly complex. This is interpreted as higher mobility of the gly-gly complex, leading to an increased spin rotational contribution to the relaxation. This contribution is independent of the nuclear spin quantum number m_I and leads therefore to more symmetric spectra [14, 24]. In contrast to that, the anisotropic dipolar and g-tensor interaction contribution to the relaxation depends on the value of m_I and leads to an anisotropic shape of the spectrum [14, 24].

b) The gly-L-trp complex shows an additional splitting of the peaks at high field, which is caused by interaction with nitrogen atoms of the ligand [12 25]. The resolution of this additional splitting is also mobility dependent [25].

c) The gly-L-trp complex shows a higher hyperfine coupling constant A than the gly-gly complex, which can be attributed to an electronic influence of the tryptophan side chain. Increased A values in copper complexes were observed with increasing substitution in amines [26] and with increasing aliphatic side chains in dipeptides [12].

Dependence of the Spectral Shape on the Position of Tryptophan and Aliphatic Side Chains in Dipeptides

The mobility of the copper complexes, which influences the asymmetry of the spectral shape, can be assumed not to depend much on the position of a bulky side chain in the complex. For the electronic contribution, which results in an increased A value, two cases can be distinguished:

(i) If copper is bound to the deprotonated peptide nitrogen (equilibria 4, 5, 6, 7, 9; Table I) a stronger influence is exerted when the side chain is in the carboxyterminal position of the dipeptide.

(ii) If copper is not bound to the peptide nitrogen (the amino end is then the coordination site), an influence is visible whenever the side chain is in the aminoterminal position (equilibria 3, 7, 8; Table I).

Figure 2 shows an example of the first case (i), the (peptide)Cu(OH)(−) complexes. Compared to the gly-gly complex, the spectra of both tryptophan containing peptides show the influence of the lower mobility in their more asymmetric line and the better resolution of the coupling with ligand nitrogen atoms. The increased A value, which shifts the feature at the highest field to the right, is only observed in the gly-L-trp complex, because in the species (peptide)Cu(OH)(−) copper is bound to the deprotonated peptide nitrogen (Fig. 2).

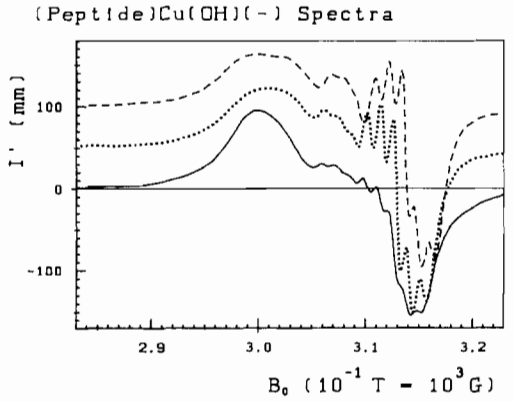

Fig. 2. The concentration of all species is 0.00490 *M*, —— gly-gly, − − − gly-L-trp (shifted 100 units in positive I' direction). . . . L-trp-gly (shifted 50 units in positive I' direction).

Figure 3 shows the spectra of the $(peptide)_2Cu(-)$ complexes. For this species, both cases (i) and (ii) are relevant, because one peptide molecule is bound with deprotonated, the other one with protonated peptide nitrogen [27, 28].

The same trends as outlined here for copper complexes of tryptophan containing dipeptides are also found for the corresponding complexes of aliphatic dipeptides (Table II).

One of the Two Ligands of a Ternary Complex Determines the Shape of the ESR Spectrum in Solution

Figure 4 shows a comparison of the spectra of $(ATP)_2Cu(OH)_n(9-)$ and (gly-L-pro)Cu(ATP)(5−).

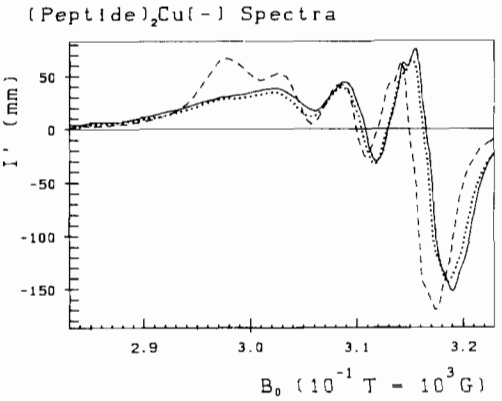

Fig. 3. The concentration of all species is 0.00490 *M*, − − − gly-gly, · · · gly-L-trp, —— L-trp-gly.

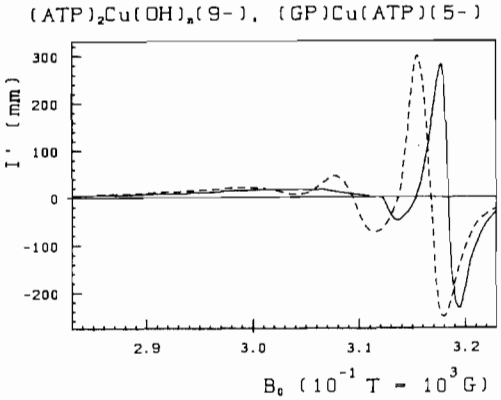

Fig. 4. The concentration of both species is 0.00490 *M*, —— $(ATP)_2Cu(OH)_n(9-)$, − − − (gly-L-pro)Cu(ATP)(5−).

Clearly, one bound ATP molecule determines the spectral shape, whereas the second ligand shows only a minor influence. Figure 5 shows a comparison of the spectra of the (gly-gly)Cu(ATP)(4−) and the $(gly\text{-}gly)_2Cu(-)$ complex. Here the gly-gly molecule, which is bound *via* the deprotonated peptide nitrogen, determines the spectral shape.

It is straightforward to assume that the molecule which is bound more strongly to the Cu atom is the spectral shape determining ligand. As a 'first order' approximation it can be assumed that in the ternary complex the ligand, which itself forms stronger binary complexes with the metal ion, is also bound

TABLE II. Selected A_{Cu} Values (in G) of Copper Complexes of Aliphatic Dipeptides (Taken from ref. 12).

Dipeptide	(PE)Cu(+)	(PE)Cu(O)	(PE)Cu(OH)(−)	$(PE)_2Cu(-)$
gly-gly	52	69	41	57
gly-leu	52	73	47	64
leu-gly	55	69	42	62
leu-leu	–	73	49	65
'case' (see text)	(ii)	(i)	(i)	(i) + (ii)

'PE' denotes the neutral peptide molecule.

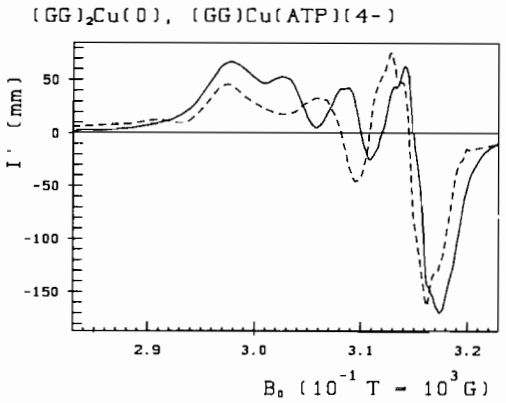

Fig. 5. The concentration of both species is 0.00490 *M*, – – – (gly-gly)Cu(ATP)(4–), —— $(gly\text{-}gly)_2Cu(-)$.

more strongly to the copper atom. This view is supported by the comparison of the effective stability of the compounds (Fig. 6). As the deprotonation constants of the ribose hydroxyls of ATP as well as of the peptide proton cannot be determined with sufficient accuracy, single equilibrium constants cannot show which of the two ligands is the stronger binding one. Such a comparison is possible using the effective stability constants. The effective stability constant is defined to be the stability constant of the equilibrium M + A = MA (where M is the metal ion and A is a hypothetical compound) to form a complex as stable as all the equilibria of the real ligand, *i.e.* to yield the same final concentration of free metal ion. The two complexes compared in Fig. 4 occur at pH > 10 (6, 11). As can be seen from Fig. 6, at pH above 10 ATP binds copper much more strongly than gly-L-pro and ATP determines the shape of the ESR spectrum of the ternary complex (gly-L-pro)Cu(ATP)(5–) (Fig. 4). The complexes compared in Fig. 5, however, occur at pH ~ 8 (6), where gly-gly binds copper much more strongly than ATP (Fig. 6). In the two spectra of Fig. 5 gly-gly determines the spectral shape.

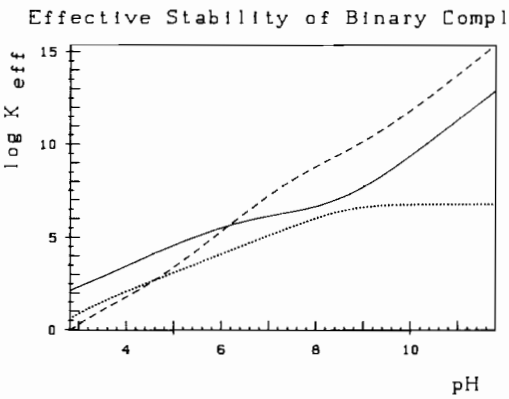

Fig. 6. The concentration of the metal and all ligands is 0.00500 *M*, —— ATP, – – – gly-gly, · · · gly-L-pro.

Conclusions

As mentioned above, the present discussion about the shape of ESR spectra of copper complexes in aqueous solution is based on the comparatively few available ESR spectra of copper complexes in solution. Further work must show whether the conclusions drawn about the shape of ESR spectra are valid for ATP, dipeptide Cu(II) complexes only or can be generalized. In the latter case, the method used in this paper will prove to be a valuable tool for investigations of complex structures in solution and for the classification of metal–ligand bonds in ternary or even more complicated complexes.

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References

1 H. C. Freeman in 'Bioinorganic Chemistry', Vols 1 and 2, edited by G. L. Eichhorn, p. 159, Elsevier, Amsterdam, 1973.
2 Z. A. Karcioglu and R. M. Sarper, 'Zinc and Copper in Medicine', Charles C. Thomas, Springfield, Illinois, USA, 1980.
3 L. Pickart, *Lymphokines, 8*, 425 (1983).
4 L. Pickart in 'Chemistry and Biochemistry of Amino Acids, Peptides and Proteins', Vol. 6, ed. by B. Weinstein, p. 75, Marcel Dekker Inc., New York and Basel, 1982.
5 G. H. Burrows and A. Barnea, *Endocrinology, 110* (4), 1456 (1982).
6 E. R. Werner and B. M. Rode, *Inorg. Chim. Acta, 91* (3), 217 (1984).
7 H. Sigel, *Angewandte Chemie, 87* (11), 391 (1975).
8 H. Sigel, B. E. Fischer and B. Prijs, *J. Am. Chem. Soc., 99* (13), 4489 (1977).
9 P. Mitchell and H. Sigel, *J. Am. Chem. Soc., 100* (5), 1564 (1978).
10 P. Mitchell, B. Prijs and H. Sigel, *Helv. Chim. Acta, 62* (6), 1723 (1979).
11 E. R. Werner and B. M. Rode, *Inorg. Chim. Acta, 80*, 39 (1983).
12 W. S. Kittl and B. M. Rode, *J. Chem. Soc. Dalton Trans., 3*, 409 (1983).
13 M. J. A. Rainer and B. M. Rode, *Inorg. Chim. Acta, 92* (1), 1 (1984).
14 M. Noack, G. F. Kokoszka and G. Gordon, *J. Chem. Phys., 54* (3), 1342 (1971).
15 D. H. Bouisson and H. Sigel, *Biochim. Biophys. Acta, 343*, 43 (1974).
16 S. H. Laurie, T. Lund and J. B. Raynor, *J. Chem. Soc. Dalton Trans., 14*, 1389 (1975).
17 P. Kroneck, C. Naumann and P. Hemmerich, *Inorg. Nucl. Chem. Lett., 7*, 695 (1971).
18 C. E. Brown, W. E. Antholine and W. Froncisz, *J. Chem. Soc. Dalton Trans.*, 590 (1980).
19 T. D. Smith and A. E. Martell, *J. Am. Chem. Soc., 94* (9), 3029 (1972).

20 B. M. Hursthouse, S. S. A. Jayaweera, G. H. W. Milburn and A. Quick, *J. Chem. Soc. Dalton Trans.*, 2569 (1975).
21 B. M. Hursthouse, S. A. A. Jayaweera, G. H. W. Milburn and A. Quick, *Chem. Commun.*, 207 (1971).
22 H. Sigel and C. F. Naumann, *J. Am. Chem. Soc.*, *98* (3), 730 (1976).
23 G. Arena, R. Cali, V. Cucinotta, S. Musumeci, E. Rizarelli and S. Sammartano, *J. Chem. Soc. Dalton Trans.*, 1271 (1983).
24 R. Poupko and Z. Luz, *J. Chem. Phys.*, *57* (8), 3311 (1972).
25 D. C. Gould and H. S. Mason, *Biochemistry*, *6*, 801 (1967).
26 T. C. Chiang, *J. Chem. Phys.*, *48* (4), 1814 (1968).
27 G. Brookes and D. L. Pettit, *J. Chem. Soc. Dalton Trans.*, *20*, 2106 (1975).
28 I. Nagypal and A. Gergely, *J. Chem. Soc. Dalton Trans.*, *11*, 1104 (1972).