ELSEVIER

Coordination Chemistry Reviews
184 (1999) 319–346

COORDINATION CHEMISTRY REVIEWS

Specific structure–stability relations in metallopeptides

Henryk Kozłowski *, Wojciech Bal, Marcin Dyba, Teresa Kowalik-Jankowska

Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland

Received 7 August 1998; accepted 2 November 1998

Contents

* Corresponding author. Tel.: +48-71-204251; fax: +48-71-3282348.
E-mail address: henrykoz@wchuwr.chem.uni.wroc.pl (H. Kozłowski)

PII: S0010-8545(98)00261-6

Abstract

This review discusses the modes of coordination of oligopeptides by Cu(II) and Ni(II). Special attention is given to two general classes of peptides. The first part of the review deals with indirect effects introduced by special sequences of non-bonding side-chains. Unusual coordination modes resulting from the introduction of the break-point proline residues are also discussed. The second part of the review describes the binding properties of histidine peptides. The effects of the positioning of a His residue are discussed in the terms of cooperation and competition between potential metal anchoring sites. Special attention is given to His-3 peptides, modeling the biologically relevant albumin-like metal binding site. Finally, the coordination-related specific hydrolysis processes in histidine peptides are briefly discussed.

Keywords: Metallopeptides; The proline break-point; Structure–stability relations

1. Introduction

Peptides are very effective and often specific ligands for a variety of metal ions. They contain a range of potential donor atoms and the complexes formed exist in a variety of conformations [1,2]. Among metal ions, Cu(II) and Ni(II) have been widely studied and seem to have the most interesting chemistry. In particular, these two metal ions share the peptidic binding site at the N-terminus of human serum albumin, which is their transport form in the human body [3–6].

Peptides with non-coordinating side-chains possess amino and amide nitrogens and carbonyl and carboxyl oxygens as donor sites. Oligoalanine (Fig. 1) is a good example of such a ligand. Although the basic binding mode provided by a peptide with non-coordinating side-chains is simple, a number of variations can occur, when particular residues are inserted into the peptide sequence. The residues containing aromatic rings, like Tyr or Phe, may contribute to stability of the complex or its structure through hydrophobic interactions or ring stacking. The interactions between peptide residues may favour a particular peptide conformation, which in turn may have an essential impact on metal–peptide coordination equilibria, both in a thermodynamic and a structural sense. As the number of biologically important amino acid residues exceeds twenty, the side chains thus available may be involved in a variety of intramolecular interactions, making metallopeptide systems structurally very specific. This may result in metal-assisted modulation of biological activity, e.g. when a neuropeptide interacts with its receptor.

Conformational consequences of metal ion binding to peptide ligands may also have a critical impact on the peptide folding processes. Protein folding, and in particular hydrophobic effects, although receiving much attention, are only partly understood [7]. The existence of a relation between the binding of metal ions to proteins and the local hydrophobicity at the binding site has been recognised only recently [8]. Thus, detailed studies on the relations between the peptide sequence, complex structure and thermodynamical stability are instrumental for the under-

standing of biological functions of peptides as well as the impact of metal ions on protein folding and conformation.

The main aim of this review is the presentation of specific interactions in Cu(II) and Ni(II)–peptide systems, like unusual binding modes or very high complex stabilities that involve interactions additional to the direct coordination of a metal ion to a peptide donor system. The first part is devoted to oligopeptides of four or more amino acid residues, because they are principally capable of providing the saturated (four nitrogen, or 4N)[1] equatorial binding site. The shorter peptides have been extensively reviewed [9,10]. We made an exception for α-hydroxymethyl-L-serine (HmS)-containing tripeptides for the striking complex stability-enhancing capabilities of this amino acid. The second part of this review describes coordination properties of peptides containing histidine residues, for their unique binding efficacy and biological relevance of their Cu(II) and Ni(II) complexes.

2. Metal ion binding to simple peptides with non-coordinating side-chains

Oligopeptides composed of glycine or alanine are good examples of simple peptides.

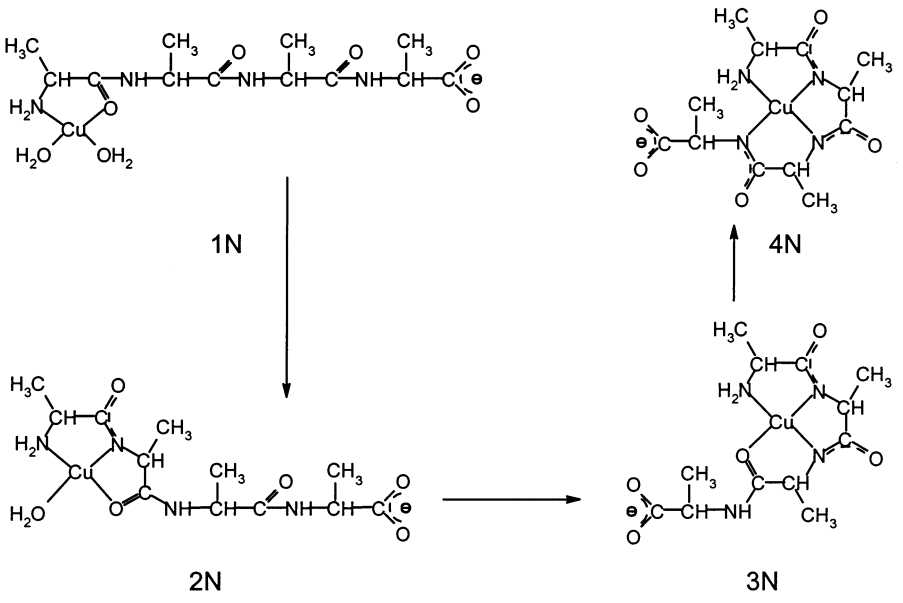

Fig. 1. The stepwise complex formation between Cu(II) and tetraalanine.

[1] Abbreviations, formulae and constants used throughout the text are explained in Appendix A.

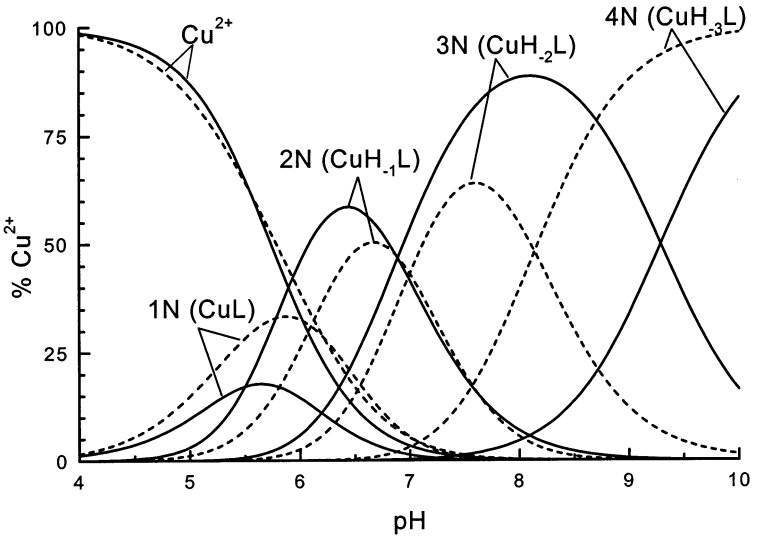

Fig. 2. Comparison of species distribution curves for Cu(II) complexes with: (—) Gly–Gly–Gly–Gly and (---) Gly–Gly–Gly–Gly–Gly: initial metal ion concentrations 1 mmol dm^{-3}, and metal-to-ligand molar ratios 1:1.

Coordination of Cu(II) or Ni(II) ions to oligoglycine or oligoalanine starts at the N-terminal amino nitrogen, which acts as an anchoring binding site, preventing metal ion hydrolysis. The adjacent carbonyl oxygen is the second donor, completing the chelate ring [1,2,9,10]. As the pH is raised, both metal ions are able to deprotonate successive peptide nitrogens, forming $M-N^-$ bonds, until eventually a $[MH_{-3}L]^{2-}$ species (4N complex) is formed around pH 9–10. Fig. 1 provides the structures of particular complexes with tetraglycine or tetraalanine and Fig. 2 presents a species distribution diagram for Cu(II) ions with tetra- and pentaglycine. The solution structures of oligoglycine complexes presented in Fig. 1 gain support in single crystal X-ray studies of Cu(II) complexes of diglycine [11], triglycine [12,13], tetraglycine [14] and pentaglycine [15].

The formation of stable five-membered chelate rings by consecutive nitrogens is the driving force of the coordination process, lowering the pK value of the first amide nitrogen by as much as 10 log units [1,9,10]. In the case of Cu(II) complexes, the deprotonations of particular amide nitrogens are usually well separated from each other. This indicates the lack of cooperativity in the binding process. Table 1 contains protonation-corrected stability constants for Gly- and Ala-containing tetra- and pentapeptides. All-glycine peptides form stronger complexes than their alanine counterparts. The alanine methyl substituent is not bulky enough to directly interfere with the complex formation. Apparently, the flexibility of Gly residues reduces strain in chelate rings and thereby stabilises the complex. The extension of the peptide chain with an additional residue results in an increase of stability of the final 4N complex at the expense of the 3N species [1,9,10,16–18]. This interesting

effect has been interpreted as evidence for the presence of a particular conformation of the C-terminal part of the peptide in the 4N complex, stabilised indirectly by Cu(II) [19].

Unfortunately, the data on complexes of still longer Gly or Ala peptides, that might shed more light on such phenomena, are not available in the literature.

Ni(II) coordination to tetrapeptides is more complicated [10,20]. The detailed discussion of cooperative deprotonation in Ni(II)–tetraglycine systems was presented by Martin [20], so only an outline is given here. Initial coordination of Ni(II) aqua ion to a simple peptide occurs through the N-terminal amine, same as with Cu(II). The complex is, however, octahedral rather than tetragonal. The deprotonation of the adjacent amide nitrogen occurs at a higher pH than in corresponding Cu(II) complexes (8–9 vs. 5). However, the further two amide donors (when available) deprotonate cooperatively. This is indicated by a lowering of formal pK values for successive deprotonations below the value of the initial one. This event is accompanied by a transition from a hexacoordinate (octahedral) to a planar geometry, which causes this reaction to be distinctly slow. The species distribution diagram for the Ni(II)–tetraglycine system is given in Fig. 3. It clearly illustrates the cooperativity of amide deprotonations. Although the stoichiometric species are analogous to those obtained for Cu(II), the concentrations of the intermediate 2N and 3N complexes are much lower than the respective Cu(II) species. The cooperative transition from an octahedral NiL (1N) to a planar $MH_{-3}L$ (4N) species causes that only around 2% of total Ni(II) forms the $NiH_{-1}L$ (2N) intermediate complex.

The solution structures of oligoglycine complexes are supported by single crystal X-ray studies of an octahedral Ni(II) complex with diglycine [21] and a square-planar complex of tetraglycine [22].

Table 1
Values of log $*K$[a] for Cu(II) complexes of simple tetra- and pentapeptides: data for Met-enkephalin and the ANF pentapeptide are given for comparison

Peptide	1N	2N	3N	4N	Ref.
Gly–Gly–Gly–Gly	−2.89	−8.39	−15.28	−24.57	[1]
Ala–Gly–Gly–Gly	−2.89	−8.75	−15.73	−24.99	[18]
Ala–Ala–Ala–Ala	−3.36	−8.58	−16.22	−25.48	[16]
Ala–Ala–Ala–Ala–Ala–NH_2	−3.11	−8.70	−16.44	−24.41	[17]
Gly–Gly–Gly–Gly–Gly	−2.66	−8.76	−15.76	−23.90	[18]
Ala–Gly–Gly–Gly–Gly	−2.58	−8.58	−15.58	−23.79	[18]
Gly–Gly–Gly–Gly–Ala	−2.63	−8.64	−15.69	−23.94	[18]
Tyr–Gly–Gly–Phe–Met	−2.86	−8.01	−15.13	−23.62	[31]
Asn–Ser–Phe–Arg–Tyr–NH_2	−1.93	−6.91	−13.35	−20.08	[17]

[a] For notation and definitions of constants see Appendix A.

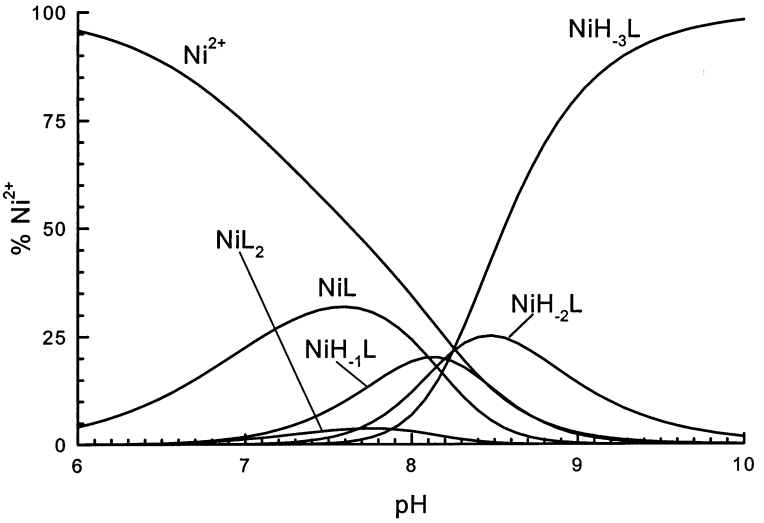

Fig. 3. Species distribution curves for Ni(II) complexes with Gly–Gly–Gly–Gly: initial metal ion concentration 1 mmol dm^{-3}, and metal-to-ligand molar ratio 1:1.

3. Non-bonding amino acid side-chains can influence the stability and structure of metal complexes with peptides

3.1. Superstability through indirect interactions

In the Cu(II)–Asn–Ser–Phe–Arg–Tyr–NH_2 (NSFRY–NH_2) system [17] the coordination modes are analogous to those shown in Fig. 1. The Cu(II) binding begins at the N-terminus and, with pH increase, the 1N, 2N, 3N and 4N species are formed. However, the comparison of the species distribution of Cu(II)–NSFRY–NH_2 with that of Cu(II)–pentaalanine amide (Fig. 4) indicates clearly that the coordination equilibria in both systems are very different. The formation of a 4N complex is observed at much lower pH in the case of NSFRY–NH_2, indicating an extreme stability enhancement. The stability constants collected in Table 1 indicate that the 4N Cu(II) complex of NSFRY–NH_2 is almost five orders of magnitude more stable than that of pentaalanine. This exceptionally high complex stability (according to our knowledge, NSFRY–NH_2 is the most effective ligand for Cu(II) ions among oligopeptides with non-coordinating side-chains) must result from the involvement of the amino acid side chains. Since the metal-free peptide has a completely random conformation in aqueous solution, the impact of the side-chains on the complex stability derives from the metal-ion promoted conformational organisation. The other unusual feature found for the Cu(II)–NSFRY–NH_2 system is the cooperative transition from 2N to 4N complex.

In order to gain better understanding of the metal ion-assisted peptide organisation in NSFRY–NH_2, the synthesis and coordination studies were performed for a series of systematically Ala-substituted NSFRY analogues [23]. The stability con-

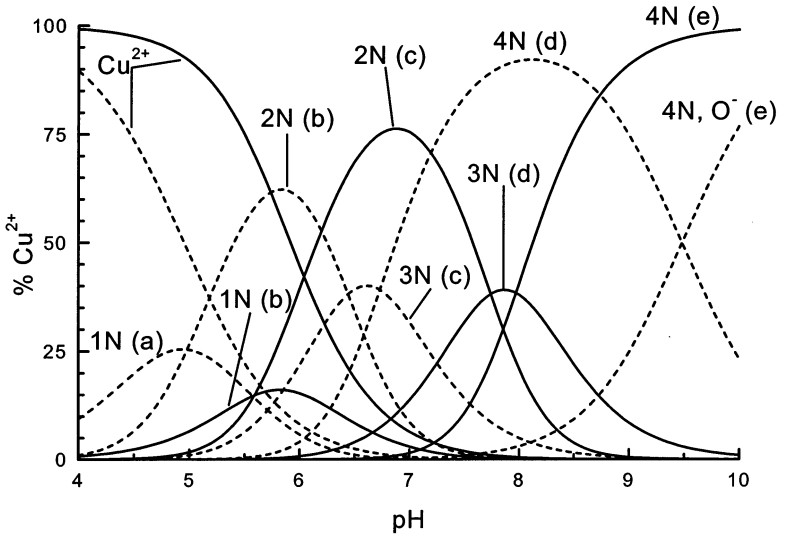

Fig. 4. Comparison of the species distribution curves for Cu(II) complexes with: (—) Ala–Ala–Ala–Ala–Ala–NH_2 and (---) Asn–Ser–Phe–Arg–Tyr–NH_2: metal ion concentrations 1 mmol dm^{-3}, and metal-to-ligand molar ratios 1:1; (a) CuHL, (b) CuL, (c) $CuH_{-1}L$, (d) $CuH_{-2}L$, (e) $CuH_{-3}L$.

stants obtained for this series allows one to estimate the effect of each of the five amino acid residues on the stability gain in NSFRY–NH_2 analogues. The values of pK of formation of particular complexes and log *K (which has to be used instead of pK for 1N complexes) are good measures of the relative stability constants of a particular complex species when coordination occurs through deprotonation. Differences of these values between NSFRY–NH_2 and other analogues collected in Table 2 allows one to evaluate the effects of amino acid substitutions on the formation of particular complexes. These data indicate that the substitution of Asn-1 with Ala has the biggest effect on the stability of the 1N complex. A sizeable effect from Tyr-5 is also seen. Substitution of other residues with Ala have smaller, but uniformly diminishing impact on the stability of the complexes. The formation

Table 2
Values of log *K for Cu(II) complexes of the analogues of the ANF pentapeptide

Peptide	1N	2N	3N	4N	Ref.
Asn–Ser–Phe–Arg–Tyr–NH_2	−1.93	−6.91	−13.35	−20.08	[17]
Ala–Ser–Phe–Arg–Tyr–NH_2	−3.36	−8.26	−15.55	−21.52	[23]
Asn–Ala–Phe–Arg–Tyr–NH_2	−2.23	−7.40	−14.24	−20.59	[23]
Asn–Ser–Ala–Arg–Tyr–NH_2	−2.07	−6.97	−13.93	−20.56	[23]
Asn–Ser–Phe–Ala–Tyr–NH_2	−2.33	−7.24	−14.31	−20.88	[23]
Asn–Ser–Phe–Arg–Ala–NH_2	−2.84	−7.56	−14.65	−21.75	[23]
Asn–Ser–Phe–Arg–Tyr–OH	−1.82	−6.89	−14.06	−20.47	[23]
Ala–Ala–Ala–Ala–Ala–NH_2	−3.11	−8.70	−16.44	−24.41	[17]

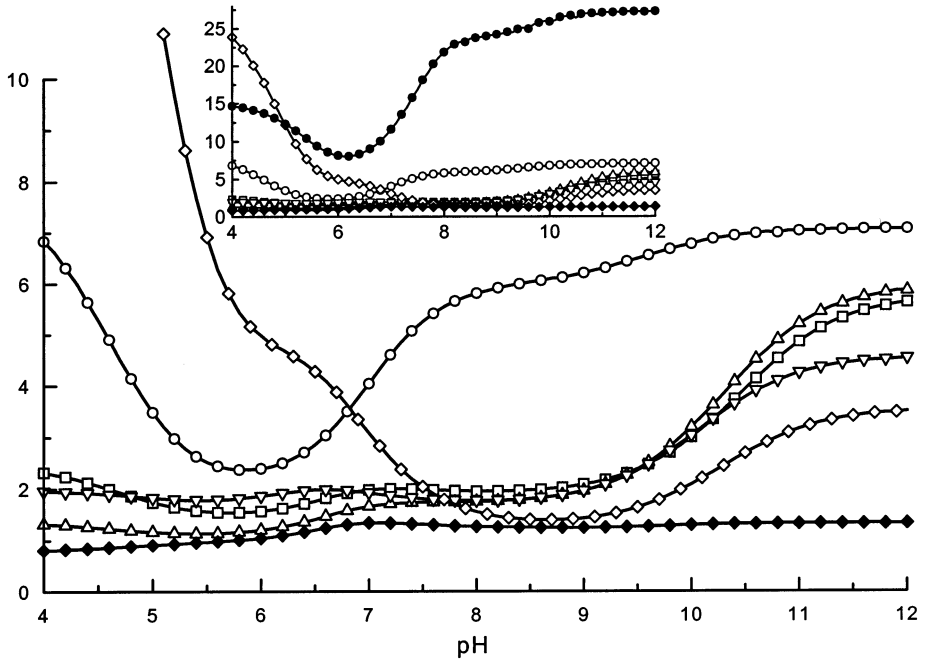

Fig. 5. Plot of pairwise ratios of Cu(II) sequestering, obtained by dividing total concentrations of Cu(II) bound to NSFRY–NH_2 by total concentrations of Cu(II) bound to a competing peptide. Calculations were done for 1 mmol dm^{-3} Cu(II), NSFRY–NH_2 and a competing ligand: ◇, ASFRY–NH_2; ▽, NAFRY–NH_2; △, NSARY–NH_2; □, NSFAY–NH_2; ○, NSFRA–NH_2; ◆, NSFRY–OH; ●, AAAAA–NH_2.

of the 2N species is the least sensitive to amino acid substitutions. Comparison with AAAAA–NH_2 and other simple peptides indicates that the 1N→2N transition in NSFRY–NH_2 analogues is less enhanced than the 1N or 4N formation, but still the 2N species form at pH approximately 0.5 log unit lower than with typical oligopeptides.

Values of pK of formation of 3N complexes (Table 2) are usually higher than respective values of 4N complexes. This phenomenon is well known from complexes of His-containing peptides, and reflects a cooperativity of binding of the third and fourth nitrogens to Cu(II) [1]. This cooperative transition from 2N to 4N complexes is the prominent feature of NSFRY–NH_2 and its analogues. The values collected in Table 2 indicate clearly that Asn-1 and Ser-2 do not significantly contribute to this effect. Contributions of Phe-3, Arg-4, and C-terminal amide are approximately equal, and the largest effect is conveyed by the Tyr-5 residue. Compared with AAAAA–NH_2, contributions of all amino acid residues in NSFRY–NH_2 to the stability gain are additive within the experimental error of stability constant determinations (-2.54 vs. -2.46). Plots of Cu(II) sequestering ratios (Fig. 5) reveal another specific effect in the Tyr residue, seen only in NSFRY–NH_2 and NSFRY–OH. In other analogues complexation results in the

elevation of pK_{Tyr} by 0.7–0.8 log units, but in these two, this value is even slightly decreased, and the deprotonation of the Tyr ring results in the several-fold increase of the affinity to Cu(II), seen in the elevation of the curves above pH 10.

The phenomena described above have several origins. There is a clear electronic effect from the Asn side chain, seen also in the lowering of the amino pK, that increases the Cu–N bond strength, and also a secondary effect from Ser oxygen, stabilising 1N and 2N complexes. A fence around Cu(II) is provided by Asn and Phe side chains. The 4N complex stabilisation also results from the formation of a main chain loop around Cu(II) by hydrogen bonding between polar atoms of residues one and five. Such an effect is responsible for the stability gain in pentapeptide versus tetrapeptide complexes [2]. The most striking and novel effect, however, comes from the presence of the Arg and Tyr side chains in positions four and five, away from the Cu(II) site. The only explanation for this effect is the formation of a secondary fence above the one formed by side chains of residues one to three. It is located over the coordination plane so that an edge to face interaction between Phe and Tyr chains is possible, but this is not required for the fence stability. In fact, all shielding contributions are additive rather than cooperative, as reflected by the values of pK (3N + 4N) in Table 2. The secondary fence has a larger effect on stability than the primary one, probably by effectively limiting the access of water molecules to Cu(II) over a larger surface of approach.

3.2. General effects of aromatic rings on complexation equilibria

Aromatic rings of Phe, Tyr, or Trp can enhance complex stability through direct electronic interactions with the metal ion, the stacking between two rings, or general hydrophobic effects. There are numerous examples of such effects in complexes of amino acids [24,25], dipeptides [26,27] and tripeptides [27–30]. However, the longer the peptide, the less pronounced the stability enhancement from these interactions. In the Met-enkephalin peptapeptide no complex stabilisation from aromatic rings could be detected (Table 1) [31]. Therefore, the participation of aromatic residues in the stabilisation of NSFRY analogues seems unique.

3.3. Pre-conformation in the peptide molecule

Arginine vasopressin (AVP) and oxytocin (OXT), important neurohypophyseal hormones, are nonapeptides. Their molecules contain a loop resulting from the disulphide bridge between cysteines in positions one and six. We have found that this loop provides and excellent pre-formed coordination site for Cu(II) ions, with the stability increase for the 4N complex of ca. four orders of magnitude over the ones with oligoalanines having the same $\{NH_2, 3 \times N^-\}$ donor set [32,33] (Table 3). This effect stems from a particular conformation of these hormones, locked into a ring by the above mentioned disulphide bridge. Positions of the first four nitrogen atoms are apparently suited for Cu(II) coordination. An extension of the peptide at the N-terminus by a single Ala residue completely removes the stabilisation for all complexes [33], and a substitution of an L-amino acid in position four of AVP with

a D-residue decreases specifically the stability of the 4N complex by two orders of magnitude [32]. This sensitivity of AVP and OXT to substitutions further exposes the subtlety of indirect conformational interactions between Cu(II) and peptides.

It is worthwhile to mention here that the metal ion binding to oxytocin and the resulting conformational effects may have a distinct impact on the activity of the hormone [34].

3.4. Unusual effect of an α-hydroxymethylserine residue on the binding ability of peptides

The HmS is an N-terminal residue in several peptidic antibiotics. Its role is poorly understood, but it is likely that its distinct impact on peptide conformation could be of biological relevance. Conformations of peptides, on the other hand, are critical for their binding abilities towards metal ions. Indeed, HmS with its bulky side-chain affects considerably the coordination equilibria in the metal–peptide systems [35,36]. The HmS residue has two hydroxyl groups in its side-chain which may bind to metal ion in strongly basic solution. Below pH 9, tri-HmS binds Cu(II) ions via a $\{NH_2, 2 \times N^-\}$ donor set as does trialanine. However, the presence of HmS residues increases stability constant of the 3N ($CuH_{-2}L$) species by almost four orders of magnitude when compared to trialanine (Fig. 6). This indicates that there are very effective indirect interactions involving α-hydroxymethyl side-chains, stabilising the complexes formed. It is interesting to note that even a single HmS residue inserted in an oligopeptide may change distinctly its coordination ability for Cu(II) [36,37]. No structural information on the Cu(II) complexes with HmS peptides has been available to date, but it is likely to assume that the stability enhancement results in part from the efficient shielding of the coordination plane from the hydrolytic attack from the bulk of solution (cf. histidine complexes, below).

4. Impact of the proline break-point on coordination abilities of oligopeptides

Proline (Pro) is the only protein-building amino acid having a secondary amino nitrogen. While Pro residue inserted as the N-terminal residue into the peptide

Table 3
Values of log $*K$ for Cu(II) complexes of AVP, its analogues and tetraalanine

Log $*K$	1N	2N	3N	4N	Ref.
Ala–AVP	−2.87	−8.04	−15.42	−24.68	[33]
OXT	−2.14	−7.94	−14.24	−21.34	[33]
AVP	−2.48	−8.26	−14.36	−20.51	[32]
D-Val^4–AVP	−2.68	−8.06	−13.75	−22.17	[32]
Ala–Ala–Ala–Ala	−3.36	−8.58	−16.22	−25.48	[16]

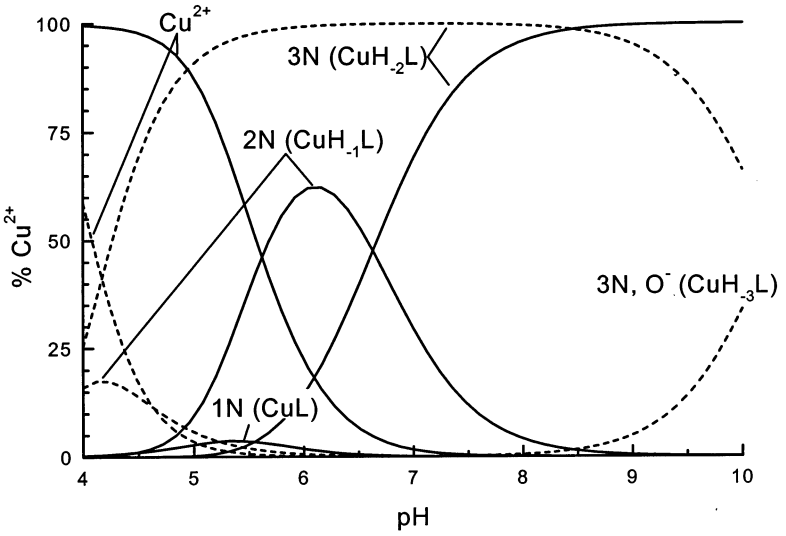

Fig. 6. Comparison of species distribution curves for Cu(II) complexes with: (—) Ala–Ala–Ala and (---) HmS–HmS–HmS: initial metal ion concentrations 1 mmol dm^{-3}, and metal-to-ligand molar ratios 1:1.

sequence is an effective anchoring site for metal ions, its introduction into the peptide chain in position two, three or further, results in a peptide bond that does not have an amide proton that may be displaced by metal ions. As a consequence, the simple stepwise coordination of consecutive amide nitrogens, discussed above, is no longer possible. On the other hand, the presence of a Pro residue in the peptide sequence increases the propensity of a peptide chain to bend. For these reasons we named this phenomenon break-point [38] and studied numerous examples of this particular coordination mode in a variety of the Cu(II) proline-containing peptide systems.

The most interesting feature of these systems is the inducing the formation of large macrochelate loops, with Cu(II) bound to the N-terminal amino group and a distant donor. The latter might be either a main chain amide or a donor group of a normally non-coordinating side chain, like the Tyr phenolate, the ε-NH_2 of Lys or the lateral carboxylate of Glu. Pro-2 tetrapeptides seem to be the most specific ligands among all studied systems. In such tetrapeptides with no potential metal-binding groups in side chains, the amide nitrogen of the fourth residue coordinates to the metal ion. When Tyr or Lys is present in the sequence at position four, its side chains is involved in coordination, forming large macrochelates. If the formation of an appropriate macrochelate is sterically impossible (Tyr-1 or Tyr-3), then Cu(II) dimeric species with $\{NH_2, O_{Tyr}\}$ donor sets predominate, utilising the bent peptide molecule as a bridge between metal ions. Pro-3 tetrapeptides are more stable due to the $\{NH_2, N^-\}$ binding. Macrochelates are formed in this case as well, with the chelate-closing group occupying the third coordination site [39–43].

Two Pro residues in Pro-2-Pro-3 pentapeptides form the most stable macrochelates in their class (Table 4). This is due to a very rigid conformation imposed on the peptide by the pair of Pro residues, increasing the entropic contribution to complex stability [44,45]. The exotic structure of the Gly–Pro–Pro–Gly–Gly complex of Cu(II) is presented, together with its speciation in Fig. 7a. The behaviour of the Pro-2–Xaa-3–Pro-4 moiety in pentapeptides is perhaps the most unusual of all: in Gly–Pro–Gly–Pro–Gly there is no macrochelate formation. Only weak, amino-bound mono and bis complexes were found, with no evidence for, e.g. C-terminal carboxylate coordination (Fig. 7b). However, in Gly–Pro–Gly–Pro–Glu, the lateral carboxylate binds Cu(II), as evidenced both in spectroscopy and potentiometry (increase of complex stability by 0.6 log units, see Table 4, Fig. 7c). A Lys residue introduced in position three of such a pentapeptide readily coordinates, forming yet another macrochelate [45,46]. A study of peptide analogues of antigenic surfaces of the hepatitis B envelope proteins, indicates a possibility that even the binding of a usually very basic Arg guanidinium group may be promoted by the presence of a Pro residue [47]. Namely, pS2, of a sequence LQDPRVRGLTL binds Cu(II) at neutral pH through a donor set $\{NH_2, 2 \times N^-, \beta\text{-}COO^-\}$ typical for Asp-3 peptides. However, in alkaline conditions further deprotonations take place. Their spectroscopic effects, and a comparison with a shorter analogue, LQDPR, are indicative of the binding of the Arg-7 residue.

The potential importance of such interactions for the biological function of proline-containing neurohormones has previously been postulated [48]. The bent conformations assumed by many proline peptides upon Cu(II) binding through macrochelate formation resemble β-turns, which are believed to be essential for the receptor binding by Pro-containing neurohormones and neuromodulators, like casomorphins or substance P. In this way, Cu(II) coordination at the receptor may activate these bioligands. A possibility that Cu(II) coordination enhances activity of a peptide effector molecule was presented recently with amylase inhibitors [49].

Table 4
Values of log $*K$ for Cu(II) complexes of selected peptides containing one or two proline residues

Log $*K$	1N	2N	Ref.
Phe–Pro–Gly–Gly	−2.51	−9.29	[46]
Gly–Pro–Gly–Phe	−3.18	−10.04	[46]
Phe–Gly–Pro–Phe	−2.87	−7.50	[43]
Phe–Gly–Pro–Tyr	−2.74	−7.46	[43]
Gly–Pro–Pro–Gly–Gly	−2.92	−9.66	[45]
Glu–Pro–Pro–Gly–Gly	−2.79	−9.79	[45]
Gly–Pro–Gly–Pro–Glu	−1.96	–	[45]
Gly–Pro–Gly–Pro–Gln	−2.56	–	[45]
Gly–Pro–Lys–Pro–Gly	−2.91	−9.37	[45]
Arg–Pro–Lys–Pro–Gln	−2.82	−9.28	[45]
Substance P.	−2.93	−9.55	[45]

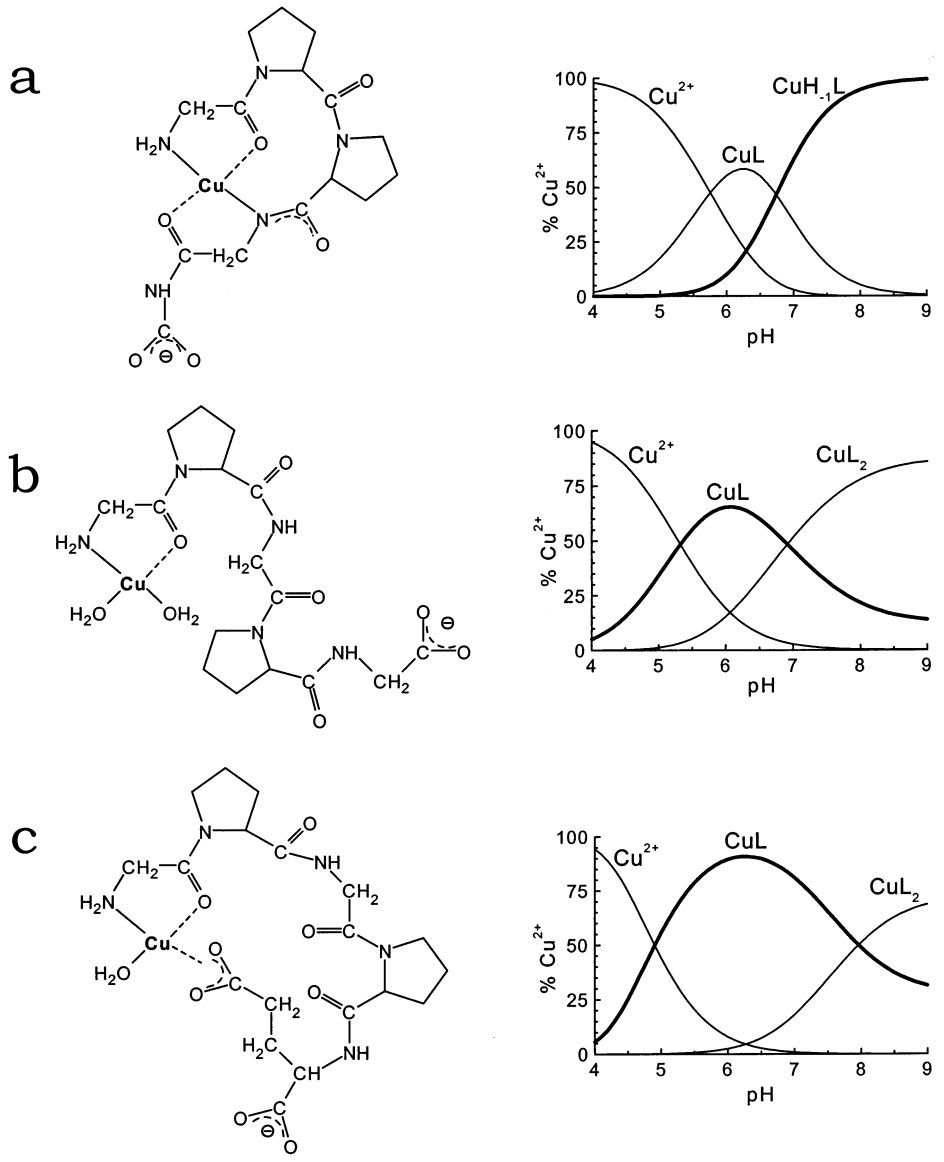

Fig. 7. Species distribution curves and selected structures for Cu(II) complexes of proline peptides: (a) Gly–Pro–Pro–Gly–Gly ($CuH_{-1}L$); (b) Gly–Pro–Gly–Pro–Gly (CuL); and (c) Gly–Pro–Gly–Pro–Glu (CuL); initial metal ion concentrations 1 mmol dm^{-3}, and metal-to-ligand molar ratios 1:2.

5. Coordination of Cu(II) and Ni(II) to histidine peptides

The histidine residue possesses a very efficient nitrogen donor in its side chain

imidazole ring. The cooperativity of all three donor groups of this amino acid in metal binding is made possible by the formation of two fused chelate rings: the five-membered {NH_2, COO^-} (amino acid-like) and the six-membered {NH_2, N_{im}} (histamine-like). The high thermodynamic stability of five- and six-membered rings versus larger ones results in the selection of the N-1 rather the N-3 imidazole nitrogen (Fig. 8). Such terdentate binding makes histidine a primary low molecular weight chelator in living systems. The specificity of histidine in metal ion binding is preserved in His-containing peptides. The His residue provides two nitrogen donors and a six-membered chelate ring for the coordination. However, the coordination properties of a His residue within a peptide sequence depend enormously on the position of this residue in a peptide chain.

5.1. His-1 complexes

Simple peptides with His in the N-terminal position bind Cu(II) and Ni(II) differently from ordinary peptides. The {NH_2, N_{im}} chelate of the His-1 residue, analogous to histamine [50], is so efficient, that it wins competition with the amide bonds for Ni(II), and hinders their deprotonation for Cu(II). Thus, Ni(II) forms only pseudooctahedral ML and ML_2 complexes with peptides like His–Gly [51–53], His–Met [54] or His–Gly–Gly [55]. With Cu(II), apart from such species, also monomeric and dimeric complexes with deprotonated amides have been detected. The pH of the first amide deprotonation is, however, elevated from ca. 5 with oligoglycines to well above 6 with these peptides [52–56].

5.2. His-2 complexes

The insertion of histidine in position two of the peptide chain allows for the simultaneous participation of the amine, the imidazole and the intervening His amide nitrogens in the binding. Gly–His is the simplest peptide of this group of peptides. The predominant complex is $CuH_{-1}L$ detected at pH as low as 4, and remaining as the most important species through pH 10 [52,57,58]. A very high stability of this complex results from the formation of another pair of fused chelate

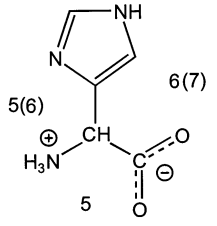

Fig. 8. Chelating abilities of histidine. Numbers are denote sizes of potential chelate rings.

rings, the five-membered {NH_2, N^-} and the six-membered {N^-, N_{im}}. This flat chelate system uses only three of four equatorial coordination positions around the Cu(II) ion. The fourth position can be occupied by a second Gly–His molecule, or a molecule of another ligand (Fig. 9a). At high pH this position can also taken up by the deprotonated N-3 nitrogen of another Gly–His unit. A unique tetrameric complex $Cu_4H_{-8}L_4$ with four imidazole bridges is thus formed [57,59].

Similar prominence of the $CuH_{-1}L$ complex was also found for Cu(II) complexes of Ala–His [60], Gly–His–Gly [61,62], glycylhistamine [63], and Gly–His–Lys [60,64,65]. The latter peptide is a human tissue growth factor. It requires complexation by Cu(II) for its physiological activity [66]. This fact confirms a very high specificity and biological relevance of the tridentate Xaa–His coordination of Cu(II). On the other hand, the presence of the ε-amino function of the Lys residue in Gly–His–Lys does not affect complexation equilibria.

X-ray structures of Cu(II) complexes with Gly-His [67], Gly–His–Gly [68] and Gly–His–Lys [69] support the 3N coordination in the $CuH_{-1}L$ complex. The crystal structure of the Cu(II)–Tyr–His complex [70] reveals an interaction between the Cu(II) atom and the aromatic ring. This indicates the ability of the amino acid in position 1 to fine-tune the complex formation, quite similarly to simple peptides.

The major difference between Cu(II) and Ni(II) in Xaa–His complexation stems from the lesser ability of Ni(II) to promote amide deprotonation. Thus, the major $NiH_{-1}L$ complex, of octahedral character, forms at pH 6–7 [51,52,71].

Carnosine, β-alanylhistidine, provides a special case of a His-2 peptide, because the presence of a β-residue in position one makes both potential chelate rings six-membered (a $6+6$ system). A complex species of $CuH_{-1}L$ stoichiometry, possessing these rings, is indeed proposed by some authors [52,72] in dilute solutions at neutral and alkaline pH. At millimolar concentrations, however, the major complex species is an interesting dimeric complex $Cu_2H_{-2}L_2$ [52,59,72,73]. The X-ray structure of this symmetrical dimer [74] indicates that each of the two imidazole donors binds to the fourth equatorial position of the Cu(II) ion coordinated by the {NH_2, N^-, COO^-} donor set of the other carnosine molecule. Such an arrangement provides a $5+6$-membered chelate ring system which is more efficient than the $6+6$ one.

5.3. His-3 complexes

The presence of the His residue in position three of the peptide chain allows for the simultaneous formation of three fused chelate rings, and thus the saturation of the coordination plane. The Cu(II) complexation reaction with the simplest representative of this class of peptides, Gly–Gly–His, proceeds cooperatively, with both amide groups deprotonating and bonding to Cu(II) between pH 4 and 5 [75–77] (Fig. 9b). The 4N complexes formed in this way are the most stable ones formed by peptides. Table 5 presents the results of calculations of the idealised (i.e. with hydrolysis neglected) equilibrium concentrations of free Cu(II) at pH 7.4 in 1:1 systems with 1 mM peptides based on published stability constants. These data indicate that Gly–Gly–His is ten-fold more effective in Cu(II) sequestrating than

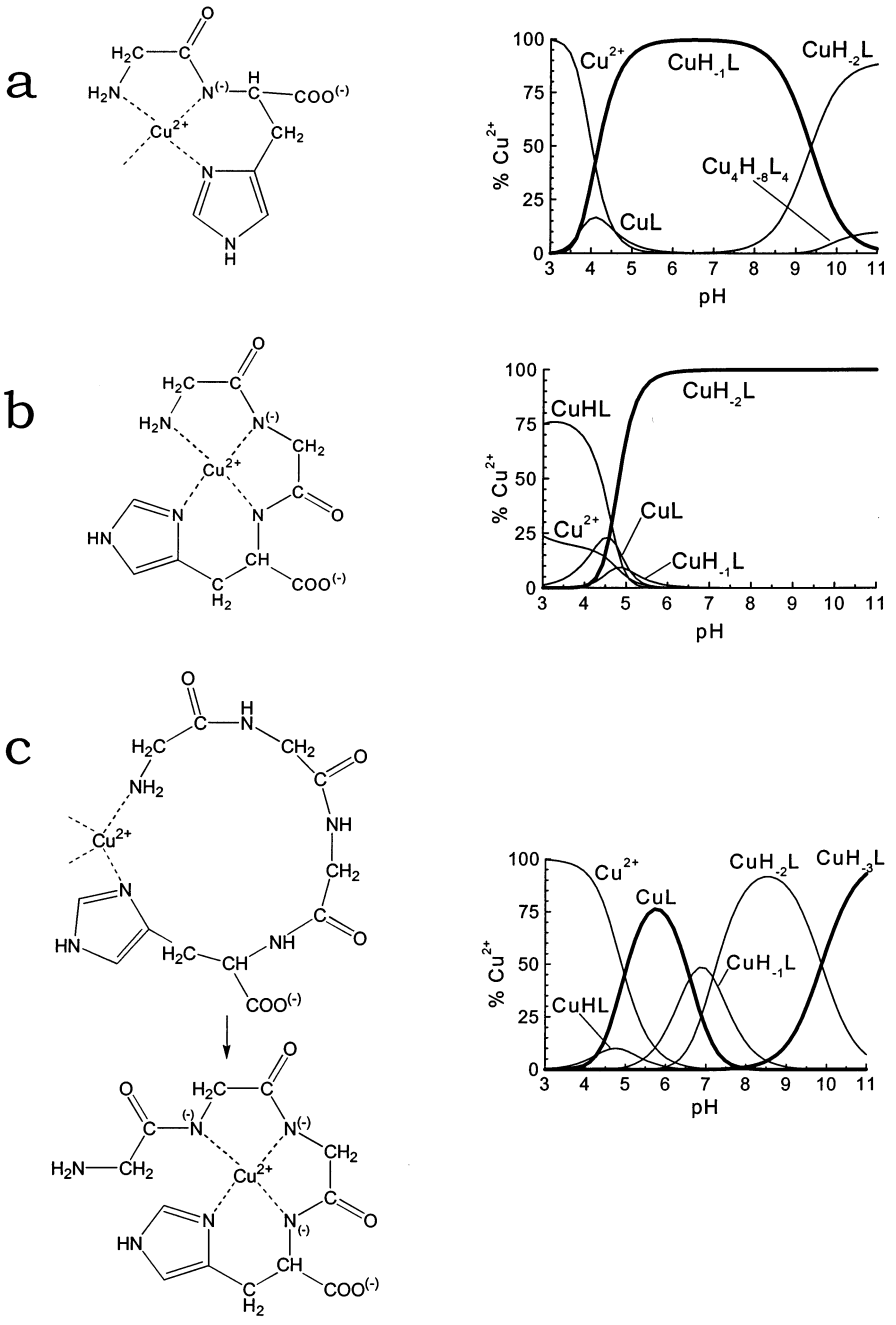

Fig. 9. Species distribution curves and structures of major complex species for Cu(II) complexes of histidine peptides: (a) Gly–His ($CuH_{-1}L$); (b) Gly–Gly–His ($CuH_{-2}L$); and (c) Gly–Gly–Gly–His (CuL and $CuH_{-3}L$); initial metal ion concentration 1 mmol dm^{-3} and metal-to-ligand molar ratio 1:1.

Table 5
Equlibrium concentrations of copper(II) aqua ion at pH 7.4 in the presence of representative peptides, calculated from stability constants for total peptide and Cu(II) concentrations of 1 mmol dm^{-3}

Peptide	$p[Cu^{2+}]$ at pH 7.4	Ref.
Gly–Gly–Gly–Gly	4.98	[1]
Ala–Ala–Ala–Ala–Ala–NH_2	4.66	[17]
Asn–Ser–Phe–Arg–Tyr–NH_2	5.85	[17]
Gly–His	7.16	[52]
Gly–Gly–His	8.11	[77]
$HP2_{1-15}$	9.15	[78]

Gly–His, and ca. 200-fold more effective than NSFRY–NH_2, the strongest ligand among non-His peptides.

As a result of the binding cooperativity, the intermediate complexes, with 1N, 2N, and 3N coordination, are minor. Some authors assume the presence of at least some of these complexes in the Cu(II)–Gly–Gly–His system at the level of 10–20% of total Cu(II) at pH 4–5 [75,77], while others do not [76]. The existence of these species is usually derived from the computer analysis of potentiometric titrations, as their introduction into the model of complexation improves the numerical fit. However, a careful spectroscopic study revealed the absence of such species, despite the better fit, among the Cu(II) complexes of RTHGQ–NH_2 [78]. This finding indicates that the reported presence of these minor complexes may be just an artifact of the potentiometric method. On the other hand, the analysis of line broadening patterns in the NMR measurements of Cu(II)–glycylglycylhistamine solutions suggests the short-lived existence of various partially coordinated species [79].

The cooperative formation of fused chelate rings by Gly–Gly–His around the Ni(II) ion is associated with a transition from the octahedral and high spin to the square-planar and low spin geometry. The resulting 4N complex is very similar to those formed by Cu(II). The process of spin-pairing is induced by the increased ligand field strength of further in-plane nitrogen donors. Similarly to Ni(II) complexes with tetraglycine, four nitrogen donors are required. It seems that the turning point is located closer to the 4N than the 3N coordination. For example, histidine forms only high-spin complexes, despite the 4N coordination [80], and the spin-pairing transition was observed between 4N complexes of its hydroxamic analogue upon the increase of electronic density in the hydroxamic group [81].

Despite the existence of two reasons for the cooperative formation of the 4N complexes between Ni(II) and Gly–Gly–His and analogous peptides, the conformational one and the electronic one, the presence of the intermediate complexes seems to be a fact, at least for some peptides. Minor complexes of an octahedral character were detected in the UV–vis spectra of Asp–Ala–His–NHMe [82]. An interaction between the paramagnetic Ni(II) ion and the imidazole moiety of Gly–Gly–His was also detected in the NMR spectra [83]. On the other hand, such complexes were not detected in UV–vis and CD studies of the HP2 peptides [78]

(see below). The likely reason for this difference originates in kinetic properties of Cu(II) and Ni(II) complexes. The Cu(II) complexes are labile, and the stepwise binding events are swift. In contrast, the formation of a square-planar Ni(II) complex from an octahedral predecessor requires the rearrangement of the electronic structure as well as the multiple ligand exchange and dissociation events [84]. This process is slow, and thereby leaves an open time window for the accumulation of detectable amounts of intermediate octahedral complexes, which form much faster.

The structure of the 4N binding mode of Xaa–Yaa–His complexes was confirmed by single crystal X-ray studies. Analogous structures were found for Cu(II) complexes with Gly–Gly–His–*N*-methylamide [85], glycylglycyldehydrohistamine [86], and glycylglycylhistamine [79]. There is only one such structure known for Ni(II), with glycylgycyl-α-hydroxy-D,L-histamine [87].

5.4. Coordination properties of peptides with His in position four

Separation of the N-terminal amine and the imidazole donors by two or more intervening amino acid residues removes the possibility of concerted formation of the fused chelate system, because there are five or more potential nitrogen donors for four equatorial sites around the metal ion. A study of Cu(II) complexation by Gly–Gly–Gly–His, Ala–Gly–Gly–His, and their analogues with particular donor groups selectively blocked, provided insight into this situation [88]. That study provided evidence that, for 1N complexes, the anchoring of Cu(II) at the C-terminal His residue is preferred over the N-terminal binding. For tetrapeptides with both terminal donors available, the next complex species (2N), present at neutral pH contain 15-membered macrochelate loops with the $\{NH_2, N_{im}\}$ coordination (Fig. 9c). At higher pH amide deprotonations occur. Again, the imidazole anchoring is preferred. A system of two (3N), and subsequently three (4N) chelate rings is formed, and the amine donor remains uncoordinated. Note that there is no cooperativity of formation of these rings, similarly to simple peptides. Interestingly, the presence of a bulky substituent in the N-terminal residue (a Boc protection on the amine) hinders the formation of the final 4N complex. This fact indicates that there are specific conformational or steric constraints in the complex molecule that have to be satisfied for the 4N coordination anchored at the His residue to occur. They probably result from the bulkiness of the imidazole ring that allows only small ligands in the position taken by the fourth nitrogen. Such effects have not been observed in Xaa–Yaa–His complexes, with [89] or without the acetylation of the terminal amine, but are responsible for the particular coordination properties of angiotensin II (AT2), described below [89].

Similar tendencies: preferential anchoring at His, formation of a macrochelate loop in the intermediate pH, and of a square-planar 4N complex without the participation of the terminal amine, were found for Ni(II) complexes with Ala–Gly–Gly–His [90]. In contrast to the Cu(II) case, Boc–Ala–Gly–Gly–His formed the square-planar 4N complex similar to Ala–Gly–Gly–His. However, the sterical hindrance of the bulky terminal residue was in operation as well, lowering the

stability of this complex by as much as four orders of magnitude compared to Ala–Gly–Gly–His.

The ability of tetrapeptides to form macrochelate loops with the sole participation of terminal donors was confirmed in studies of Ni(II) binding to Ac–Cys–Ala–Ile–His–NH_2, in which the coordination occurs through the $\{S^-, N_{im}\}$ donor set [91,92]. The latter peptide is a succesful model for Ni(II) binding to histones, thereby supporting the biological relevance of such a mode of coordination [93].

5.5. Coordination properties of peptides with His in position five and six; the case of angiotensin II

AT2, a human hormone regulating blood pressure, has a sequence Asp–Arg–Val–Tyr–Ile–His–Pro–Phe (DRVYIHPF). It contains the N-terminal and the imidazole binding sites separated by four amino acid residues. Studies of coordination of Cu(II) to AT2 and its truncated analogues, DRVY, AcYIH, and AcHPF [89], as well as RVYIH and DRVYIH [94] revealed that when the separation is bigger than two residues, then the His residue is the anchoring site for Cu(II) as well. The major complex formed with AT2, RVYIH and DRVYIH at neutral pH is of 3N type, involving nitrogen donors of Ile and His residues. The 4N coordination at the His residue is not attained by these peptides. Apparently, the bulky aromatic substituent of the Tyr residue is responsible for that. Instead, at pH ca. 9, the Cu(II) ion is transferred to the N-terminal site, and forms the classical 4N complex, identical to the one formed by DRVY. This is a clear example of the subtle effect of a non-bonding side chain that results in a complete rearrangement of the complexation mode. Another striking effect of this kind, which is difficult to assign to a particular interaction, is the presence of the axial binding of the imidazole ring to the N-terminal 4N template. Such binding, which clearly enhances complex stability, was detected for DRVYIH, but not for RVYIH and the whole AT2.

The complexation of Ni(II) by AT2 [95] provides further evidence for general similarities, but also fine differences between Cu(II) and Ni(II) coordination by histidine peptides. In this case only the square-planar N-terminal complex, identical to that of DRVY could be characterised spectroscopically, due to precipitation of complexes between pH 5 and 9. However, the comparison of species distributions of the two functional subunits of AT2, DRVY and AcYIH, (Fig. 10) clearly shows that the former peptide anchors Ni(II) much more efficiently than the latter.

5.6. Introduction of more than one His residue in the oligopeptide

His–His is the simplest peptide meeting the above criterion. Its coordination properties towards Cu(II) was studied [96]. Three major coordination modes were found, depending on pH. At pH 4 a CuHL species formed, with histidine-like binding through the N-terminal $\{NH_2, N_{im}\}$ donor set. At pH 5–6 the complex rearrangement takes place, and the resulting CuL species has a structure analogous to the $CuH_{-1}L$ complex of Gly–His (Fig. 9a). The imidazole ring of the His-1

residue becomes protonated, to deprotonate again with a pK value of ca. 7. A dimeric $CuH_{-2}L_2$ complex results, related to that of carnosine (Fig. 11).

Extension of the His–His sequence in His–His–Gly–Gly does not alter its coordination properties towards Cu(II), as indicated by spectroscopic studies at weakly alkaline pH [97]. The complex formed in the same conditions by His–Gly–His–Gly seems to have an identical donor set with that of Gly–Gly–His.

On the other hand, the complexation pattern of Gly–His–Gly–His towards Cu(II) [98] is quite complicated. The initial tetragonal Gly–His-like coordination mode of the CuL complex, present at pH 4–5, turns into a square pyramid upon the axial binding of the His-4 imidazole at pH 6 (a $CuH_{-1}L$ complex). A further rearrangement follows at pH 7, yielding a $CuH_{-2}L$ species with the proposed structure of a distorted trigonal bipyramid. The equatorial coordination in this complex is provided by a triglycine-like $\{NH_2, 2 \times N^-\}$ donor set, and both imidazole donors provide the axial binding.

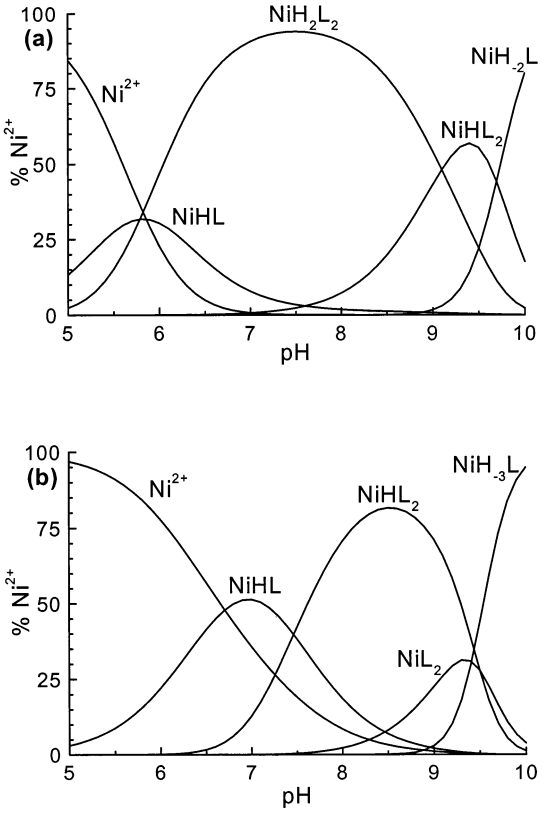

Fig. 10. Species distribution curves for Ni(II) complexes of: (a) Asp–Arg–Val–Tyr; and (b) MeCO–Tyr–Ile–His; initial metal ion concentration 1 mmol dm^{-3} and metal-to-ligand molar ratio 1:6.

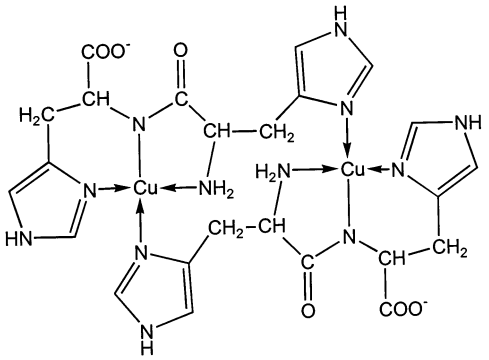

Fig. 11. The proposed structure of the $[(CuH_{-1}L)_2]$ dimer His–His.

The complex-forming properties of the further extended alternate Gly–His sequence, Gly–His–Gly–His–Gly–His–Gly–His [99], provided more evidence that the selection of coordination mode by the Cu(II) ion depends on subtle alterations of the peptidic environment. At pH 5 Cu(II) is thought to bind through imidazole nitrogen donors of all four His residues (Fig. 12), while at higher pH some (not identified) binding through deprotonated amides takes place. This tendency is found in polyhistidine as well [100], where both imidazole and deprotonated nitrogen donors were found to participate in Cu(II) binding at neutral pH.

Ni(II) complexes of peptides possessing His residues in positions 1 and 3 (pNiXa-1, His–Arg–His–Arg–His–Glu–Gln–Gln–Gly–His–His–Asp–Ser–Ala–Lys–His–Gly–His [101]), as well as 2 and 3 (Ser–His–His–Lys–NH_2, [102]) were found to be analogous to the square planar complex of Xaa–Yaa–His. Quite naturally, the N-terminal extension of the peptide, in Ac–Thr–Glu–Ser–His–His–

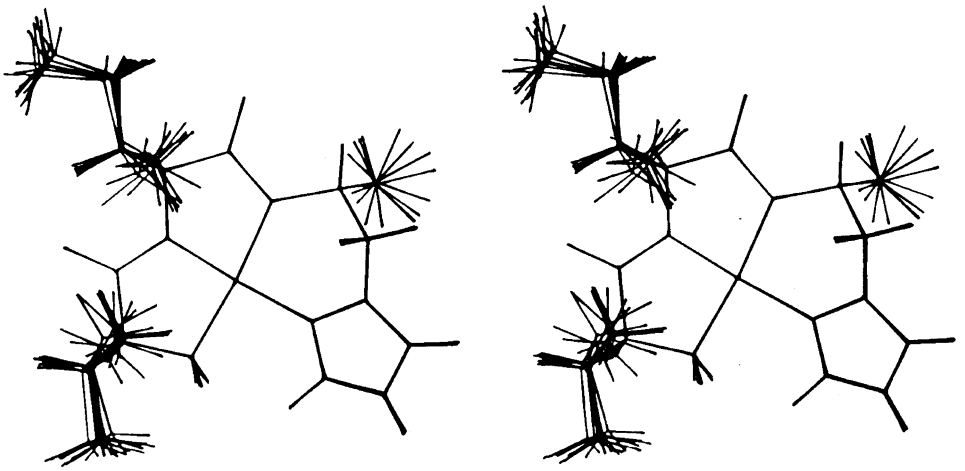

Fig. 12. Fig. 1, Ref [104]—need permission.

Lys–NH_2, results in a very different Ni(II) binding, and the formation of an octahedral 1:1 complex at neutral pH, with the binding through the side chains of His and Glu residues [102]. A similar tendency is found for Gly–His–Gly–His–Gly–His–Gly–His, which was shown to coordinate Ni(II) with imidazole nitrogens of all four His residues [99]. Because of the higher number of donor atoms available, this complex is square-planar. On the other hand, the Ni(II) complexes of pNiXa peptides, Ac–Lys–His–Arg–His–Arg–His–Glu and *N*-acetylated pNiXa-2 are octahedral in weakly acidic solution. Most likely, the bigger number of available imidazole donors results in 5N and 6N complexes [101].

The lesser ability of Ni(II) to deprotonate amide nitrogens, compared to Cu(II), together with the formation of a rigid square-planar complex, may result, somewhat paradoxically, in a higher specificity of Ni(II) to produce a particular (and foreseeable) bent conformation of the peptide at physiological pH. This effect was proposed, on the basis of CD studies, for Gly–His–Gly–His–Gly–His–Gly–His [99]. The examples gathered in this review allow to support this view. It is clear that Cu(II) will generally bind locally, within two, three, or maximally four residues. Due to its geometrical plasticity, it has an ability to form rather irregular structures, and equatorial and axial ligands can easily swap. Ni(II) complexes with oligopeptides containing more than one histidine studied so far present only two coordination styles at physiological pH: either an imidazole-only binding, or a Gly–Gly–His-like coordination, when the $\{NH_2,\ 2\times N^-,\ N_{im}\}$ donor set is available.

5.7. Secondary effects in complexes with Xaa–Yaa–His ligands

The 4N environment around Cu(II) provided by Xaa–Yaa–His ligands creates a situation similar to that of simple oligopeptides with non-bonding side chains. Due to the extreme stability of this binding mode, even the incorporation of potentially binding side chains in position one or two does not influence the donor set. This effect can be seen in the N-terminal binding site of human serum albumin, Asp–Ala–His–Lys–. The Asp carboxylate does not participate in the binding of Cu(II) or Ni(II) [103]. Not even His residues in position one or two can affect this coordination mode (see above). However, secondary interactions, markedly influencing complex stability, are possible. The stability constant of the square-planar $NiH_{-2}L$ complex of Val–Ile–His–Asn [104] is 100-fold higher than that of Gly–Gly–His (Table 6). The complex structure in solution determined by NMR techniques revealed the presence of a hydrophobic fence of bulky aliphatic side chains of Val and Ile residues (Fig. 13). This fence effectively shields one side of the complex plane from the access of water molecules from the bulk of solution. As a result, the rate of water-assisted hydrolysis of N^-–Ni(II) bonds slows down, thereby increasing complex stability. The phenomenon of side chain ordering induced by main chain coordination, found also for NSFRY analogues described above, may provide a tool for designing functional metal binding sites in proteins.

The presence of arginine in position one in $HP2_{1-5}$ (Arg–Thr–His–Gly–Asn–NH_2, the N-terminal pentapeptide of human protamine HP2) [78], increased the

Table 6
Comparison of log β and log $*K$ values for selected 4N complexes of Xaa–Yaa–His peptides with Cu(II) and Ni(II)[a]

Peptide	Cu(II)		Ni(II)		Ref.
	Log β	Log $*K$	Log β	Log $*K$	
Gly–Gly–His	−1.73	−16.43	−6.93	−21.81	77
Gly–Gly–His	−1.55	−16.33			76
Gly–Gly–hist	−2.48	−17.14	−7.99	−22.65	79
Val–Ile–His–Asn			−5.39	−19.75	104
$HP2_{1-5}$	−1.11	−14.24	−5.95	−19.23	78
$HP2_{1-15}$[b]	−0.96	−13.13	−5.95	−19.29	78

[a] Unless stated otherwise, log β values correspond to $MH_{-2}L$ complexes, M denotes Cu(II) or Ni(II).
[b] CuH_2L and NiHL complexes, respectively.

stability of Cu(II) and Ni(II) complexes even more than the hydrophobic effect in Val–Ile–His–Asn. One reason for the stability gain was ascribed to selective electronic and/or electrostatic effects of the guanidinium group (lowering of the pK of the terminal amine) that do not affect the metal coordination site (the net charge of which is zero). Further effects are also possible. The stability of the equivalent Cu(II) complex formed by the C-terminally extended pentadecapeptide $HP2_{1-15}$ is

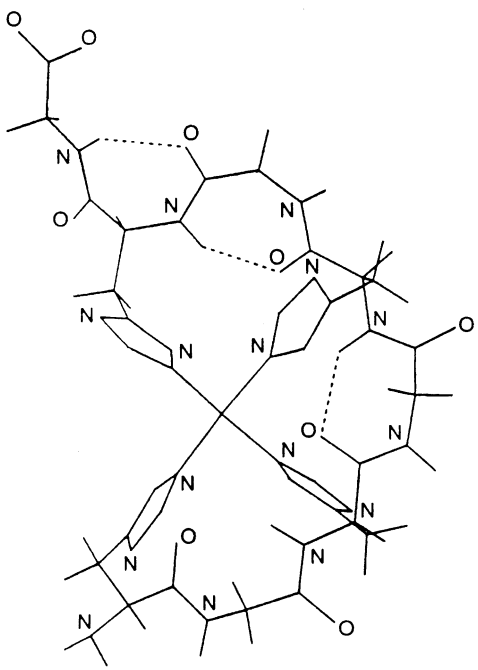

Fig. 13. Fig. 2b, Ref [99]—need permission.

higher than that of pentapeptide, while Ni(II) complexes do not differ in this respect. This fact, together with the partial ordering of the peptide seen in CD spectra, may indicate the presence of further long-range interactions in this complex. Similarly interpreted CD spectral effects were presented for neuromedin C, a neurohormone sharing the Xaa–Yaa–His motif [105], although no thermodynamical data were presented to support this opinion.

The data presented in [78] allows one to propose that the presence of positively charged side-chains somewhat paradoxically increases stabilities of 4N complexes, so that they may be physiologically relevant. This fact is of particular importance for metal toxicology and carcinogenesis, while complexes of Xaa–Yaa–His peptides are able to inflict oxidative DNA damage, and the presence of positively charged residues increases their specificity [106–111].

6. Specific peptide hydrolysis in His peptides

There is emerging evidence that the coordination of Cu(II) to histidine peptides may result in a specific peptide hydrolysis reaction which does not involve oxidative reactions. An extensive study revealed a particular susceptibility of Xaa–Ser–His and Xaa–Thr–His sequences to Cu(II)-assisted hydrolysis at alkaline pH, with specific hydrolysis of the Xaa–Ser(Thr) bond [112]. The proposed driving force of this reaction is a high stability of the Cu(II) complex of the leaving Ser(Thr)–His complex, but the presence of a side-chain hydroxyl is instrumental for the formation of a transition state complex, thus providing reaction specificity. A similarly specific cleavage reaction was found for the breaking of the Gly–Sar bond in a Gly–Sar–His complex of Cu(II) [113]. The active intermediate in this case seems to be provided by the three-coordinate macrochelate structure of the alkaline pH complex, enforced by a break-point enforcing Sar (*N*-methylglycine) residue. Again, the hydrolysis product is a tight Gly–His-like complex of Sar–His.

Interestigly, Ni(II) complexation to Ac–Thr–Glu–Ser–His–His–Lys–NH_2 results in a slow, but extremely specific hydrolysis of the Glu–Ser peptide bond at physiological conditions [102]. The reaction mechanism is apparently similar to that proposed above for Cu(II). The milder reaction conditions, and consequently a higher hydrolytic activity of Ni(II) in this particular system result from the fact that the reaction product is a square-planar complex of the Ser–His–His–Lys–NH_2 peptide, in which a 4N coordination yields a higher product stabilisation than does a 3N coordination of Ser(Thr)–His peptides. The Ni(II) reaction may be of particular importance for molecular mechanisms of nickel carcinogenesis [102].

Appendix A

1. Protonation reaction below can be quantitatively described by the equilibrium constant, K, as well as by the stability constant, β:

$$L^{2-} + H^{+} \overset{K_1}{\leftrightarrow} HL^{-}$$

$$\beta_1 = K_1 = \frac{[HL^{-}]}{[L^{2-}][H^{+}]}$$

In a general case of a molecule binding i hydrogen ions:

$$\beta_i = \frac{[H_iL]}{[L][H^{+}]^i}$$

2. Similar constants can be defined for metal ion coordination:

$$M^{a+} + H_2L \overset{K_I}{\leftrightarrow} MH_2L^{a+}$$

$$\beta_I = \frac{[MH_2L^{a+}]}{[M^{a+}][H^{+}]^2[L]}$$

In general, for a complex containing i metal ions, j hydrogen ions and k ligand molecules:

$$\beta_{ijk} = \frac{[M_iH_jL_k]}{[M]^i[H^{+}]^j[L]^k}$$

Note that for reactions involving proton displacement from amide groups by a metal ion, j can assume negative values. This is because amide protons do not dissociate freely, and therefore cannot be introduced into the ligand formula.

Reaction of a metal ion with a ligand can be written as a proton competition reaction:

$$M^{a+} + H_nL \overset{{}^*K}{\leftrightarrow} [MH_jL]^{(a-n+j)+} + (n-j)\ H^{+}$$

The equilibrium constant for such reactions is denoted $^*K$. Values for $^*K$ constants can be easily derived from stability constants:

$$\log\ {}^*K = \log(\beta(CuH_jL)) - \log(\beta(H_nL))$$

References

[1] L.D. Pettit, J.E. Gregor, H. Kozłowski, in: R.W. Hay, J.R. Dilworth, K.B. Nolan (Eds.), Perspectives on Bioinorganic Chemistry, vol. 1, JAI Press, London, 1991, pp. 1–41.
[2] W. Bal, M. Dyba, H. Kozłowski, Acta Biochim. Pol. 44 (1997) 467.
[3] R.A. Bradshaw, W.T. Shearer, F.R.N. Gurd, J. Biol. Chem. 243 (1968) 3817.
[4] J.D. Glennon, B. Sarkar, Biochem. J. 203 (1982) 15.
[5] P.J. Sadler, A. Tucker, J.H. Viles, Eur. J. Biochem. 220 (1994) 193.
[6] S.U. Patel, P.J. Sadler, A. Tucker, J. Viles, J. Am. Chem. Soc. 115 (1993) 9285.
[7] R.H. Pain (Ed.), Mechanisms of Protein Folding, IRL Press, Oxford, 1994.
[8] M.M. Yamashita, L. Wesson, G. Eisenman, D. Eisenberg, Proc. Natl. Acad. Sci. 87 (1990) 5648.
[9] I. Sovago, in: K. Burger (Ed.), Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems, Ellis Horwood, Chichester, 1990, pp. 135–184.
[10] H. Sigel, R.B. Martin, Chem. Rev. 82 (1982) 385.

[11] B. Strandberg, I. Lindqvist, R. Rosenstein, Z. Kristallogr. 116 (1961) 266.
[12] H.C. Freeman, G. Robinson, J.C. Schoone, Acta Crystallogr. 17 (1964) 719.
[13] H.C. Freeman, J.C. Schoone, J.G. Sime, Acta Crystallogr. 18 (1965) 381.
[14] H.C. Freeman, M.R. Taylor, Acta Crystallogr. 18 (1965) 939.
[15] J.F. Blount, H.C. Freeman, R.V. Holland, G.H.W. Milburn, J. Biol. Chem. 245 (1970) 5177.
[16] B. Decock-Le Reverend, L. Andrianarijaona, C. Livera, L.D. Pettit, I. Steel, H. Kozłowski, J. Chem. Soc. Dalton Trans. (1986) 2221.
[17] W. Bal, H. Kozłowski, G. Kupryszewski, Z. Maækiewicz, L.D. Pettit, R. Robbins, J. Inorg. Biochem. 52 (1993) 79.
[18] C.R. Hartzell, F.R.N. Gurd, J. Biol. Chem. 244 (1969) 147.
[19] J.J. Czarnecki, D.W. Margerum, Inorg. Chem. 16 (1977) 1997.
[20] R.B. Martin, in: H. Sigel (Ed.), Metal Ions in Biological Systems, vol. 23, Marcel Dekker, New York, 1987, p. 123.
[21] H.C. Freeman, J.M. Guss, Acta Crystallogr. B34 (1978) 2451.
[22] H.C. Freeman, J.M. Guss, R.L. Sinclair, Acta Crystallogr. B34 (1978) 2459.
[23] W. Bal, M. Dyba, F. Kasprzykowski, H. Kozłowski, R. Latajka, L. Kankiewicz, Z. Maćkiewicz, L.D. Pettit, Inorg. Chim. Acta 283 (1998) 1.
[24] O. Yamauchi, A. Odani, J. Am. Chem. Soc. 107 (1985) 5938.
[25] G. Liang, R. Tribolet, H. Sigel, Inorg. Chim. Acta 155 (1989) 273.
[26] L. Xiao, M. Jouini, B.T. Fan, G. Lapluye, J. Chem. Soc. Dalton Trans. (1990) 1137.
[27] B. Radomska, T. Kiss, J. Coord. Chem. 21 (1990) 81.
[28] L.D. Pettit, S.Pyburn, B. Decock-Le Reverend, A. Lebkiri, J. Chem. Soc. Dalton Trans. (1989) 235.
[29] H. Kozłowski, Inorg. Chim. Acta 31 (1978) 135.
[30] T. Kiss, J. Chem. Soc. Dalton Trans. (1987) 1263.
[31] G. Formicka-Kozłowska, L.D. Pettit, I. Steel, C. Livera, J. Inorg. Biochem. 24 (1985) 299.
[32] H. Kozłowski, B. Radomska, G. Kupryszewski, B. Lammek, C. Livera, L.D. Pettit, S. Pyburn, J. Chem. Soc. Dalton Trans. (1989) 173.
[33] W. Bal, H. Kozłowski, B. Lammek, L.D. Pettit, K. Rolka, J. Inorg. Biochem. 45 (1992) 193.
[34] G. Valensin, A. Maccotta, E. Gaggelli, Z. Grzonka, F. Kasprzykowski, H. Kozłowski, Eur. J. Biochem. 240 (1996) 118.
[35] T. Kowalik-Jankowska, H. Kozłowski, M. Stasiak, M.T. Leplawy, J. Coord. Chem. 40 (1996) 113.
[36] T. Kowalik-Jankowska, M. Stasiak, M.T. Leplawy, H. Kozłowski, J. Inorg. Biochem. 66 (1997) 193.
[37] E. Chruścińska, G. Micera, D. Sanna, H. Kozłowski, K. Kaczmarek, J. Olejnik, M.T. Leplawy, J. Inorg. Biochem., submitted for publication.
[38] H. Kozłowski, Proceedings of the 9th Conference on Coordinaton Chemistry, Smolenice, Bratislava, 1983, p. 201.
[39] H. Kozłowski, M. Bezer, L.D. Pettit, M. Bataille, B. Hecquet, J. Inorg. Biochem. 18 (1983) 231.
[40] L.D. Pettit, I. Steel, B. Hartrotd, K. Neubert, P. Rekowski, G. Kupryszewski, J. Inorg. Biochem. 22 (1984) 155.
[41] L.D. Pettit, I. Steel, T. Kowalik, H. Kozłowski, M. Bataille, J. Chem. Soc. Dalton Trans. (1985) 1201.
[42] G. Formicka-Kozłowska, D. Konopinska, H. Kozłowski, B. Decock-Le Reverend, Inorg. Chim. Acta 78 (1983) 47.
[43] C. Livera, L.D. Pettit, M. Bataille, J. Krembel, W. Bal, H. Kozłowski, J. Chem. Soc. Dalton Trans. (1988) 1357.
[44] L.D. Pettit, C. Livera, I. Steel, M. Bataille, C. Cardon, G. Formicka-Kozłowska, Polyhedron 6 (1987) 45.
[45] L.D. Pettit, W. Bal, M. Bataille, C. Cardon, H. Kozłowski, M. Leseine-Delstanche, S. Pyburn, A. Scozzafava, J. Chem. Soc. Dalton Trans. (1991) 1651.
[46] M. Bezer, L.D. Pettit, I. Steel, M. Bataille, S. Djemil, H. Kozłowski, J. Inorg. Biochem. 20 (1984) 13.

[47] L. Chruściński, M. Dyba, M. Jeżowska-Bojczuk, H. Kozłowski, G. Kupryszewski, Z. Maíkiewicz, A. Majewska, J. Inorg. Biochem. 63 (1996) 49.
[48] H. Kozłowski, G. Formicka-Kozłowska, L.D. Pettit, I. Steel, in: A.V. Xavier (Ed.), Frontiers in Bioinorganic Chemistry, VCH, Weinheim, 1986, p. 668.
[49] Z.-Q. Tian, P.A. Bartlett, J. Am. Chem. Soc. 118 (1996) 943.
[50] S Sjoberg, Pure Applied Chem 69 (1997) 1549.
[51] E. Farkas, I. Sovago, A. Gergely, J. Chem. Soc. Dalton Trans. (1983) 1545.
[52] G. Brookes, L.D. Pettit, J. Chem. Soc. Dalton Trans. (1975) 2112.
[53] A. Yokoyama, H. Aiba, H. Tanaka, Bull. Chem. Soc. Japan 47 (1974) 112.
[54] I. Sovago, G. Petocz, J. Chem. Soc. Dalton Trans. (1987) 1717.
[55] R. Agarwal, D. Perrin, J. Chem. Soc. Dalton Trans. (1975) 268.
[56] P. Daniele, O. Zerbinati, R. Aruga, G. Ostacoli, J. Chem. Soc. Dalton Trans. (1988) 1115.
[57] P. Daniele, O. Zerbinati, V. Zelano, G. Ostacoli, J. Chem. Soc. Dalton Trans. (1991) 2711.
[58] P.J. Morris, R.B. Martin, J. Inorg. Nucl. Chem. 33 (1971) 2913.
[59] I. Sovago, E. Farkas, A. Gergely, J. Chem. Soc. Dalton Trans. (1982) 2159.
[60] M. Rainer, B. Rode, Inorg. Chim. Acta 107 (1985) 127.
[61] E. Farkas, I. Sovago, T. Kiss, A. Gergely, J. Chem. Soc. Dalton Trans. (1984) 611.
[62] K. Takehara, Y. Ide, Inorg. Chim. Acta 183 (1991) 195.
[63] T. Gajda, B. Henry, J.-J. Delpuech, J. Chem. Soc. Dalton Trans. (1993) 1301.
[64] P. May, J. Whittaker, D. Williams, Inorg. Chim. Acta 80 (1983) 5.
[65] S. Lau, B. Sarkar, Biochem. J. 199 (1981) 647.
[66] L. Pickart, S. Lovejoy, Methods Enzymol. 147 (1987) 314.
[67] J.F. Blount, K.A. Fraser, H.L. Freeman, J.T. Szymanski, C.-H. Wong, Acta Crystallogr. 22 (1967) 396.
[68] R. Osterberg, B. Sjoberg, R. Soderqvist, J. Chem. Soc. Chem. Commun. (1972) 983.
[69] C.M. Perkins, N.J. Rose, B. Weinstein, R.E. Stenkamp, L.H. Jensen, L. Pickart, Inorg. Chim. Acta 82 (1984) 93.
[70] H. Masuda, A. Odani, O. Yamauchi, Inorg. Chem. 28 (1989) 624.
[71] T. Gajda, B. Henry, J.-J. Delpuech, Inorg. Chem. 34 (1995) 2455.
[72] C.E. Brown, W.E. Antholine, J. Phys. Chem. 83 (1979) 3314.
[73] J. Baran, B.S. Parajon-Costa, T. Rojo, R. Saez-Puche, F. Fernandez, R.M. Totaro, M.C. Apella, S.B. Etcheverry, M.H. Torre, J. Inorg. Biochem. 58 (1995) 279.
[74] H.C. Freeman, J.T. Szymanski, Acta Crystallogr. 22 (1967) 406.
[75] H. Aiba, A. Yokoyama, H. Tanaka, Bull. Chem. Soc. Jpn. 47 (1974) 1437.
[76] E. Farkas, I. Sovago, T. Kiss, A. Gergely, J. Chem. Soc. Dalton Trans. (1984) 611.
[77] R Hay, M Hassan, C. You-Quan, J. Inorg. Biochem. 52 (1993) 17.
[78] W. Bal, M. Jeżowska-Bojczuk, K.S. Kasprzak, Chem. Res. Toxicol. 10 (1997) 906.
[79] T. Gajda, B. Henry, A. Aubry, J.-J. Delpuech, Inorg. Chem. 35 (1996) 586.
[80] P.L. Meredith, R.A. Palmer, Inorg. Chem. 10 (1971) 1049.
[81] B. Kurzak, W. Bal, H. Kozłowski, J. Inorg. Biochem. 38 (1990) 9.
[82] J.D. Glennon, B. Sarkar, Biochem. J. 203 (1982) 15.
[83] W. Bal, P.J. Sadler, unpublished results.
[84] C.E. Bannister, J.M.T. Raycheba, D.W. Margerum, Inorg. Chem. 21 (1982) 1106.
[85] N. Camerman, A. Camerman, B. Sarkar, Can. J. Chem. 54 (1976) 1309.
[86] P. de Meester, D.G. Hodgson, Inorg. Chem. 17 (1978) 440.
[87] W. Bal, M.I. Djuran, D.W. Margerum, E.T. Gray, Jr., M.A. Mazid, R.T. Tom, E. Nieboer, P.J. Sadler, J. Chem. Soc. Chem. Commun. (1994) 1889.
[88] L.D. Pettit, S. Pyburn, W. Bal, H. Kozłowski, M. Bataille, J. Chem. Soc. Dalton Trans. (1990) 3565.
[89] B. Decock-Le Reverend, F. Liman, C. Livera, L.D. Pettit, S. Pyburn, H. Kozłowski, J. Chem. Soc. Dalton Trans. (1988) 887.
[90] W. Bal, H. Kozłowski, R. Robbins, L.D. Pettit, Inorg. Chim. Acta 231 (1995) 7.
[91] W. Bal, M. Jeżowska-Bojczuk, J. Lukszo, K.S. Kasprzak, Chem. Res. Toxicol. 8 (1995) 683.
[92] W. Bal, J. Lukszo, K.S. Kasprzak, Chem. Res. Toxicol. 9 (1996) 435.

[93] W. Bal, K.S. Kasprzak, in: N. Hadjiliadis (Ed.), Cytotoxic, Mutagenic and Carcinogenic Potential of Heavy Metals Including Metals Related to Human Environment, NATO ASI series, Kluwer, Dordrecht, 1997, pp. 107–121.
[94] W. Bal, M. Jeżowska-Bojczuk, H. Kozłowski, L. Chruściński, G. Kupryszewski, B. Witczuk, J. Inorg. Biochem. 57 (1995) 235.
[95] L.D. Pettit, S. Pyburn, H. Kozłowski, B. Decock-Le Reverend, F. Liman, J. Chem. Soc. Dalton Trans. (1989) 1471.
[96] C. Livera, L.D. Pettit, M. Bataille, H. Kozłowski, B. Radomska, J. Chem. Soc. Dalton Trans. (1987) 661.
[97] J. Ueda, N. Ikota, A. Hanaki, K. Koga, Inorg. Chim. Acta 135 (1987) 43.
[98] R. Bonomo, F. Bonsignore, E. Conte, G. Impellizzeri, G. Papalardo, R. Purrello, E. Rizzarelli, J. Chem. Soc. Dalton Trans. (1993) 1295.
[99] R.P. Bonomo, L. Casella, L. De Gioia, H. Molinari, G. Impellizzeri, T. Jordan, G. Pappalardo, R. Purrello, E. Rizzarelli, J. Chem. Soc. Dalton Trans. (1997) 2387.
[100] A. Levitzki, I. Pecht, A. Berger, J. Am. Chem. Soc. 94 (1972) 6844.
[101] F.W. Sunderman Jr., A.H. Varghese, O.S. Kroftova, S. Grbac-Ivankovic, J. Kotyza, A.K. Datta, M. Davis, W. Bal, K.S. Kasprzak, Mol. Reprod. Dev. 44 (1996) 507.
[102] W. Bal, J. Lukszo, K. Bialkowski, K.S. Kasprzak, Chem. Res. Toxicol. 11 (1998) 1014.
[103] P.J. Sadler, A. Tucker, J.H. Viles, Eur. J. Biochem. 220 (1994) 193.
[104] W. Bal, G.N. Chmurny, B.D. Hilton, P.J. Sadler, A. Tucker, J. Am. Chem. Soc. 118 (1996) 4727.
[105] C. Harford, B. Sarkar, Biochem. Biophys. Res. Commun. 209 (1995) 877.
[106] W. Bal, J. Lukszo, K.S. Kasprzak, Chem. Res. Toxicol. 10 (1996) 915.
[107] D.F. Shullenberg, P. Denney Eason, E.C. Long, J. Am. Chem. Soc. 115 (1993) 11038.
[108] Q. Liang, P. Denney Eason, E.C. Long, J. Am. Chem. Soc. 117 (1995) 9625.
[109] Q. Liang, D.C. Ananias, E.C. Long, J. Am. Chem. Soc. 120 (1998) 248.
[110] D.P. Mack, P.B. Dervan, Biochemistry 31 (1992) 9399.
[111] K.C. Brown, S.-H. Yang, T. Kodadek, Biochemistry 34 (1995) 4739.
[112] G. Allen, R.O. Campbell, Int. J. Pept. Protein Res. 48 (1996) 265.
[113] J. Ueda, M. Miyazaki, Y. Matsushima, A. Hanaki, J. Inorg. Biochem. 63 (1996) 29.