Proton and Copper(II) Complexes of the Pentapeptide Thymopoietin$_{(32-36)}$

IMRE SÓVÁGÓ, TAMÁS KISS and ARTHUR GERGELY

Institute of Inorganic and Analytical Chemistry, Lajos Kossuth University, 4010 Debrecen, Hungary

Received June 20, 1984

The pentapeptide Arg–Lys–Asp–Val–Tyr (TP5) is a fragment of thymopoietin, which is a 49 amino acid polypeptide hormone of the thymus. It has been shown that thymopoietin influences the differentiation of various cells. The synthetic fragment corresponding to positions 32–36 in the peptide chain is also biologically active [1], and TP5 therefore appears to be the active centre of thymopoietin.

Concerning the coordination chemistry of TP5, only its lanthanide complexes have so far been studied [2]. Lanthanides serve as useful analogues of calcium and permit fluorescence and NMR characterization of the metal complexes. It has been found that the arginine guanidino and aspartate carboxylate groups take part in coordination with lanthanide(III) ions. On the other hand, it is well known that certain transition metal ions (palladium(II), copper(II), nickel(II), *etc.*) induce the deprotonation of amide groups in pentaglycine, the coordination occurring exclusively via N-donors [3]. This suggests that complex formation with transition metal ions can significantly change the solution conformation of TP5 [4], which may well be reflected in an altered biological activity. Accordingly it seemed worthwhile to study complex formation between TP5 and transition metal ions. Equilibrium analysis of these systems requires a knowledge of the acid–base properties of the ligand. Because of the large number of functional groups in TP5, the dissociation processes of the protons are not separated, and various structures can correspond to the same stoichiometric composition.

In this work the pK values of TP5 and the protonation microconstants for the lysyl amino and tyrosyl phenolate groups will be discussed together with the possible complex formation reactions with copper(II).

Experimental

THe acetate form of TP5 was obtained from Gedeon Richter Ltd., Budapest. Solutions with a concentration of 10^{-3} mol dm^{-3} in a volume of 10 cm^3 were used for pH-metric titrations. Copper(II) complexes were investigated at metal ion–ligand ratios of 1:1, 1:2 and 1:3. All pH-metric measurements were made at 298 K, at a constant ionic strength of 0.2 mol dm^{-3} KCl. The details of the pH-metric method and the calculations of stability constants were reported earlier [5]. For the determination of microconstants the UV absorption band of the phenolate group was measured as a function of pH with a Beckman Acta MIV double-beam recording spectrophotometer.

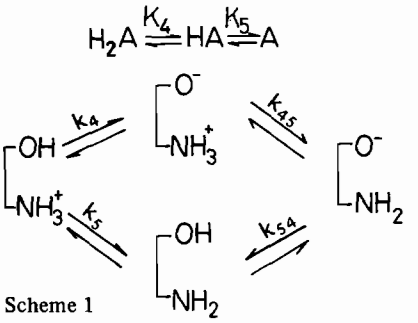
Results and Discussion

The pentapeptide Arg–Lys–Asp–Val–Tyr contains five dissociable protons in the measurable pH range. From a consideration of the pK values listed in Table I, it can be suggested that at least two sets of deprotonation processes overlap each other. Deprotonation of the aspartyl and tyrosyl carboxylate groups takes place at around pH 2 to 4, which means that the pK values cannot be separated. It should also be noted that deprotonation of acetic acid occurs in this pH range too, and in the calculations this was taken into account with a pK value reported in the literature [6].

TABLE I. The Dissociation Macro- and Microconstants of TP5 (I = 0.2 mol dm^{-3} KCl, T = 298 K).

		Acidic group
pK_1	10.38	lysyl amino and
pK_2	9.75	tyrosyl phenolic
pK_3	7.20	arginyl amino
pK_4	3.25	aspartyl and
pK_5	1.92	tyrosyl carboxylate
pk_4 = pk_{54}	9.99	tyrosyl phenolic
pk_5 = pk_{45}	10.20	lysyl amino

It is a reasonable assumption that deprotonation of the arginyl amino group is separated almost completely from the overlapping acid–base reactions of the lysyl amino and tyrosyl OH groups, and thus the microconstants for the latter two groups can be determined from a combined spectrophotometric and pH-metric measurement [7]. The dissociation macro- and microprocesses of this bifunctional residue of TP5 can be described by scheme 1:



Scheme 1

0020-1693/84/$3.00

where K_4 and K_5 are macroprotonation constants, while k_5, k_{45} and k_4, k_{54} are the microconstants for the dissociation of the amino and phenolate groups, respectively. Since the molar absorptivities of the protonated and deprotonated forms of the phenolate group are different, the degree of dissociation can be monitored specifically. The details of the method have been published elsewhere [7]. The microconstants obtained are listed in Table I.

It can be seen from Table I that $pk_4 = pk_{54}$ and $pk_5 = pk_{45}$. In other words, this means that the acidic groups undergo dissociation simultaneously, but the degree of protonation of one group does not affect the acid–base properties of the other group. This result is not surprising if the relatively large distance between the lysyl and tyrosyl residues in the molecule is taken into account. The independence of the dissociation processes of the lysyl amino and tyrosyl phenolate groups is supported by the literature data: the protonation constant of the phenolate group in glycyltyrosine is $pK = 9.96$ [8], and that of the end-chain amino group in lysine is $pK = 10.21$ [9]. These values are in very good agreement with the data for TP5, and suggest that the dissociation processes of the various acidic groups in TP5 do not affect each other.

The data from pH-metric titrations on copper(II)–TP5 systems at different ratios revealed that complex formation takes place in the pH range 4 to 6. At a ratio of 1:1 altogether five equivalents of proton/ligand can be titrated up to pH 6. There is an inflexion point at around pH 7.5, and two further acidic groups can be titrated in basic solution. At higher ligand–metal ion ratios, the excess of ligand is measured as free ligand, suggesting the formation of 1:1 complexes exclusively. Computer analysis of these pH-metric titration curves leads to the results in Table II. The corresponding concentration–distribution curves are depicted in Fig. 1.

It can be seen from Table II and Fig. 1 that only 1:1 complexes are formed in the copper(II)–TP5 system. At lower pH (<5) various protonated complexes are present, in which the coordination occurs via the N-terminal amino and neighbouring carbonyl groups, as for other peptides [3], while the lysyl

TABLE II. Stability Constants of the Species Formed in the Copper(II)–TP5 System (I = 0.2 mol dm^{-3} KCl, T = 298 K):

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

Species	$\log\beta_{pqr}$
$CuAH_3^{2+}$	30.88
$CuAH_2^{+}$	27.81
CuA^{-}	17.71
$CuAH_{-1}^{2-}$	9.03
$CuAH_{-2}^{3-}$	−1.00

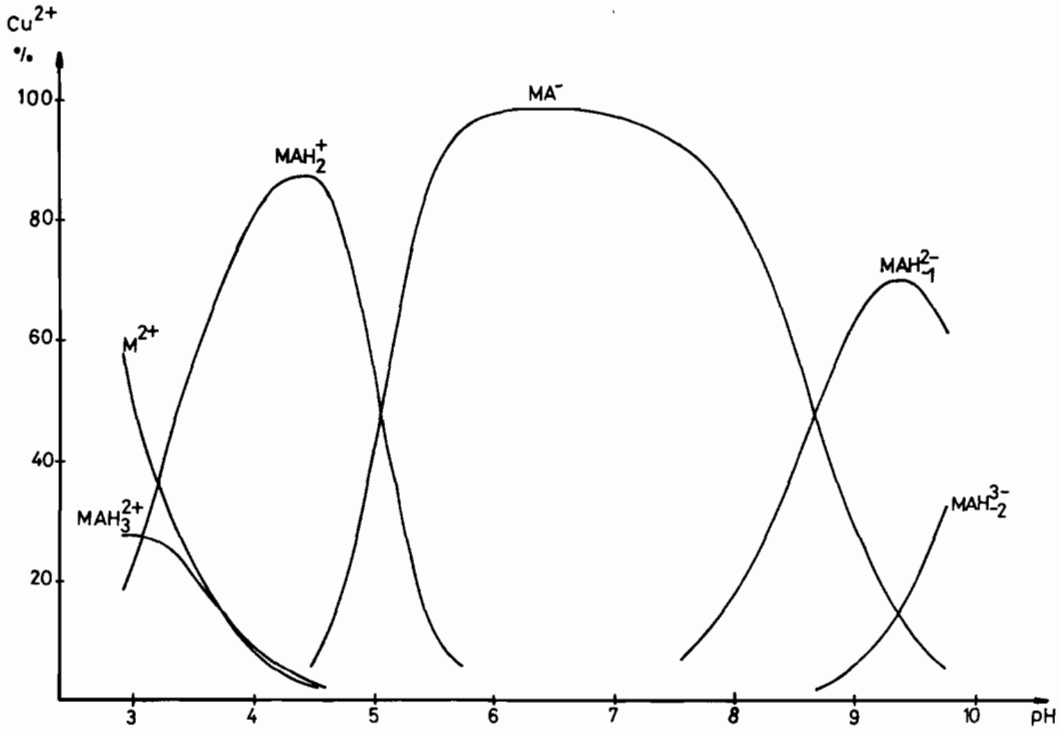


Fig. 1. Concentration–distribution curves of the complexes formed in the Cu(II)–TP5 system as a function of pH. $C_{Cu^{2+}} = 10^{-3}$ mol dm^{-3}; $C_{TP5} = 2 \times 10^{-3}$ mol dm^{-3}.

amino and tyrosyl phenolate (for $CuAH_2^+$) and one of the carboxylate groups (for $CuAH_3^{2+}$) remain protonated. Deprotonation and binding of two peptide-N donors occur in a cooperative manner at around pH ~5, which results in the formation of CuA^-. Since the complex has two pK values (pK_4' = 8.68 and pK_4' = 10.03) in alkaline solution, the species CuA^- probably corresponds to $[Cu(AH_{-2})H_2]^-$, in which the coordination takes place via the arginyl amino and two neighbouring peptide nitrogens, while the lysyl amino and tyrosyl phenolate groups are protonated.

This assumption is supported by the spectrophotometric results: by pH ~6 an intense absorption band with λ_{max} = 540 nm and ϵ_{540} = 150 mol^{-1} dm^3 cm^{-1} develops at any copper(II)–TP5 ratio. These spectral parameters correspond well to the coordination of three nitrogen donors around the copper(II) ion in a heavily distorted tetragonal structure [3, 10, 11]. The position of the absorption band is not altered in basic solution, suggesting that TP5 acts only as a tripeptide in the copper(II) complexes. Accordingly, the formation of $CuAH_{-1}^{2-}$ and $CuAH_{-2}^{3-}$ does not change the binding sites of the ligand. Hence, these species are formed by the deprotonation of tyrosyl phenolic and/or lysyl amino groups. The corresponding pK values are lower than those listed in Table I. A similar decrease in phenolate pK values was observed with other tyrosyl peptide complexes of copper(II) [12].

To summarize it may be stated that during complex formation TP5 acts as a tripeptide through coordination of the arginyl amino and two peptide-N donors. Coordination of the fourth nitrogen is in all probability hindered by the γ-carboxylate group of the aspartic acid residue, and by the steric requirements of the bulky side chains.

Acknowledgement

The authors wish to thank Gedeon Richter Ltd., Budapest, for the pentapeptide.

References

1 G. Goldstein, M. P. Scheid, E. A. Bape, D. H. Schlesinger and J. Van Wauwe, *Science, 204,* 1309 (1979).
2 J. B. Vaughn, R. L. Stephens, R. L. Lenkinski, N. R. Krishna, G. A. Heavner and G. Goldstein, *Biochim. Biophys. Acta, 671,* 50 (1981).
3 H. Sigel and R. B. Martin, *Chem. Rev., 82,* 385 (1982).
4 N. R. Krishna, D. H. Huang, D. M. Chen and G. Goldstein, *Biochemistry, 19,* 5557 (1980).
5 A. Gergely and I. Nagypál, *J. Chem. Soc., Dalton Trans.,* 1104 (1977).
6 D. D. Perrin, 'Stability Constants of Metal Ion Complexes, Part B, IUPAC Chemical Data Series, No. 22', Pergamon, Oxford, 1979.
7 a) J. R. Edsall, R. B. Martin and B. R. Hollingworth, *Proc. Natl. Acad. Sci., U.S.A., 44,* 505 (1958).
b) T. Kiss and B. Tóth, *Talanta, 29,* 539 (1982).
8 T. Kiss, unpublished results.
9 D. L. Rabenstein and T. L. Sayer, *Anal. Chem., 48,* 1141 (1976).
10 J. M. Tsangaris, R. B. Martin, *J. Am. Chem. Soc., 92,* 4255 (1970).
11 G. Formicka-Kozłowska, B. Jezowska-Trzebiatowska and H. Kozłowski, *Inorg. Chim. Acta, 25,* 1 (1977).
12 R. J. W. Hefford and L. D. Pettit, *J. Chem. Soc., Dalton Trans.,* 1331 (1981).