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Review

Chemical and biological aspects of Cu^{2+} interactions with peptides and aminoglycosides

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Contents

Abstract

The coordination ability of histidine containing peptides towards Cu^{2+} ion is discussed. The binding ability of the peptide strongly depends on the position and number of histidine residues in the peptide sequence, the vicinal amino acid residues, and in poly-histidine peptides on the distance between the His residues within the peptide chain. The imidazole nitrogen of the His residues usually act as an anchoring site and multi-histidine Cu^{2+} binding is extremely effective; this results in the stabilization of very specific peptide structures. The nitrogen atom of the N-terminal amino group may compete with imidazole to bind Cu^{2+} ion and both of them may form an effective macrochelate coordination when they are close to each other.

Amino groups of aminoglycosides are basic and very efficient binding sites for Cu^{2+} ions. The metal ion coordination to aminoglycoside antibiotics may dramatically change the pharmacological effect inducing oxidative reactions. These reactions when induced in the human body may be the reason for the side-effects caused by aminoglycosidic antibiotics.

Keywords: Cu^{2+} complexes; Histidine peptides; Aminoglycoside antibiotics; Oxidative reactions; Biological implications

1. Introduction

Cu^{2+} ion involvement in biological processes is well established although in many cases the molecular mechanisms are still unclear. In some cases, like in neurodegeneration processes, the role of metal ions, including copper, seems to be

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very obvious but details are very badly understood. Histidine residues play a critical role in the interactions of metal ions with peptides and proteins, and the details of these interactions are crucial to understand the biological consequences.

Nitrogen donors are also crucial in the binding of Cu^{2+} ion by many other biomolecules including widely used aminoglycoside antibiotics. The binding of Cu^{2+} to this family of ligands has very unexpected consequences including catalytically induced oxidative reactions. The latter reactions may be responsible for the side effects of these antibiotics including oto- and nephro-toxicity.

2. Coordination abilities of peptides containing histidine residue(s) at different positions in a peptide sequence

The histidine side chain, and in particular the imidazole moiety, is a basic binding site for the majority of metal ions including Cu^{2+} [1]. The metal ion coordination generally takes place via N(1) or N(3) atoms of imidazole as is found in a great variety of iron, zinc or copper proteins including myoglobin and other heme proteins, carbonic anhydrase, carboxypeptidase and blue copper proteins. Another group of metalloenzymes, contains the imidazole moiety as a bridging ligand (e.g. superoxide dismutases and tyrosinases) where both nitrogen atoms of the negatively charged imidazolato residue take part in metal binding [2].

The recent discoveries that mammalian prions as well as amyloid precursor proteins may involve copper binding in their function have indicated that the specific multi-histidine metal ion binding sites could also be critical for biological processes involving these proteins.

In peptides containing non-coordinating side chains, the N-terminal amino group is usually the principal binding site for biologically relevant metal ions such as Cu^{2+}, Ni^{2+}, Zn^{2+} or Pt^{2+}. This group acts as a primary anchoring site for the metal ions and, as such, is then able to promote the stepwise deprotonation and coordination of successive peptide amide nitrogen donors [3,4], leading to the formation of hydrolytically stable, fused, five-membered chelate rings with M–N bonds. However, the imidazole ring of the histidine (His, H) residue containing a pyridine-like nitrogen atom may also act as a primary ligation site for these metal ions [5]. Consequently, the very stable complexes are formed when the terminal amino and imidazole donor groups are in close proximity to form a chelate ring.

Many studies have been performed in this field and the most important observations have already been reviewed [3,4,6–9]. The results of these studies reveal that the presence of histidine in the peptide chain significantly enhances the metal binding ability of the ligand, but the extent of the increase in the stability and structure of various complexes largely depends upon the location of histidyl residues in the peptide chain and the presence of free or blocked amino group. The metal complexes of peptides containing two or more imidazole residues are also frequently used to mimic the structural and catalytic features of the active sites of metalloproteins. In this review, we report the coordination abilities of peptides containing one or more histidine residues to Cu^{2+} ions depending on the position of this residue and the presence of free or blocked amine group in a peptide chain.

2.1. Cu^{2+}–His^1—peptide complexes

Peptides containing a His residue at the N-terminal position (His^1) bind Cu^{2+} differently from ordinary peptides. The complex of Cu^{2+} ion with the histamine-like coordination $\{NH_2, N_{Im}\}$ is formed around pH 4–5 (Fig. 1) and above pH 5 the dimeric complex $Cu_2H_{-2}L_2$ is formed through Gly-Gly-like coordination with the imidazole nitrogen acting as a bridging ligand. This structure has been proposed for His-Gly and His-Met peptides [7], as well as for His-His [10] and His-Lys [11] dipeptides. The longer peptides (e.g. His-Ser-Asp-Gly-Ileu-NH_2) containing His^1 residue may form the dimeric species but the presence of this species does not prevent the deprotonation and the coordination of a subsequent amide nitrogen(s) at higher pH [12,13]. The deprotonation and coordination of the first amide nitrogen to copper(II) ion occurs above pH 6, while for oligoglycines at pH around 5 [10–13].

Much less is known about Cu^{2+} complexes with peptides having a His residue at the N-terminal position with a protected amino group. In this case, the simultaneous coordination of the imidazole nitrogen and two or three flanking deprotonated amide groups could lead to the formation of less stable seven-member and one or two five-member rings. For the Cu^{2+}–Z–His-GlyOH (Z—the protection group transforms the terminal amino group into a Ph-CH_2-O-C(O)-NH-function of the carbamate type) [14] and Cu^{2+}–Ac–His-Val-Gly-Asp-NH_2 (Ac—acetyl group, CH_3–C(O)–NH–) [15] systems, precipitates are observed over a wide pH range, although at low pH range the imidazole nitrogen atom is

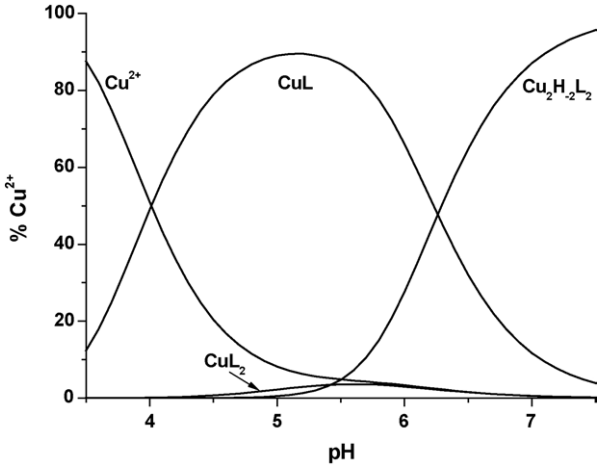

Fig. 1. Species distribution curves for Cu^{2+} complexes of His-Gly. Cu^{2+}-to-peptide molar ratio 1:1; $[Cu^{2+}] = 0.001$ M.

coordinated. In solution above pH 9, Cu^{2+} ions are coordinated by the amide nitrogen atoms while oligonuclear chain structures are proposed for complexes with Z-His-GlyOH with the imidazole nitrogen acting as a bridging moiety [14].

2.2. Cu^{2+}–His^2—peptide complexes

The histidine residue in the second position (His^2) of the peptide chain with an unprotected N-terminal amine group allows for the simultaneous involvement of the amine, the imidazole and the His amide nitrogen to bind Cu^{2+} ions. For Gly-His, Gly-Histamine, Gly-His-Gly [7], and also for HmS-His (HmS—α-hydroxymethylserine) [16], Ala-His, Ala-His-Ala, Gly-His-Leu [17], and Gly-His-Lys [18], the major complex over pH 4–9 is the $CuH_{-1}L$ monomeric species with $\{NH_2, N^-_{amide}, N_{Im}\}$ binding sites. The very high stability of this species results from the formation of the five-membered $\{NH_2, N^-_{amide}\}$ and six-membered $\{N^-_{amide}, N_{Im}\}$ chelate rings. This chelate system uses only three of four equatorial coordination positions around the Cu^{2+} ion. The fourth position can be occupied by a second molecule of a peptide or by the deprotonated N^1(imidazole) donor atom of another peptide unit leading to formation of a tetrametric $Cu_4H_{-8}L_4$ with imidazole bridges [16,17,19,20]. The X-ray structures obtained for complexes with Gly-His, Gly-His-Gly and Gly-His-Lys support the 3N coordination mode in the $CuH_{-1}L$ species [7].

In the Cu^{2+}–carnosine (β-alanylhistidine) system [7], equilibrium and spectroscopic studies in solution and X-ray analysis in the solid phase definitely prove the formation of the dimeric species $Cu_2H_{-2}L_2$, in which the metal ions are coordinated via terminal amino, deprotonated amide nitrogen, and carboxylate groups, while the imidazole–N3 donor atoms serve as the bridging units. Such coordination provides the 5 + 6-membered chelate ring system, which is more efficient than the 6 + 6 ring system. This type of coordination is also possible with β-Asp-His, however, the amino acid-like coordination of Asp results in the enhanced stability of mono and bis complexes without involvement of the amide group [21]. The complex $Cu_2H_{-2}L_2$ for β-Asp-His is the main species above physiological pH range (Fig. 2). In the Cu^{2+}–carnosine system, the dinuclear species is formed at lower pH, which shows that the simple amino acid-like binding sites of β-Asp-His slightly suppress the deprotonation and coordination of amide donors.

The structures of γ-Glu-His and homocarnosine are analogous to those of β-Asp-His and carnosine, but the aliphatic chain in the N-terminal contains one more carbon atom for Glu when compared to Asp. Consequently, the terminal amino and amide nitrogen atoms can form only a seven-membered chelate ring, which is less favored. For the Cu^{2+}–γ-Glu-His system, various mono- and bis-complexes are present up to pH 9, in which the α-amino and carboxylate groups are the main binding sites [21]. In highly basic solution (pH above 9), the formation of hydroxo complexes is suggested.

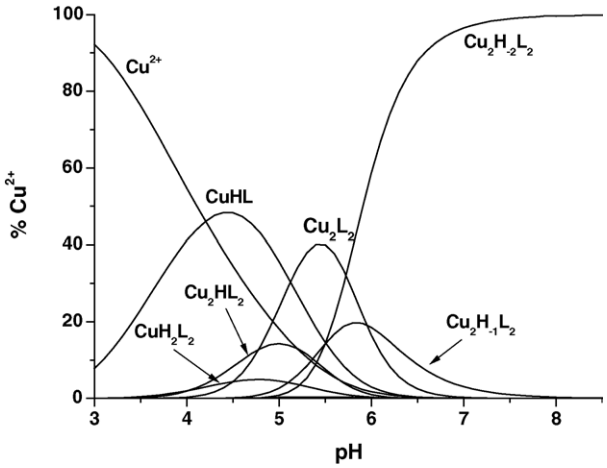

Fig. 2. Species distribution curves for Cu^{2+} complexes of β-Asp-His. Cu^{2+}-to-peptide molar ratio 1:1; $[Cu^{2+}] = 0.001$ M.

N-protected peptides, Z-Gly-His [14] and Ac-Gly-His-Gly-Gly [22] form in the pH range 6–10 the $CuH_{-2}L$ complex with $\{N_{Im}, 2N^-_{amide}\}$ coordination, including the six-membered $\{N_{Im}, N^-_{amide}\}$ and the five-membered $\{N^-_{amide}, N^-_{amide}\}$ chelate rings. The involvement of three nitrogen atoms in the binding of Cu^{2+} ions over the entire pH range studied was observed for Cu^{2+}–thyrotropin releasing factor (pGlu-His-Pro-NH_2, TRF) [23,24], and Cu^{2+}–luteinizing hormone-releasing hormone (LHRH, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) systems [25]. In the CuN_3O chromophore of the Cu^{2+} ion, the fourth position of the equatorial plane is occupied most likely by water molecule [14,22–25], which hydrolyzes at high pH giving the $CuH_{-3}L$ complex.

2.3. Cu^{2+}–His^3—peptide complexes

Peptides containing the N-terminal sequence Xaa-Yaa-His (His^3, albumin-like sequence) exhibit particular affinity towards Cu^{2+} resulting from the formation of three fused chelate rings and a flat four-nitrogen (4N) coordination sphere around the metal ion. The Cu^{2+} complexation with the simplest peptide Gly-Gly-His proceeds cooperatively, with both amide groups deprotonating and bonding to Cu^{2+} between pH 4 and 5 [7]. The 4N species formed in this way are the most stable among the complexes formed by the peptide ligands. The four nitrogen $\{NH_2, 2N^-_{amide}, N_{Im}\}$ coordination mode is observed for most peptide sequences Xaa-Yaa-His: the fragments of β-amyloid human peptide 11-16H (EVHHQK-NH_2) [26,27], the N-terminal pentadecapeptide of human protamine HP2 ($HP2_{1–15}$) [28], and the fragment of the matricellular Secreted Protein, Acidic and Rich in Cysteine (SPARC) Lys-Gly-His-Lys [29]. The $CuH_{-2}L$ species with $\{NH_2, 2N^-_{amide}, N_{Im}\}$ binding mode predominates above pH 7 for the His-Val-His tripeptide containing two His residues [30]. Insertion of α-hydroxymethylserine residues in a Xaa-Yaa-His sequence results in the strongest peptidic,

Table 1
Comparison of log K^* values for the 4N complexes of Xaa-Yaa-His peptides with Cu^{2+} ion

Peptide	log K^*[a]	Reference
Gly-Gly-His	−16.43	[33]
Gly-Gly-Histamine	−17.14	[34]
Arg-Thr-His-Gly-Asn-NH_2	−14.24	[28]
Arg-Thr-His-Gly-Asn-(15)	−13.13	[28]
Glu-Val-His-His-Gln-Lys-NH_2	−15.40	[26]
Glu-Val-His-His-Gln-Lys-(20)	−15.31	[27]
Glu-Val-His-His-Gln-Lys-(28)	−15.34	[27]
His-Val-His	−15.79	[30]
HmS-HmS-His	−13.12	[31]
HmS-HmS-His-NH_2	−11.04	[31]

[a] $\log K^* = \log \beta(CuH_jL) - \log \beta(H_nL)$ (where the index j corresponds to the number of the protons in the coordinated ligand to metal ion and n corresponds to the number of protons coordinated to ligand).

albumin-like Cu^{2+} chelator [31]. A dramatic stability gain of 3.3 log units versus Gly-Gly-His is observed for the $CuH_{-2}L$ species (Table 1) [31]. The studies have indicated that the very acidic ammonium group of the HmS residue changes distinctly the electron density within the amide bond causing the amide nitrogen to become a much more effective donor than those derived from amino acids with more basic amino functions. This unusual gain in the 4N complex stability in the Cu^{2+}–HmS-HmS-His complex seems to derive from enhancement of the π-electron contribution to the metal–amide nitrogen bond [32].

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^{2+} complexes with Gly-Gly-His-N-methylamide, Gly-Gly-Histamine and Gly-Gly-dehydro-Histamine [7].

For the Z-Gly-Gly-His [14] and Ac-Gly-Gly-His-Gly [22] peptides the set of complexes: CuL, $CuH_{-1}L$, $CuH_{-2}L$ and $CuH_{-3}L$, is formed with the $\{N_{Im}\}$, $\{N_{Im}, N^-_{amide}\}$, $\{N_{Im}, 2N^-_{amide}\}$ and $\{N_{Im}, 3N^-_{amide}\}$ coordination modes, respectively. For the peptide containing two His residues, Ac-His-Val-His-NH_2 the $CuH_{-3}L$ complex with $\{N_{Im}, 3N^-_{amide}\}$ binding mode was also observed above pH 8 [15]. The data show that the first pK_1 (amide) value (deprotonation and coordination of amide nitrogen) for the N-protected peptide is significantly higher than that found for the peptides coordinating by the N-terminal amino group [14,27,35]. This derives from the involvement of the carbonyl group (binding via the $\{NH_2, CO\}$ donor set) inducing an electron-withdrawing effect, which favors the deprotonation and coordination of the adjacent amide nitrogen. Instead, in the case of Z-Gly-Gly-His or Ac-Gly-Gly-His-Gly, the ligand is coordinated through the imidazole nitrogen only and such effect favoring deprotonation of the amide nitrogen is lacking.

2.4. Cu^{2+}–His^n ($n \geq 4$)—peptide complexes

When the peptides contain two potential binding sites: the terminal amino group and the imidazole side chain being at the other end of the molecule, separated by 3, 4 and more amino acid residues (His^n where $n \geq 4$), two possible patterns of metal ion binding can occur: a competition between these sites and a cooperativity, i.e. simultaneous coordination by both "sticky" nitrogen atoms. The latter possibility is always observed when there is 0 or 1 spacer residue. In these cases, exemplified by Gly-His and Gly-Gly-His (see above), a synergetic deprotonation of an intermediate amide nitrogen(s) takes place, resulting in the simultaneous formation of fused chelate rings and a very high complex stability. With two amino acid residues spacer, a macrochelate complex $\{NH_2, N_{Im}\}$ is formed [26,27,36–40]. The stability of this macrochelate is decreased with an increase in size of the macrochelate ring. An increase of pH of the solution results in deprotonation and coordination of amide nitrogen atoms adjacent to a terminal amino group. The studies indicate that below pH 10 the following peptides: Gly-Gly-Gly-His, Gly-Gly-Gly-Gly-His and Gly-Gly-Gly-Gly-Gly-His have very similar metal ion speciation with similar binding modes, including the presence of the $\{NH_2, N_{Im}\}$ macrochelate coordination [37]. As one of the consequences of macrochelate formation, the deprotonation and coordination of the third amide nitrogen is significantly disfavored (pK_3 (amide) > 10) when compared to that of pentaglycine (pK_3(amide) = 7.89 [37,38,40]. The peptides containing a His residue at position 4 or further away from the N-terminal, form the $CuH_{-3}L$ complex above pH 9 with $\{NH_2, 3N^-_{amide}\}$ or $\{N_{Im}, 3N^-_{amide}\}$ bonding modes. The penta- and hexapeptides are characterized by pentaglycine-like coordination with an uncoordinated imidazole moiety $\{NH_2, 3N^-_{amide}\}$ [26,27,37,38]. For the larger peptides like squash trypsin inhibitor (small protein containing 29 amino acids) [41], the human prion protein fragments $PrP_{106–113}$, $PrP_{106–126}$ [39] and the 1–16 and 1–28 fragments of human and mouse β-amyloid peptide [40], above pH 8 the complex with $\{N_{Im}, 3N^-_{amide}\}$ coordination mode was proposed.

The protection of the N-terminal amino group (by Z- or Ac-groups) influences both the speciation and the structures of the complexes formed. The N-protected peptides containing one His residue in a peptide sequence bind Cu^{2+} ions through the imidazole nitrogen around pH 3–4 giving 1N complex; when the pH increases the metal ion deprotonates successive peptide nitrogen atoms, forming M–N^- bonds, till a $CuH_{-3}L$ species (4N complexes) is formed above pH 8 [22,26,27,35,38,42–44].

2.5. Cu^{2+} ion binding by peptides having multi-histidine sites

His-His is the simplest peptide having more than one His residue [10]. At pH 4, a CuHL species with histamine-like binding is formed. Around pH 5–6, the CuL complex with binding mode similar to the $CuH_{-1}L$ complex of Gly-His $\{NH_2, N^-_{amide}, N_{Im}\}$ predominates. About pH 7, the carnosine-like dimer $Cu_2H_{-2}L_2$ was found. Simple extension of the His-His sequence towards C-terminal in

His-His-Gly-Gly does not change the coordination properties towards Cu^{2+} ions [7].

The hexapeptide amide EVHHQK-NH_2, (11-16H) [26], and its longer analogues 11-20H and 11-28H [27], which are human fragments of β-amyloid peptide containing the N-terminal sequence Xaa-Yaa-His form very stable complexes in the pH range 4.5–10.5, typical for the albumin-like sequence.

The acetylation of the N-terminal amino group has a major influence on both the speciation and structures of the complexes formed. In all peptides, Ac-11-16H (Ac-EVHHQK-NH_2) [26], the Ac-11-20H and Ac-11-28H [27] human fragments of β-amyloid peptide the imidazole nitrogen acts as an anchoring site. In pH range 4–7, Cu^{2+} ion is bound through both imidazole, $\{N_{Im}, N_{Im}\}$, and with increasing pH successive amide nitrogen donors are also involved.

Studies of the interaction of Cu^{2+} ions with hexapeptides of the C-terminal region of the histone H2A, Ac-Thr-Ala-Ser-His-His-Lys-NH_2 (Ac-TASHHK-NH_2), Ac-Thr-Glu-Ala-His-His-Lys-NH_2 (Ac-TEAHHK-NH_2) [44] show the presence of species with coordinated histidine imidazole nitrogen atoms at pH 3.5–6.5.

The insertion of an amino acid between two histidine residues, His-Xaa-His or Xaa-His-Yaa-His- sequences, changes the coordination abilities of these peptides depending on amino acid sequence and on the position of the histidine residue from the N-terminal amino group. The dominant complex formed by His-Gly-His-Gly seems to have an identical donor set with that of Gly-Gly-His [7]. The $CuH_{-2}L$ species with $\{NH_2, 2N^-_{amide}, N_{Im}\}$ coordination was also found for the Cu^{2+}–His-Val-His system [30], but the presence of the N-terminal His residue shifted the formation of 4N-complexes to higher pH. The coordination pattern of Gly-His-Gly-His towards Cu^{2+} was already discussed [7]. 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 ($CuH_{-1}L$ complex). A further rearrangement follows at pH 7, yielding a $CuH_{-2}L$ species with a structure suggested to be a distorted trigonal bipyramid. The equatorial coordination in this complex is provided by a triglycine-like $\{NH_2, 2 \times N^-_{amide}\}$ donor set, and both imidazole donors are involved in the axial binding.

For the Cu^{2+}–Ac–His-Gly-His-Gly [45] and Cu^{2+}–Ac–His-Val-His-NH_2 [15] systems, the CuL, $CuH_{-2}L$ and $CuH_{-3}L$ are the major species in solution. The binding sites in these complexes were assumed to be $\{N_{Im}, N_{Im}\}$, $\{N_{Im}, 2N^-_{amide}, N_{Im}\}$ and $\{N_{Im}, 3N^-_{amide}\}$, respectively. Among them, the species $CuH_{-2}L$ predominates at physiological pH 7.4, but the CuL complex stabilized by the macrochelate formation is also present.

For the pentadecapeptide containing -His-Xaa-Yaa-His- sequence, such as that in TLEDTKKGHKLHLDY, the 114–128 protein fragment of SPARC (secreted protein, acidic and rich in cysteine) the involvement in copper complex formation of both the terminal amino and imidazole groups has been suggested [46]. Potentiometric and spectroscopic investigations on the Cu^{2+} complexes with protected fragments 122–126, 121–126, 120–126 [47] and 114–128 [48] of SPARC indicated complex formation with two His residues involved. As the pH increases, deprotonation and coordination of three amide nitrogen atoms may also occur. At pH 9.5, all the fragments mentioned above form species with Cu^{2+} coordinated by Lys^{123}, Leu^{124} and His^{125} amide nitrogen atoms and the imidazole ring of the His^{125} bound in equatorial position, while His^{122} interacts with metal ion at axial position. A very similar structure hypothesis has been suggested for some protected oligopeptides containing two or more His residues, linked by Gly residues [49].

The fragments of amyloid precursor protein (APP) protected at the N- and C-terminals, 147–150 [50], 145–155 and 145–157 [51] having the His-Xaa-His-Yaa-His motif form species with three imidazoles coordinated to the Cu^{2+} ion. This binding mode predominates around pH 5. At neutral pH, the metal ion induces ionization of Leu^{148} and His^{149} amide nitrogen atoms and the complex with $\{N_{Im}, N_{Im}, N_{Im}, 2N^-_{amide}\}$ binding mode is formed giving a

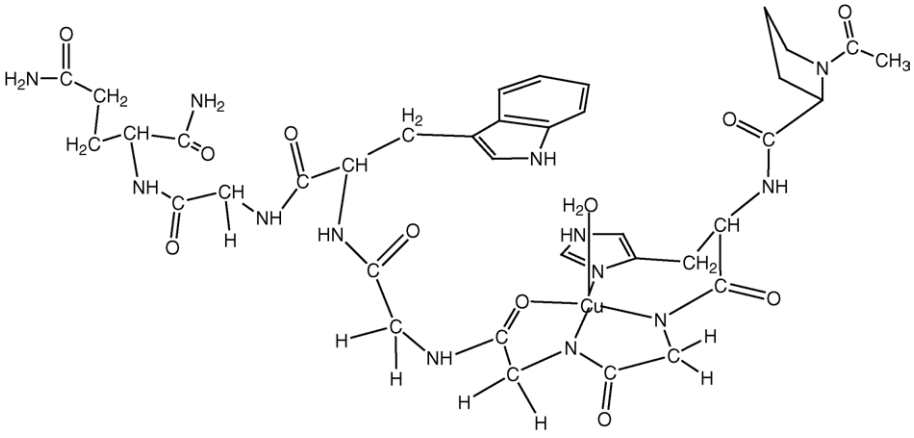

Fig. 3. The proposed structure of the Cu^{2+}–Ac-PHGGGWGQ-NH_2 complex.

geometry distinctly different from the tetragonal one usually observed for Cu^{2+} peptide complexes.

Residues 60–91 of human prion protein (PrP) are composed of four repeats of a rather unusual eight-residue sequence PHGGGWGQ (Pro-His-Gly-Gly-Gly-Trp-Gln), which according to recent studies, may be responsible for the binding of copper [52–54]. Solution and solid-state studies have shown that at around pH 7.4 a Cu^{2+} complex with metal ion coordinated to imidazole and two Gly amide nitrogen atoms dominates, both in solution [55,56] and in the solid state [56] (Fig. 3). The binding of Cu^{2+} ion to the imidazole of His and the amide nitrogen atoms of Gly residues on the C-terminal side of His results rather in the unusual coordination mode with a less favorable seven-membered $\{N_{Im}, N^{-}_{Gly3}\}$ chelate ring [57,58]. The presence of three Gly residues on the C-terminal side of His could be structurally favored due to the greater flexibility derived from the Gly_3 insert. To test this hypothesis, potentiometric, spectroscopic and calorimetric studies for two octarepeat analogues having Ala_3 and Lys_3 instead of Gly_3 were performed. The insertion of bulkier Ala or Lys residues instead of a Gly_3 unit makes the $\{N_{Im}, 2N^{-}_{amide}\}$ complex distinctly less stable [59]. Thus, the glycine rich region in the octarepeat fragment of prion may play two basic roles (i) maintaining high peptide flexibility for the unstructured prion N-terminal region and (ii) allowing the very specific coordination of Cu^{2+} ions in the pH range important for binding and release of metal ion during the biological transportation process. The binding of Cu^{2+} results in partial protein organization, which could be biologically relevant [56]. Modeling studies of Cu^{2+} binding ability of the 1-, 2-, and 4-Hex peptides of chicken prion [60] strongly suggests the cooperative effect, which is also observed for the human variant [61]. The human octa-repeat tetrameric unit is, however, much more effective in binding Cu^{2+} ions than the chicken hexapeptide analogue [60,61].

3. Chemical and biological implications of Cu^{2+} binding to aminoglycoside antibiotics

Due to the widespread presence of pathogenic bacteria and their rapid adaptation even to the most hostile conditions, special attention is paid to the design of new or improved drugs and to studies of their chemical properties.

Aminoglycosides are among the antibiotics of highest interest since they are recognized as life-rescuing as well as health-threatening agents. They are highly effective against Gram-negative and some Gram-positive bacteria. Their therapeutic action consists in binding to the bacterial ribosome, decreasing the fidelity of mRNA translation and further preventing protein synthesis [62]. The subsequent damage of cytoplasmic membrane has also been proven [63,64]. Patients subjected to therapy with aminoglycosides should be monitored permanently for the drug concentration in the blood plasma. This results from the coexistence of serious toxic effects like ototoxicity and nephrotoxicity [65]. Therefore, detailed insight into the chemical basis of their action is required.

Aminoglycosides interfere with most molecules of the RNA group, including ribosomal RNA [66], HIV-1 viral Rev responsible element [67], group I introns [68], yeast $tRNA^{Phe}$ [69], hammerhead ribozyme [70] and RNase P RNA [71]. One of them, neomycin B, displaces essential metal ions from major regions in intron RNA, inhibits splicing, and protects the catalytic core from cleavage induced by selected metal ions like Fe^{2+} or Pb^{2+} [72,73].

The specific arrangement of amine and hydroxyl groups in molecules of aminoglycosides, as well as the flexibility of glycosidic bonds, became the reason for the studies of coordination behaviour of almost all their representatives. Numerous studies were focused on their interaction with Cu^{2+} ions. Moreover, the formation of neomycin and gentamicin complexes with metal ions like Ni^{2+}, Co^{2+} or Zn^{2+} has been investigated [74,75]. Coordination of selected divalent metal ions by sisomicin was used in order to synthesize derivatives resistant to bacterial enzymes [76,77]. In clinical studies, gentamicin [78,79] and neomycin [80] were suspected to interact with iron and thus contribute to the molecular basis of aminoglycoside-induced ototoxicity. Aminoglycosides as well as their cupric complexes were examined previously for their possibility to cleave both DNA [81,82] and RNA [83,84], even in the in vivo studies [85].

3.1. Acid–base properties of aminoglycosidic antibiotics

Positive charge, which the aminoglycosides carry on their amine functions, has an influence for their chemical and therapeutic behaviour. In the latter case, the proton binding abilities of amino groups determine and make possible their interactions with the negatively charged residues of nucleic acids and also with biological membranes. Individual aminoglycosides differ from each other with the amount of the groups undergoing ionization; however, not all of them are protonated at physiological pH. Small values of the first deprotonation constant were found for all aminoglycoside antibiotics studied to-date: 6.0 for geneticin [86] and 6.2 for kanamycin A [87], which carry a +4 charge at low pH, and ca. 5.7 for kanamycin B [88] and tobramycin [89], which have a +5 charge. In the case of sisomicin, also a +5 polycation, the value of this pK_a is a little bit higher (6.4), which is the result of a certain rigidity of the antibiotic molecule. This is caused by the presence of a double bond in one of its aminosugar constituents [90]. Amikacin, on the other hand, the antibiotic with one amino groups at the deoxystreptamine ring acylated, is also a +4 ion and has a much higher value (6.8) of this pK_a [91]. We have recently concluded that the reason for the lowering of the first pK_a is a local electrostatic interaction between the neighbouring NH_3^+ groups of deoxystreptamine ring, rather than the overall charge of the whole molecule [87–90]. The rest of the pK_a values fit the range of 7–9.5, which is typical for amino functions.

Table 2
Comparison of the summary stability constants log βH_xL (ΣpK_x) for selected aminoglycoside antibiotics with the number of amino groups of particular types indicated

Antibiotic	log β (H_xL)	x	1°	2°	RCH_2NH_2	Aglycon	Reference
Amikacin	33.433	4	2	0	1	1	[91]
Kanamycin A	30.80	4	3	0	1	0	[87]
Kanamycin B	37.637	5	4	0	1	0	[88]
Tobramycin	38.121	5	4	0	1	0	[89]
Geneticin	30.127	4	3	1	0	0	[86]
Sisomicin	39.404	5	3	1	1	0	[90]
pK_{av}[a]	–	–	7.2(2)	8.6(2)	9.3(3)	9.8(3)	–

Table was reproduced from reference [90].
x represents the complete number of protonating functions.
[a] pK_{av} negative logarithm of average dissociation constant.

The amines in these antibiotics may be divided into four groups with respect to their chemical properties: the primary ones, bound directly to the aminosugar ring (RNH_2); the aminomethylene functions (RCH_2NH_2); the primary amines in the aliphatic aglycon chains and the secondary ones, attached to the sugar rings. Results, based on potentiometric and ^{1}H NMR studies, suggest that the most acidic ones are the primary amines, situated directly at the sugar rings [90]. Comparison of the summary stability constants logβ H_xL (ΣpK_x) for selected antibiotics allowed for estimation of the average values of protonation constants for the groups, as distinguished above (Table 2).

3.2. Cu^{2+} coordination by aminoglycosides

The foregoing observations are crucial to understand the coordination properties of aminoglycoside antibiotics. In order to obtain a complete description of the complexation process, it was advisable to use a combination of potentiometric and spectroscopic techniques (CD, EPR, electronic absorption and NMR). Our recent investigations have shown that aminoglycosides form the strongest complexes with Cu^{2+}, which was revealed in studies of kanamycin interactions with both toxic and basic metal ions [92]. Kanamycin A, a typical representative of these antibiotics, coordinates cupric ion with both terminal aminosugar rings. The complex dominating at physiological pH has a specific coiled structure, quite dissimilar to that of the ligand. The metal binding process engages two deprotonated amino groups and two hydroxyl functions, which also dissociate protons [87]. Several mutually incompatible binding modes were proposed previously for kanamycin A. The earliest studies suggested that coordination occurs through both nitrogen atoms of the deoxystreptamine ring [93]. The alternative binding mode, proposed on the basis of spectroscopic investigations, involves the donors of the terminal aminosugar [94–96]. However, another way of coordination was also proposed with use of the ring oxygen, even though it would require a twisted boat conformation for this ring and unfavorably high potential energy [96]. Only in one such study was the deprotonation of the coordinated hydroxyl taken into consideration [97], although this is a well proven feature of all aminosugar and their derivatives [98–104]. The very recent NMR studies confirmed that nitrogen and oxygen donors of both terminal aminosugar rings bind Cu^{2+} ion, however the involvement of the deoxystreptamine oxygen is likely [105].

Analogous coordination properties, assuming the involvement of terminal aminosugar donors, are exhibited by geneticin [86], kanamycin B [88], tobramycin [89] or gentamicin C [106] (Fig. 4). Distinctly different is the coordination pattern of Cu^{2+} complexes of amikacin [91,107]. This antibiotic is a modified version of kanamycin A, having one amine group acylated in order to avoid inactivation by bacterial enzymes. The newly formed amide, together with adjacent amino group constitute very strong binding site for Cu^{2+} and coordinate the metal ion at physiological pH with a peptide fashion. This rare complex structure is responsible for its exceptional properties. It was shown that at relatively high concentrations of the complex the dimeric species coexist in the solution [108] (Fig. 5).

A completely distinct coordination pattern is represented by the Cu^{2+}–kasugamycin complex [109]. This antibiotic is the only two-ring molecule with an additional carboxyamidyne moiety. That unique group appeared to be essential for the coordination properties of the drug. At physiological pH, it anchors Cu^{2+} with nitrogen and oxygen atoms allowing the nitrogen of the aminosugar ring to occupy the axial posi-

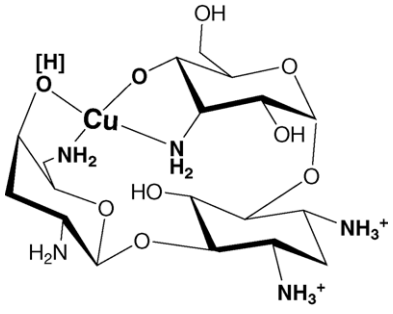

Fig. 4. The molecule of Cu^{2+}–tobramycin complex existing at physiological pH as an example of the coordination mode of an unsubstituted antibiotic.

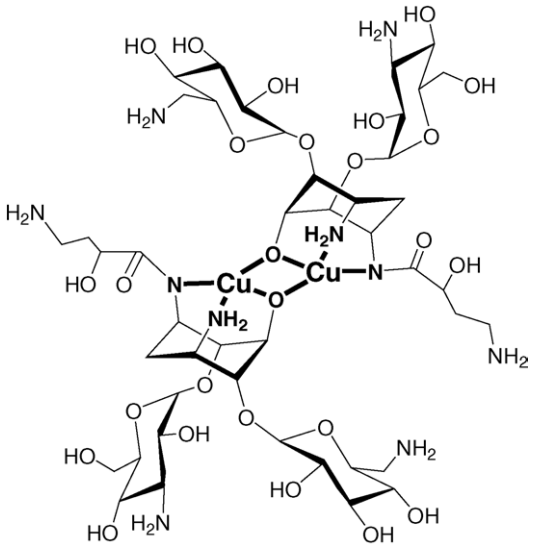

Fig. 5. The structure of Cu^{2+}–amikacin dimer proposed on the basis of XAS results.

tion. At higher pH, the hydroxyl group of a cyclitol residue deprotonates and enters the coordination sphere. This results in a completely distorted complex molecule, when compared to the free antibiotic (Fig. 6). Undoubtedly, such process influences the stereochemistry of the drug, which is essential in the interactions with the intracellular targets.

Very interesting, from a biological point of view, was the experiment of a possible effect of aminoglycoside antibiotic on Cu^{2+} homeostasis. In order to examine whether the drug could be capable of disturbing Cu^{2+} content in blood plasma, the ability to remove metal ions from saturated N-terminal binding site of human serum albumin was tested for kanamycin A [92]. The formation of an $NH_2 \rightarrow Cu^{2+}$ charge transfer transition characteristic for Cu^{2+} complex of aminosugar antibiotics [86–89] was observed. This fact, simultaneously with the decrease of the d–d band intensity of Cu^{2+}–HSA, would suggest that under favorable conditions Cu^{2+}–aminoglycoside complex formation might be possible, especially in the case of high antibiotic concentration in plasma, which may occur during intravenous or intramuscular injection.

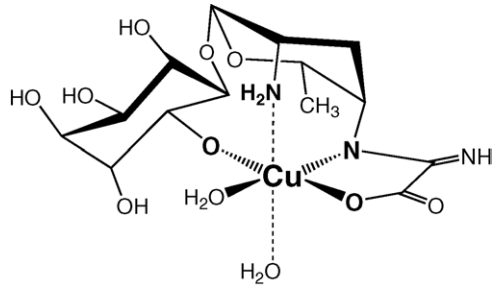

Fig. 6. The proposed structure of the fully deprotonated Cu^{2+}–kasugamycin complex. Figure was reproduced with permission of the copyright holders [109].

3.3. Oxidative action of Cu^{2+}–aminoglycoside complexes

The natural consequence of Cu^{2+} coordination studies were experiments aimed to characterize the possible oxidation states of the metal center, the behavior of the complexes in the presence of physiological amounts of hydrogen peroxide, as well as a description of the reactive intermediates of the oxidation processes. H_2O_2 is an endogenous substance, a product of a respiratory system, and as such may participate directly or indirectly in the reactions with xenobiotics. Cyclic voltammetry data proved that the antibiotics discussed are redox-inactive, while their cupric complexes undergo reduction and oxidation in a wide pH range [87,108,109]. Reduction proceeds to Cu^0, and is accompanied by a complex decomposition, as indicated by the irreversibility of the process. Oxidation of the species studied appeared to proceed from Cu^{2+} to Cu^{3+} species (Fig. 7). The CV studies demonstrated that only the complex form, which exists around physiological pH is susceptible to reduction and oxidation processes [87,108].

As a result of their electrochemical lability, Cu^{2+} complexes of aminosugar antibiotics disproportionate hydrogen peroxide to dioxygen and water, through radical intermediates [87,108–110]. Two types of reactive oxygen species were distinguished. Superoxide was found in the case of kasugamycin [109], while for the rest of the antibiotics the hydroxyl radical was detected. Further experiments showed that the complex, which maximizes around physiological pH is the most efficient $^{\bullet}OH$ generator (Fig. 8) [87].

Another significant observation was the interdependence between the amount of hydroxyl radical and the resulting charge of the complex [110]. This charge is the highest in the case of Cu^{2+}–neomycin B, since this antibiotic has six amino groups and its complex generates $^{\bullet}OH$ most effectively. Hydroxyl radicals are the most powerful oxidizing

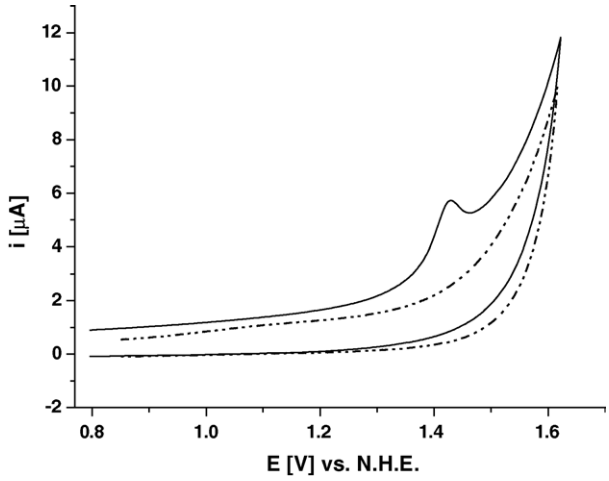

Fig. 7. Anodic scans of Cu^{2+}–kanamycin A complexes at pH 8.0. Scan rate 25 mV s^{-1}, background scan indicated. Figure was reproduced with permission of the copyright holders [87].

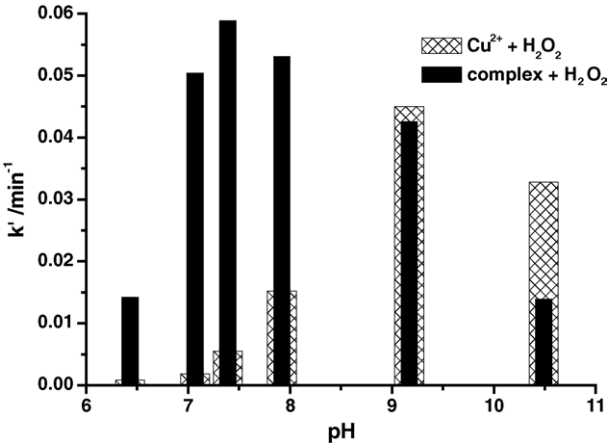

Fig. 8. The comparison of pH influence on the rates of hydroxyl radicals formation by Cu^{2+}–kanamycin A complex and Cu^{2+} ions, both in H_2O_2 presence. Figure was reproduced with permission of the copyright holders [87].

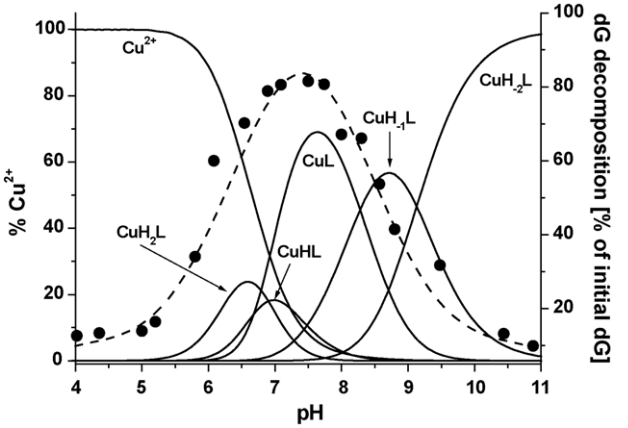

Fig. 9. The extent of dG destruction in the presence of Cu^{2+}–kanamycin A–H_2O_2 system, after 24 h incubation, overlaid with species distribution diagram for Cu^{2+}–kanamycin A. Figure was reproduced with permission of the copyright holders [112].

agents in biological systems. Therefore the stimuli of their formation constitute the species of potential pro-mutagenic and pro-carcinogenic properties. The initial target of oxidation promoted by Cu^{2+}–aminoglycosides is the complexed antibiotic molecule, as was indicated by mass spectra. Fragmentation occurred predominantly at glycosidic bonds between the constituent rings of the antibiotic molecule. Single and double oxygen adducts were also seen and their presence exhibited a lack of specificity, which is characteristic for hydroxyl radical-like agents [111].

However, self-oxidation of the complex does not prevent biomolecules from being damaged. This was proven by the observation that the complexes discussed induce the formation of 8-oxo-2′-deoxyguanosine [91,109,111,112]. Such a derivative, when present within DNA, may cause local impairment and result in initiation of pro-mutagenic and pro-carcinogenic processes. This reaction, as was presented for the cupric complex of kanamycin A accompanied by hydrogen peroxide, attains its highest yield under physiological conditions reaching as high as 80% (Fig. 9) [112].

Nevertheless, the DNA bases are not exposed in nucleic acids molecules, thus a primary attack occurs at the DNA backbone. This may result in two different phenomena, single strand damage or the double one (see forms II and III on lane 6, Fig. 10). Cu^{2+} complexes of aminoglycosides induce a high number of single nicks within the plasmid DNA in a very short reaction period [81,82,111,112]. Accumulation of these cleavages led to the double strand scission and the linearized product was already recorded after only 5 min [112]. The yield of DNA damage was found, similarly to NDMA (*N*,*N*-dimethyl-*p*-nitrosoaniline) bleaching, to be dependent on the resultant charge of the complex [110].

Some physiologically ubiquitous compounds like histidine or ATP appeared to disturb complex formation between Cu^{2+} and aminoglycoside and thus prevent DNA from damage. Both amino acid and nucleotides at physiological concentrations were found to drastically decrease the amount of strand breakages. Similar observations were drawn from the experiment including magnesium ions. Their presence also limited the yield of DNA destruction, but the chemical basis of this phenomenon is completely dissimilar. Spectroscopic parameters showed that in the presence of Mg^{2+} ions Cu^{2+}–aminoglycoside coordination remains unaffected, contrary to experiments with histidine or ATP. Decrease in the DNA destructive activity is, in this case, a result of magnesium binding to the phosphate residues and thereby protecting DNA against the complex activity [113].

Since the natural action of aminoglycosides focuses on RNA, participation of Cu^{2+} ions in this field was also investigated. The copper–aminoglycosides were shown recently to interact with HIV-1 viral Rev responsible element [83] and in vivo, with R23 mRNA [85]. They are capable of interacting with $tRNA^{Phe}$ [110–112]. Two types of reactions were distinguished. Both complexes and antibiotics were found to induce highly effective hydrolysis in the anticodon loop, at the hypermodified guanine (Y37). However, accompanied by hydrogen peroxide, the complex was shown to produce multiple oxidative breakages of various intensities in the anticodon loop as well as in D-arm. No other antibiotics (e.g. lincosamides or glycopeptides) or their Cu^{2+} complexes were

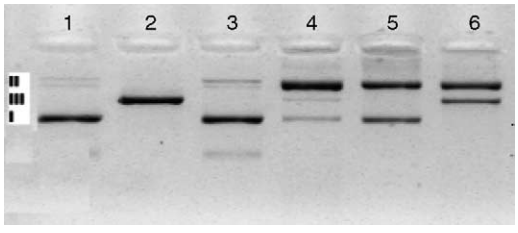

Fig. 10. Agarose gel electrophoresis of pBR322 plasmid DNA cleavage by Cu^{2+}–amikacin complex. Lane 1, plasmid; lane 2, plasmid linearized with *Xho*I endonuclease; lane 3, plasmid + H_2O_2; lane 4, plasmid + Cu^{2+} + H_2O_2; lane 5, plasmid + amikacin + H_2O_2; lane 6, plasmid + complex + H_2O_2. Figure was reproduced with permission of the copyright holders [111].

found to hydrolyze $tRNA^{Phe}$, so this feature was recognized as characteristic only for aminoglycosides [112]. Moreover, the hydrolysis appeared not to occur in the case of tRNA transcript devoid of hypermodification [110]. Thus the naturally modified molecules, including human $tRNA^{Phe}$, which carries similar bulky residue at position 37, in anticodon loop, may be susceptible to the cleavage induced by these drugs. This issue has just been subjected to in-depth analysis and the new results suggest that depurination of the polynucleotide chain at Y37 (position occupied by wybutine, a hypermodified base) is the crucial factor of the RNA cleavage mechanism [114].

Similar to the oxidative activity of Cu^{2+}–aminoglycosides, the hydrolytic behaviour was also found to be dependent on the resultant charge of the complex molecule. A possible explanation of this phenomenon is that the cleavage efficiency is a function of the complex binding strength in the vicinity of Y37, which rises with increase of the positive charge of the complexes studied [110].

Since the antibacterial agents may act as immunity system stimuli, studies of cytokines generation were undertaken [92]. The data revealed that the complexes might play an important role for the host as stimulators of immune cells and phagocytes. Three types of cytokines were recognized: interferon (IFN), tumor necrosis factor (TNF) and interleukine-10 (IL-10). The most prominent result was the interdependence between generation of pro-inflammatory and anti-inflammatory cytokines. The enhanced levels of TNF and IFN were accompanied by a drop of IL-10 concentration, which suggests that in vivo Cu^{2+} coordination by the drugs may support the pro-inflammatory processes [92].

References

[1] P. Deschamps, P.P. Kulkarni, M. Gautam-Basak, B. Sarkar, Coord. Chem. Rev. 249 (2005) 895.
[2] P.J. Hart, M.M. Balbirnie, N.L. Ogihara, A.M. Nersissian, M.S. Weiss, J.S. Valentine, D. Eisenberg, Biochemistry 38 (1999) 2167.
[3] H. Sigel, R.B. Martin, Chem. Rev. 82 (1982) 385.
[4] L.D. Pettit, J.E. Gregor, H. Kozłowski, in: R.W. Hay, J.R. Dilworth, K.B. Nolan (Eds.), Perspectives in Bioinorganic Chemistry, vol. 1, JAI Press, London, 1991, pp. 1–41.
[5] H. Sigel, R. Tribolet, O. Yamauchi, Comments Inorg. Chem. 9 (1990) 305.
[6] I. Sovago, in: K. Burger (Ed.), Biocoordination Chemistry Metal Complexes of Peptides and Derivatives, Ellis Horwood, Chichester, 1990, pp. 135–184.
[7] H. Kozłowski, W. Bal, M. Dyba, T. Kowalik-Jankowska, Coord. Chem. Rev. 184 (1999) 319.
[8] P. Tsiveriotis, N. Hadjiliadis, Coord. Chem. Rev. 190–192 (1999) 171.
[9] P. Tsiveriotis, G. Malandrinos, N. Hadjiliadis, Rev. Inorg. Chem. 20 (2000) 305.
[10] C.E. Livera, L.D. Petit, M. Bataille, B. Perly, H. Kozłowski, B. Radomska, J. Chem. Soc., Dalton Trans. (1987) 661.
[11] M. Remelli, Ch. Conato, A. Agarossi, F. Pulidori, P. Młynarz, H. Kozłowski, Polyhedron 19 (2000) 2409.
[12] T. Kowalik-Jankowska, M. Jasionowski, L. Łankiewicz, J. Inorg. Biochem. 76 (1999) 63.
[13] A. Maryi, G. Malandrinos, J.C. Plakatouras, N. Hadjiliadis, I. Sovago, Bioinorg. Chem. Appl. 1 (2003) 99.
[14] D. Sanna, C.G. Agoston, I. Sovago, G. Micera, Polyhedron 20 (2001) 937.
[15] B. Boka, A. Myari, I. Sovago, N. Hadjiliadis, J. Inorg. Biochem. 98 (2004) 113.
[16] P. Młynarz, T. Kowalik-Jankowska, M. Stasiak, M.T. Leplawy, H. Kozłowski, J. Chem. Soc., Dalton Trans. (1999) 3673.
[17] S. Bruni, F. Cariati, P.G. Daniele, E. Prenesti, Spectrochim. Acta, Part A 56 (2000) 815.
[18] Ch. Conato, R. Gavioli, R. Guerrini, H. Kozłowski, P. Młynarz, C. Pasti, F. Pulidori, M. Remelli, Biochim. Biophys. Acta 1526 (2001) 199.
[19] P.J. Morris, R.B. Martin, J. Inorg. Nucl. Chem. 33 (1971) 2913.
[20] M. Wienken, B. Lippert, E. Zangrando, L. Randaccio, Inorg. Chem. 31 (1992) 1983.
[21] I. Sovago, E. Farkas, C. Bertalan, A. Lebkiri, T. Kowalik-Jankowska, H. Kozłowski, J. Inorg. Biochem. 51 (1993) 715.
[22] M. Orfei, M.C. Alcaro, G. Marcon, M. Cheelli, M. Ginanneschi, H. Kozłowski, J. Brasuń, L. Messori, J. Inorg. Biochem. 97 (2003) 299.
[23] G. Formicka-Kozłowska, M. Bezer, L.D. Pettit, J. Inorg. Biochem. 18 (1983) 335.
[24] G. Formicka-Kozłowska, H. Kozłowski, B. Jeżowska-Trzebiatowska, G. Kupryszewski, J. Przybylski, Inorg. Nucl. Chem. Lett. 15 (1979) 387.
[25] K. Gerega, H. Kozłowski, E. Masiukiewicz, L.D. Pettit, S. Pyburn, B. Rzeszotarska, J. Inorg. Biochem. 33 (1988) 11.
[26] T. Kowalik-Jankowska, M. Ruta-Dolejsz, K. Wiśniewska, L. Łankiewicz, H. Kozłowski, J. Chem. Soc., Dalton Trans. (2000) 4511.
[27] T. Kowalik-Jankowska, M. Ruta-Dolejsz, K. Wiśniewska, L. Łankiewicz, J. Inorg. Biochem. 92 (2002) 1.
[28] W. Bal, M. Jeżowska-Bojczuk, K.S. Kasprzak, Chem. Res. Toxicol. 10 (1997) 906.
[29] Ch. Conato, H. Kozłowski, P. Młynarz, F. Pulidori, M. Remelli, Polyhedron 21 (2002) 1469.
[30] A. Myari, G. Malandrinos, Y. Deligiannakis, J.C. Plakatouras, N. Hadjiliadis, Z. Nagy, I. Sovago, J. Inorg. Biochem. 85 (2001) 253.
[31] P. Młynarz, W. Bal, T. Kowalik-Jankowska, M. Stasiak, M.T. Leplawy, H. Kozłowski, J. Chem. Soc., Dalton Trans. (1999) 109.
[32] P. Młynarz, N. Gaggelli, J. Panek, M. Stasiak, G. Valensin, T. Kowalik-Jankowska, M.T. Leplawy, Z. Latajka, H. Kozłowski, J. Chem. Soc., Dalton Trans. (2000) 1033.
[33] R.W. Hay, M.M. Hassan, C. You-Quan, J. Inorg. Biochem. 52 (1993) 17.
[34] N. Camerman, A. Camerman, B. Sarkar, Can. J. Chem. 54 (1976) 1309.
[35] M.A. Zoroddu, P. Peana, T. Kowalik-Jankowska, H. Kozłowski, M. Costa, J. Chem. Soc., Dalton Trans. (2001) 458.
[36] B. Gyurcsik, I. Vosekalna, E. Larsen, Acta Chem. Scand. 51 (1997) 49.
[37] K. Varnagy, J. Szabo, I. Sovago, G. Malandrinos, N. Hadjiliadis, D. Sanna, G. Micera, J. Chem. Soc., Dalton Trans. (2000) 467.
[38] T. Kowalik-Jankowska, M. Ruta-Dolejsz, K. Wiśniewska, L. Łankiewicz, J. Inorg. Biochem. 86 (2001) 535.
[39] B. Belosi, E. Gaggelli, R. Guerrini, H. Kozłowski, M. Łuczkowski, F.M. Mancini, M. Remelli, D. Valensin, G. Valensin, Chembiochem 5 (2004) 349.
[40] T. Kowalik-Jankowska, M. Ruta, K. Wiśniewska, L. Łankiewicz, J. Inorg. Biochem. 95 (2003) 270.
[41] P. Młynarz, D. Valensin, H. Kozłowski, T. Kowalik-Jankowska, J. Otlewski, G. Valensin, N. Gaggelli, J. Chem. Soc., Dalton Trans. (2001) 645.
[42] M.A. Zoroddu, T. Kowalik-Jankowska, H. Kozłowski, H. Molinari, K. Salnikow, L. Broday, M. Costa, Biochim. Biophys. Acta 1475 (2000) 163.

[43] M.A. Zoroddu, T. Kowalik-Jankowska, H. Kozłowski, K. Salnikow, M. Costa, J. Inorg. Biochem. 85 (2001) 47.
[44] M. Mylonas, J.C. Plakatouras, N. Hadjiliadis, A. Krężel, W. Bal, Inorg. Chim. Acta 339 (2002) 60.
[45] M. Casolaro, M. Chelli, M. Ginanneschi, F. Laschi, L. Messori, M. Muniz-Miranda, A.M. Papini, T. Kowalik-Jankowska, H. Kozłowski, J. Inorg. Biochem. 89 (2002) 181.
[46] Ch. Conato, W. Kamysz, H. Kozłowski, M. Łuczkowski, Z. Mackiewicz, F. Mancini, P. Młynarz, M. Remelli, D. Valensin, G. Valensin, Eur. J. Inorg. Chem. (2003) 1694.
[47] M. Remelli, M. Łuczkowski, A.M. Bonna, Z. Mackiewicz, Ch. Conato, H. Kozłowski, New J. Chem. 27 (2003) 245.
[48] Ch. Conato, W. Kamasz, H. Kozłowski, M. Łuczkowski, Z. Mackiewicz, P. Młynarz, M. Remelli, D. Valensin, G. Valensin, J. Chem. Soc., Dalton Trans. (2002) 3939.
[49] G. Pappalardo, G. Impellizzeri, R.P. Bonomo, T. Campagna, G. Grasso, M.G. Saita, New J. Chem. 26 (2002) 593.
[50] M. Łuczkowski, K. Wiśniewska, L. Łankiewicz, H. Kozłowski, J. Chem. Soc., Dalton Trans. (2002) 2266.
[51] D. Valensin, F.M. Mancini, M. Łuczkowski, A. Janicka, K. Wiśniewska, E. Gaggelli, G. Valensin, L. Łankiewicz, H. Kozłowski, J. Chem. Soc., Dalton Trans. (2004) 16.
[52] D.R. Brown, H. Kozłowski, J. Chem. Soc., Dalton Trans. (2004) 1907.
[53] J. Stockel, J. Safar, A.C. Wallace, F.E. Cohen, S.B. Prusiner, Biochemistry 37 (1998) 7185.
[54] J.H. Viles, F.E. Cohen, S.B. Prusiner, D.B. Goodin, P.E. Wright, H.J. Dyson, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 2042.
[55] M. Łuczkowski, H. Kozłowski, M. Sławikowski, K. Rolka, E. Gaggelli, D. Valensin, G. Valensin, J. Chem. Soc., Dalton Trans. (2002) 2269.
[56] C.S. Burns, E. Aronoff-Spencer, C.M. Dunham, P. Lario, N.I. Avdieeevich, W.E. Antholine, M.M. Olmsstead, A. Vrieelink, G.J. Gerfen, J. Peisach, W.G. Scott, G.L. Millhauser, Biochemistry 41 (2002) 3991.
[57] R.P. Bonomo, G. Impellizzeri, G. Pappalardo, E. Rizzarelli, G. Tabbi, Chem. Eur. J. 6 (2000) 4195.
[58] T. Miura, A. Hori-i, H. Mototani, H. Takeuschi, Biochemistry 38 (1999) 11560.
[59] M. Łuczkowski, H. Kozłowski, A. Łęgowska, K. Rolka, M. Remelli, J. Chem. Soc., Dalton Trans. (2003) 619.
[60] P. Stańczak, M. Łuczkowski, P. Juszczyk, Z. Grzonka, H. Kozłowski, J. Chem. Soc., Dalton Trans. (2004) 2101.
[61] D. Valensin, M. Łuczkowski, F.M. Mancini, A. Łęgowska, E. Gaggelli, G. Valensin, K. Rolka, H. Kozłowski, J. Chem. Soc., Dalton Trans. (2004) 1284.
[62] E.F. Gale, E. Cundliffe, P.E. Reynolds, M.H. Richmond, M.J. Waring, The Molecular Basis of Antibiotic Action, second ed., John Wiley & Sons, London, 1981.
[63] B.D. Davis, Microbiol. Rev. 51 (1987) 341.
[64] H.J. Busse, C. Wostmann, E.P. Bakker, J. Gen. Bacteriol. 138 (1992) 551.
[65] B.D. Davis, Microbiol. Rev. 51 (1987) 341.
[66] J. Davies, L. Gorini, B.D. Davis, Mol. Pharmacol. 1 (1965) 93.
[67] A. Sreedhara, J.A. Cowan, J. Biol. Inorg. Chem. 6 (2001) 166.
[68] U. von Ahsen, J. Davies, R. Schroeder, J. Mol. Biol. 226 (1992) 935.
[69] S.R. Kirk, Y. Tor, Bioorg. Med. Chem. 7 (1999) 1979.
[70] T.K. Stage, K.J. Hertel, O.C. Uhlenbeck, RNA 1 (1995) 95.
[71] N.E. Mikkelsen, M. Brannvall, A. Virtanen, L. Kirsebom, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 6155.
[72] I. Hoch, C. Berens, E. Westhof, R. Schroeder, J. Mol. Biol. 282 (1998) 557.
[73] N.E. Mikkelsen, K. Johansson, A. Virtanen, L. Kirsebom, Nat. Struct. Biol. 8 (2001) 510.
[74] S.M. Abu-El-Wafa, M.A. El-Ries, F.M. Abou-Attia, R.M. Issa, Anal. Lett. 22 (1989) 2703.
[75] Z.H. Chohan, M.-U.H. Ansari, Pakistan J. Pharmacol. 6 (1989) 21.
[76] T.L. Nagabhushan, A.B. Cooper, W.N. Turner, H. Tsai, S. McCombie, A.K. Mallams, D. Rane, J.J. Wright, P. Reichert, D.L. Boxler, J. Weinstein, J. Am. Chem. Soc. 100 (1978) 5253.
[77] T.L. Nagabhushan, W.N. Turner, H. Tsai, R.S. Jaret, D. Schumacher, Carbohydr. Res. 130 (1984) 243.
[78] E.M. Priuska, J. Schacht, Biochem. Pharmacol. 50 (1995) 1749.
[79] B.-B. Song, S.-H. Sha, J. Schacht, Free Radic. Biol. Med. 25 (1998) 189.
[80] B.J. Conlon, B.P. Perry, D.W. Smith, Laryngoscope 108 (1998) 284.
[81] A. Sreedhara, J.D. Freed, J.A. Cowan, J. Am. Chem. Soc. 122 (2000) 8814.
[82] A. Patwardhan, J.A. Cowan, Chem. Commun. (2001) 1490.
[83] A. Sreedhara, J.A. Cowan, J. Biol. Inorg. Chem. 6 (2001) 166.
[84] J.A. Cowan, T. Ohyama, D. Wang, K. Natarajan, Nucleic Acids Res. 28 (2000) 2935.
[85] C.-A. Chen, J.A. Cowan, Chem. Commun. (2002) 196.
[86] M. Jeżowska-Bojczuk, A. Karaczyn, W. Bal, J. Inorg. Biochem. 71 (1998) 129.
[87] W. Szczepanik, P. Kaczmarek, J. Sobczak, W. Bal, K. Gatner, M. Jeżowska-Bojczuk, New J. Chem. 26 (2002) 1507.
[88] M. Jeżowska-Bojczuk, W. Bal, H. Kozłowski, Inorg. Chim. Acta 275–276 (1980) 541.
[89] M. Jeżowska-Bojczuk, A. Karaczyn, H. Kozłowski, Carbohydr. Res. 313 (1998) 265.
[90] A. Krężel, W. Szczepanik, M. Świątek, M. Jeżowska-Bojczuk, Bioorg. Med. Chem. 12 (2004) 4075.
[91] M. Jeżowska-Bojczuk, W. Bal, J. Chem. Soc., Dalton Trans. (1998) 153.
[92] W. Szczepanik, A. Czarny, E. Zaczyńska, M. Jeżowska-Bojczuk, J. Inorg. Chem. 98 (2004) 245.
[93] S. Yamabe, Jpn. J. Pharmacol. 17 (1967) 120.
[94] S. Hanessian, G. Patil, Tetrahedron Lett. 12 (1978) 1031.
[95] S. Hanessian, G. Patil, Tetrahedron Lett. 12 (1978) 1035.
[96] I. Grapsas, I. Massova, S. Mobashery, Tetrahedron 54 (1998) 7705.
[97] A. Sreedhara, A. Patwardhan, J.A. Cowan, Chem. Commun. (1999) 1147.
[98] A. Pusino, D. Droma, P. Decock, B. Dubois, H. Kozłowski, Inorg. Chim. Acta 138 (1987) 5.
[99] H. Kozłowski, P. Decock, I. Olivier, G. Micera, A. Pusino, L.D. Pettit, Carbohydr. Res. 197 (1990) 109.
[100] M. Jeżowska-Bojczuk, H. Kozłowski, L.D. Pettit, G. Micera, P. Decock, J. Inorg. Biochem. 57 (1995) 1.
[101] G. Micera, H. Kozłowski, in: G. Berthon (Ed.), Handbook of Metal–Ligand Interactions in Biological Fluids, vol.1, Marcel Dekker, New York, 1995, pp. 707–716.
[102] M. Jeżowska-Bojczuk, E. Chruścińska, T. Trnka, G. Micera, J. Inorg. Biochem. 63 (1996) 231.
[103] M. Jeżowska-Bojczuk, S. Lamotte, T. Trnka, J. Inorg. Biochem. 61 (1996) 213.
[104] B. Gyurcsik, L. Nagy, Coord. Chem. Rev. 203 (2000) 81.
[105] N. D'Amelio, E. Gaggelli, N. Gaggelli, E. Molteni, M.C. Baratto, G. Valensin, M. Jeżowska-Bojczuk, W. Szczepanik, Dalton Trans. (2004) 363.
[106] W. Leśniak, W.R. Harris, J.Y. Kravitz, J. Schacht, V.L. Pecoraro, Inorg. Chem. 42 (2003) 1420.
[107] E. Gaggelli, N. Gaggelli, A. Maccotta, G. Valensin, D. Marini, M.E. Di Cocco, C. Manetti, M. Delfini, Spectrochim. Acta A, Mol. Biomol. Spectrosc. 55A (1999) 205.
[108] M. Jeżowska-Bojczuk, W. Leśniak, W. Bal, H. Kozłowski, K. Gatner, A. Jezierski, J. Sobczak, S. Mangani, W. Meyer-Klaucke, Chem. Res. Toxicol. 14 (2001) 1353.
[109] M. Jeżowska-Bojczuk, W. Leśniak, J. Inorg. Biochem. 85 (2001) 99.
[110] W. Szczepanik, J. Ciesiołka, J. Wrzesiński, J. Skała, M. Jeżowska-Bojczuk, Dalton Trans. (2003) 1488.

[111] M. Jeżowska-Bojczuk, W. Szczepanik, W. Leśniak, J. Ciesiołka, J. Wrzesiński, W. Bal, Eur. J. Biochem. 269 (2002) 5547.
[112] W. Szczepanik, E. Dworniczek, J. Ciesiołka, J. Wrzesiński, J. Skała, M. Jeżowska-Bojczuk, J. Inorg. Biochem. 94 (2003) 355.
[113] W. Szczepanik, M. Świątek, J. Skała, M. Jeżowska-Bojczuk, Arch. Biochem. Biophys. 431 (2004) 88.
[114] J. Wrzesiński, W. Szczepanik, J. Ciesiołka, M. Jeżowska-Bojczuk, Biochem. Biophys. Res. Commun. 331 (2005) 267.