1. Introduction

Various forms of neurodegenerative disorders represent one of the major risk for the health of the human society. There are several different diseases which belong to this category; the best known and most frequently studied disorders include the Alzheimer's disease (AD), Parkinson' disease (PD) and prion diseases. The first two diseases are rather common and the number of cases will dramatically increase with the expected increase in the lifetime of the human population. The occurence of prion diseases is very rare but its infectious nature (e.g. transmissible spongiform encephalopathy or „mad cow disease") also provides a high publicity for these disorders. As a consequence, an extremely high number of research groups are working on the understanding of various forms of neurodegenerative disorders and the number of publications is now almost innumerable. The major fields of this research include the clinical, pharmaceutical, chemical and biochemical and more recently even the bioinorganic aspects of this subject.

In spite of extensive investigation of interaction between metal ion and peptides of neurodegeneration, there are still many open questions in this research field. For example:

- the factors which able to tune finely the metal binding selectivity of peptides
- the effect of the change of redox properties of metal-protein adducts
- in addition to detailed studied copper(II), nickel(II) and zinc(II) ions the potential role of the binding of other trace and toxic elements (e.g. iron(II/III), cadmium(II) and lead(II)) to the peptides of neurodegeneration.

Our main goals were the understanding of the coordination and electrochemical background of above factors by means of studies of complex formation and redox processes of systematically planned model peptides. One of the main goals was the better understanding of the role the amino acid sequences on the coordination and redoxi properties of peptide complexes. The other aim was to clarify the potential role of the toxic (cadmium, lead) metal ions by means of studies of metal complexes of peptide fragments and their model peptides. In the frame of this project 63 peptides were synthesised and/or their complexes with numerous metal ions (essential: Cu(II), Zn(II), (Ni(II)), Fe(II), Fe(III) and toxic: Cd(II), Pb(II)) were characterized.

2. The factors which able to tune finely the metal binding affinity and selectivity of peptides

Aims of our metallopeptide chemistry research work were the synthesis, equilibrium, and structural studies of complexes of such series of histidine containing peptides, in which the systematic change of the amino acid sequence is carried out. These molecules include oligopeptides built up from 4 to 12 amino acid residues containing 2 to 4 histidines among them. The presence of positively or negatively charged and polar or bulky side chains of other amino acids in the neighbourhood of the metal binding sites, can significantly contribute to the stability of these complexes and selectivity of the peptides. To understand the specific effects of these side chains lysine, aspartic acid, serine or phenylalanine will be systematically inserted into the sequence of the histidine peptides.

The studies of the fragments of Abeta amyloid and tau peptides contributed the understanding of the difference of the metal binding sites of the proteins. The further studies of amylin peptide fragments are initiated on the one hand their similar aggregation ability to those of prion and Aβ peptides, and on the other hand the significant effect of the side chain groups on the metal binding of the molecules.

2.1. The effect of Phe and Asp side chain on the small peptides [1,2]

Structural and reactivity studies of metal complexes with amino acids and oligopeptides have provided relevant information to coordination chemists allowing them the interpretation of molecular

interactions of metals with bigger macromolecular systems such as metalloproteins. In addition, these small complexes have gained much attention due to their cytotoxic properties and their potential to be used as antitumor agents. That is why copper(II) complexes of two dipeptides, Ala-Phe and Phe-Ala were detailed characterized in solution and solid form. These results reveal that the complex formation processes are very similar to those of other dipeptides, but the position of phenyl group affects the stability and redox parameters of copper(II) complexes. These solution equilibrium studies were completed with measurements of antitumor activity. It was found that both Cu(II)-dipeptide complexes have a similar cytotoxic effect, against breast cancer cells. In addition, both complexes show minimal toxic effect in non-tumor cells compared to Cisplatin.

The effect of more polar side chain donor groups on the coordination ability of peptides was studied for metal ion complexes of di- and tripeptides containing two or more aspartic and/or glutamic acids in the sequence. The previous results obtained for studies on copper(II), nickel(II) and palladium(II) complexes of di- tri- and tetrapeptides containing Asp and/or Glu residues proved that there is significant interaction with these donor functions; they have effect on the stability constant of the complexes, occasionally modify even the stoichiometry of the formed species compared to the common peptides. These measurements were completed by the studies of complexation of various oligopeptides containing this moiety with additional metal ions, namely the biogenic iron(II), iron(III), cobalt(II), zinc(II) and the toxic cadmium(II) and lead(II) ions (see in 3. paragraph). The complexation of all the aforementioned metal ions were studied with the peptides Asp_2 and Asp_3 and usually with other Asp/Glu containing selected ligands.

The results of studies in zinc(II) complexes have shown that the presence of carboxylate groups slightly increases the stability of complexes compared to those of alanine or glycine containing peptides. This effect decreases in the AspGlu ≥ Asp_2 > Gly_2 > Glu_2 order owing to the stability difference between (NH_2, beta-COO^-) and (NH_2, CO) coordination mode. The coordination mode in the ZnL species of Glu_2 and Gly_2 is the same, however because of the repulsion between the side chain carboxylate groups, the formation of the negative charged complex is less favoured.

2.2. The effect of different factors on histidine and/or cysteine containing peptides

2.2.1. Effect of the distance and different amino acid environments of histidines on the multihistidine peptides [3]

The other field of our research was synthesis, equilibrium, and structural studies of copper(II) and nickel(II) complexes of such series of two- or three histidine containing peptides, in which the systematic change of the amino acid sequence was carried out. The studied peptides contain histidyl residues in different number and positions: Ac-HGGH-NH_2, Ac-HAAH-NH_2, Ac-HVVH-NH_2, Ac-HGGHGH-NH_2, Ac-HAAHGH-NH_2, Ac-HAAHVH-NH_2, Ac-HGGGHGH-NH_2. The results established the fact that the increasing number of histidines increases the stability of imidazole coordinated species (Figure 1). An additional stabilizing effect is the presence of HXH sequence on the C-termini of peptides. So the previously determined general trend was refined: the trend of stability of imidazole coordinated $[CuL]^{2+}$ species can be given as follows: Ac-HXXH-NH_2 < Ac-HXH-NH_2 < Ac-HXXHZZH-NH_2 ~ Ac-HHGH-NH_2 ~ Ac-HGGGHGH-NH_2 < Ac-HXHZH-NH_2 < Ac-HXXHZH-NH_2 < S3H4 (X,Z = Gly, Ala, Val, Sar, Pro).

The other group of the systematic planned peptides was the multihistidine peptides containing different amino acid side chains, namely phenylalanine, aspartic acid and lysine. The studies of copper(II), nickel(II) and zinc(II) complexes of Ac-HADH-NH_2, Ac-HDAH-NH_2, Ac-HAHDH-NH_2, Ac-HDHAH-NH_2, Ac-HADHAH-NH_2, Ac-HDHAHDH-NH_2, Ac-HAHFH-NH_2, Ac-HFHAH-NH_2, Ac-HFHAHFH-NH_2,

Ac-HAHKH-NH_2 and Ac-HKHAH-NH_2 peptides have shown that the impact on the amino acid side chains on the complex formation processes changes in the following order:

Asp ≥ Phe > Lys ~ Val (Ala).

The presence of aspartic acid and phenylalanine side chain increases the ratio of copper(II) and nickel(II) complexes in the alkaline range in which the metal ion binds to the imidazole N of the internal histidine. Moreover the phenylalanine aromatic ring has a stabilizing effect on the copper(II) complexes due to the d-π interaction between the aromatic ring of the ligand and the metal ion. Copper(II) ion binding to the HFH sequence is preferred for all three phenylalanine-containing peptides.

On the other hand the presence of aspartic acid prevents the hydrolysis of zinc(II) ion and promotes the zinc(II) induced amide nitrogen deprotonation and coordination of Ac-HADH-NH_2 and Ac-HADHAH-NH_2. However, the lysine, has no effect on the metal binding ability of the peptides .

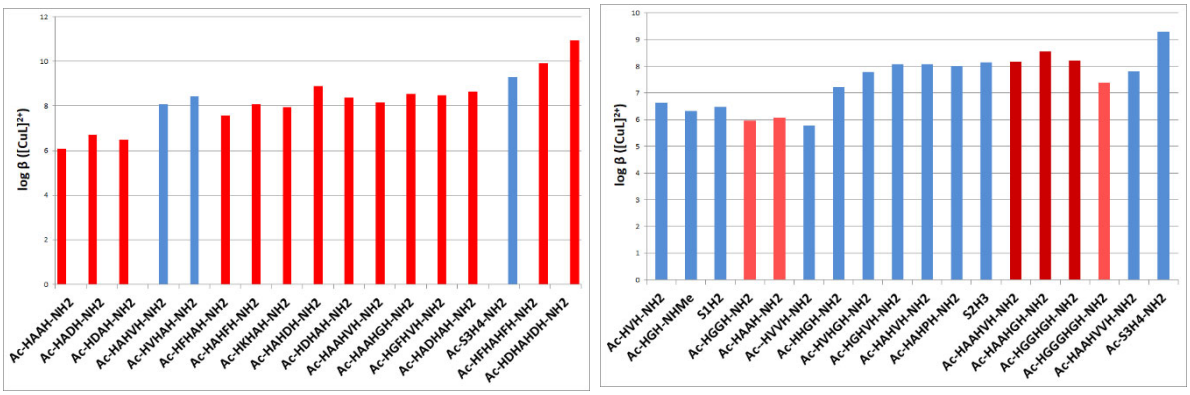


Figure 1 *Stability constants (logβ) of CuL complexes of various histidine containing peptides (Data from literature, Data of recently studied ligands)*

2.2.2. Effect of the additional coordinating side chains on peptides containing cysteine [4-7]

It is well known that for the complexation with 3d transition metal ions the carboxylate-O of aspartyl, the imidazole-N of histidyl and thiolate-S of cysteinyl residues are the most effective donor functions. Different combinations of these functions may result in very different complex formation processes.

To find suitable peptides with selective metal binding affinity, the metal complexes of peptides containing cysteine in different environments were systematically investigated. A series of hexapeptides containing separate histidyl/aspartic acid/glutamic acid and cysteinyl residues were synthesized (AAHAAC-NH_2, AHAAAC-NH_2, ADAAAC-NH_2, AADAAC-NH_2, AAEAAC-NH_2, AAASSC-NH_2) and their nickel(II), zinc(II) and cadmium(II) complexes have been studied. It was found that all peptides have outstanding metal binding ability but the speciation of the systems and the binding sites of peptides reveal a significant specificity.

(i) In the Ni(II)–AAHAAC-NH_2 system the amino terminus is the primary nickel(II) binding site in the form of the (NH_2,N^-,N^-,N_{im}) chelate. However, the C-terminal thiolate function can bind another Ni(II) ion with the involvement of amide nitrogens in metal binding. For Zn(II)- and Cd(II)-ions only mononuclear complexes are formed, in which imidazole-N and thiolate-S donors are the primary metal binding sites. In the case of AHAAAC-NH_2 both nickel(II) and zinc(II) ions can induce deprotonation and coordination of the first amide bond in the sequence. This results in the enhanced stability of the corresponding species containing a tridentate (NH_2,N^-,N_{im}) binding mode at the amino terminus supported by a macrochelate from the distant thiolate group.

(ii) The complex formation processes of the two histidine containing peptides with copper(II) and palladium(II) were also investigated and a much more pronounced effect of the distant cysteinyl residues on the metal binding of the amino terminus was obtained. In the case of copper(II)-AAHAAC-NH_2 system the (NH_2,N^-,N^-,N_{im}) coordination mode hindered the interaction between copper(II) ion and thiolate group preventing the redox reactions in acidic and neutral solution. The suppression of redox reactions between copper(II) ions and the peptide AHAAAC-NH_2 was also observed in equimolar samples but in this case the existence of Cu(II)-S(thiolate) binding was demonstrated in the alkaline pH range. Palladium(II) is known as the most effective metal ion to induce deprotonation and coordination of peptide amide groups. The results of this study unambiguously prove that the Pd-S(thiolate) binding modes dominate over the formation of the Pd-N(amide) bonds even if the thiolate residues are involved in various macrochelates only. The thiolate group of cysteinyl residue was described as the primary ligating site for both peptides and the remaining coordination sites were occupied by the amino, imidazole and one amide nitrogen donor atoms.

(iii) The presence of aspartic acid in addition to the C-terminal cysteinyl residue has also significant effect on the complex formation processes. For nickel(II), the amino terminus is the primary ligating site and aspartyl residue in position-3 in the sequence can especially enhance the thermodynamic stability of nickel(II) complexes with (NH_2,N^-,N^-,COO^-) coordination mode. However, thiolate groups of both peptides can also coordinate to nickel(II) in the slightly alkaline samples and it results in the presence of various coordination isomers. For AADAAC-NH_2 the formation of dinuclear complexes was also suggested. On the contrary, the thiolate functions are the primary binding site for zinc(II) and especially cadmium(II) ions. The formation of macrochelate complexes is the most characteristic with these metal ions including all possible donor sites. None of the side chain donor functions can, however, induce the deprotonation and zinc(II) or cadmium(II) coordination of the amide functions of these peptides.

As a conclusion it can be stated, that for nickel(II) complexes of AAHAAC-NH_2 peptide reveal an obvious binding preference of the N-terminal part of the molecule due to the formation of (NH_2,N^-,N^-,N_{im}) coordinated species. This is in a good agreement with the fact that the N-terminal domain of this peptide corresponds well to that of albumin with outstanding nickel(II) binding affinity (ATCUN motif). It results in the same mononuclear complexes as any other peptides with N-terminal XXH-sequence. However, due to the high nickel(II) binding affinity of the thiolate group, the C-terminal cysteine serves as an independent binding site for nickel(II) ion and dinuclear complexes are also formed at presence of metal ion excess.

The other two metal ions, zinc(II) and cadmium(II) form stable ML complexes with tridentate coordination of the ligands ([NH_2,N_{im},S^-] and [NH_2,COO^-,S^-], respectively). The thermodynamic stability of this coordination mode follows the Cd(II) > Zn(II) > Ni(II) order (Figure 2) which does not correspond to the usual Irving–Williams series, however, this coordination mode cannot prevent the hydrolysis and precipitation of zinc(II) and cadmium(II) hydroxide in alkali media.

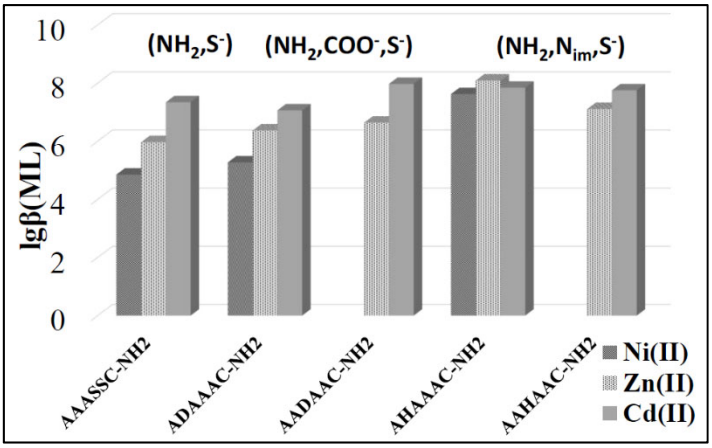


Figure 2. *The stability of ML complexes of different hexapeptides*

Comparison of the aforementioned ligands with AAASSC-NH_2 and AAEAAC-NH_2 hexapeptides it can be stated, that the ratio of coordination isomers of nickel(II) complexes can be altered by inserting strongly, weakly or non-coordinating internal amino acid residues. Priority of the C-terminal binding site enhances in an order of AAASSC-NH_2 > AAEAAC-NH_2 > AADAAC-NH_2 > AAHAAC-NH_2 forming an (N^-,N^-,N^-,S^-) fused chelate system at high pH. As a conclusion, the insertation of an internal amino acid with side chain functions alters the ratio of the coordination isomers formed in the present and previously investigated hexapeptides which is illustrated in Figure 3.

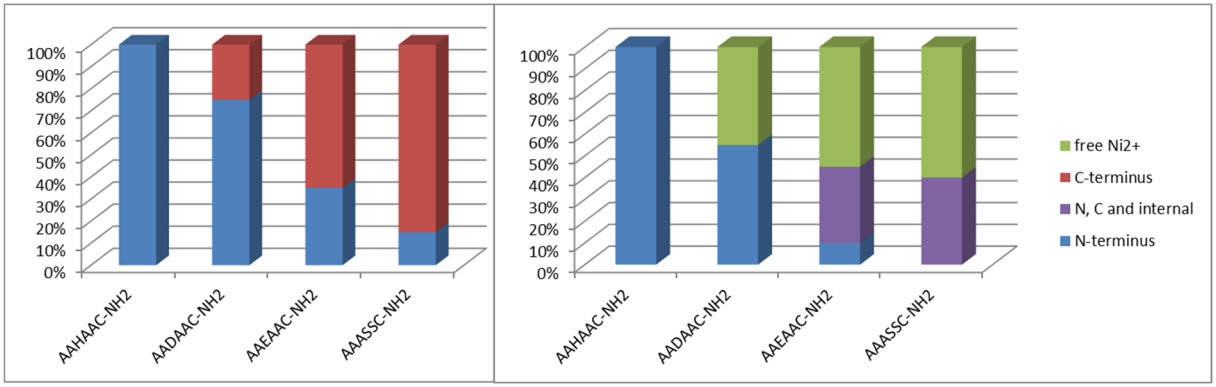


Figure 3. *Ratio of isomers of 1:1 nickel(II) complex formed in equimolar solution of hexapeptides at pH 11 (a) and pH 7 (b). Ratio of complex with C-terminal coordination mode (red); with N-terminal coordination mode (blue); with coordination of both N- and C-termini (purple).*

2.2.3. Effect of the position of cysteinyl residues on terminally free and protected multicysteine peptides [8,9]

Thiolate group of cysteine is one of the most common metal binding sites in a great variety of metalloproteins. Many of these substances contain the Ni–S bond; the nickel(II) containing superoxide dismutase (NiSOD) the metalloproteins responsible for the nickel(II) homeostasis of *Helicobacter pylori* and phytochelatins are probably the best known among them. It is also well-known from the literature that the chemistry of metal-thiolate interaction is much more complicated than those of other side chain residues of proteins because in addition to the regular complex formation processes the thiolate groups of cysteine can easily form polynuclear complexes via thiolate bridges and can take part in redox reactions with several metal ions. As a consequence, comprehensive studies on related model systems are often necessary for the better understanding of the results obtained for the interaction of nickel(II) with the native peptide fragments of proteins.

(i) We launched a systematic study for the characterization of the metal binding ability of peptides containing two strongly coordinating side chain residues but in well-separated positions. The nickel(II) complexes of three cysteine containing peptides (CSSACS-NH_2, ACSSACS-NH_2, SSCSSACS-NH_2) have been studied. All peptides have high nickel(II) binding ability in the form of square planar complexes and the stability order for the peptides is: CSSACS-NH_2 > ACSSACS-NH_2 > SSCSSACS-NH_2. The different metal binding affinity of these peptides is related to the differences in the speciation and in the binding modes of the major species. Almost exclusive formation of bis(ligand) complexes via an (NH_2,S^-) 5-membered chelate from the amino terminus is characteristic of CSSACS-NH_2. The (NH_2,N^-,S^-) tridentate chelate is the major coordination mode of ACSSACS-NH_2 but the distant cysteine can also contribute to metal binding.

The lowest metal binding affinity of SSCSSACS-NH_2 peptide is a bit surprising, because in the case of corresponding peptides of histidine the XY-His-Z is the most preferred sequence for nickel(II) (binding (ATCUN motif) (see e.g. AAHAAC-NH_2 and AHAAAC-NH_2). Contrary to the two cysteine containing peptides, the metal binding affinity of peptides with ATCUN motif is higher than that of peptide containing histidine in the second position. This difference can be well demonstrated by the Figure 4.

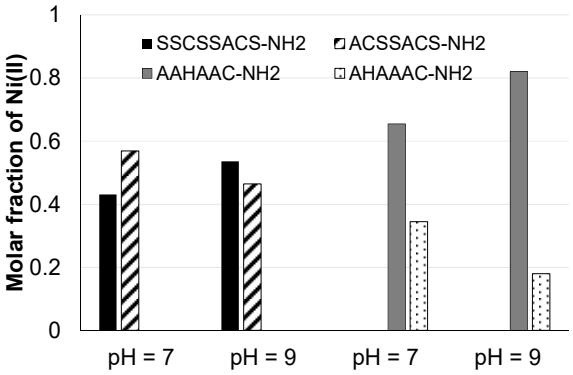


Figure 4. *The ratios of the nickel(II) complexes formed with the peptides ACSSACS-NH_2 and SSCSSACS-NH_2 or AHAAAC-NH_2 and AAHAAC-NH_2 respectively in a model system containing all components in the same concentration at pH 7 and pH 9. (c_L = 1 mM)*

(ii) We performed the systematic studies of nickel(II)- and zinc(II)-complexes of small terminally protected peptides containing CXXX, XXXC, XCCX, CX_nC (n = 1-3) sequences.

The results prove that the cysteine thiolate group of one-cysteine containing peptides is the primary binding site for zinc(II) ions, but the presence of a histidyl or aspartyl side chain in the molecule contributes to the stability of the complexes, and histidine in the ligand significantly increases the stability of zinc(II) complexes. Nickel(II) ion can induce the deprotonation of peptide amide nitrogens regardless of whether the cysteine is in the C-terminal or N-terminal position. If both cysteine and histidine are present in the molecule the C-terminal donor group is always the primary anchor group, and the N-terminal donor group is replaced by the third amide nitrogen, which means that these two side chain groups are practically equivalent with respect to nickel(II) ion, and their positions determine the coordination modes.

For two-cysteine containing peptides the bidentate coordination via two sulfur atoms of the peptide is characteristic of all metal ions in the physiological pH range, the coordinated thiolate group, however, can behave as a bridging ligand resulting in complexes with polynuclear structures. The formation of polynuclear complexes can be detected for nickel(II) ion, and the closer the two cysteines are in the peptide, the more prone it is to form polynuclear species. With increasing the distance between the cysteinyl residues the dominance of mononuclear complex increases with (S^-,N^-,N^-,S^-) coordination mode. This structure stabilizes the complexes and shifts the deprotonation and coordination of the third amide nitrogen above pH 11.

2.2.4. Effect of the replacing cysteinyl residue with penicillamine residues [10]

Oligopeptide complexes modelling the coordination modes of Cu(II), Ni(II), Zn(II) ions are useful for the better understanding of the mechanism of enzymatic reactions, transfer, storage and distribution of metal ions and their disorder. Study of peptides containing cysteine residues with nickel(II) ions was hindered by polynuclear complex formation. Replacement by penicillamine enables the investigation of Ni (II) complexes with the contribution of thiolate functions. That is why we focused on the peptides Pen-SSACS-NH_2 and CSSA-Pen-S-NH_2. It can be concluded from the results that the complex formation starts with the coordination of the N-terminus with (NH_2,S^-) chelate. The highest influence of the cysteine/penicillamine mutation was obtained for the nickel(II) complexes. L-penicillamine in N-

terminal position (Pen-SSACS-NH_2) enhances the thermodynamic stability of all nickel(II) containing species as compared to the cysteine containing counterpart. L-penicillamine in internal position work as a much more effective anchor for the deprotonation and metal ion coordination of peptide amide bonds. In the case of cysteine in N-terminal position, redistribution of nickel(II) ion is preferred, thiolate of penicillamine acts as an anchoring group and enables the presence of dinuclear species (Figure 5). Modification of the local environment of the thiol group resulted in the change of species distribution of zinc(II) and cadmium(II) ions, too.

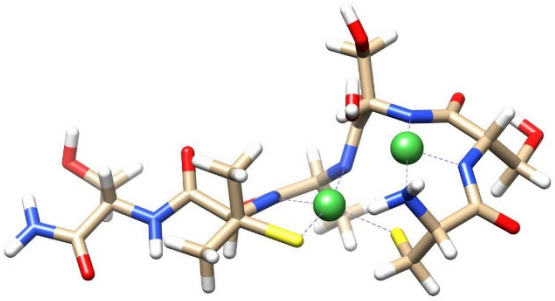


Figure 5. *Optimized structure of $[Ni_2H_{-4}L]^{2-}$ complex of CSSA-Pen-S-NH_2 using DFT method at B3P86/def2-TZVP level of theory.*

2.3. The effect of the amino acid sequence on the metal binding affinity of peptides related to the proteins of neurodegenerative deseases.

2.3.1. The effect of the presence of polar side chains on the nickel(II) and zinc(II) complexes of amylin fragments [11]

It is widely accepted that all forms of neurodegeneration have a common molecular base: they are related to the abnormal conformational changes and subsequent aggregation of otherwise natural proteins. Human amylin peptide is independent from neurodegeneration, but similar conformational changes of amylin and tendency to formation of plaques were observed. The deposition of islet amyloid polypeptide (IAPP or amylin) is a pathological feature of type 2 diabetes. The human form (hIAPP) is built up from 37 amino acid residues containing an internal histidine in positions 18. Rat amylin (rIAPP) does not show tendency for self-aggregation and is not toxic to islet cells. Rat amylin is highly homologues to human amylin, but differs in six amino acids. Our previous studies of copper(II) complexes of rat amylin fragments and its mutants unambiguously proved the outstanding role of asparagine in the copper(II) binding of those peptides, in which the asparagine and other polar side chains are present in a specific sequence; these peptides can be effective copper(II) binding ligands even in the absence of a strongly coordinating side chain like histidine.

These findings, however, are very interesting from point of view of peptide coordination chemistry, as well. Generally nickel(II) ion is able to induce the deprotonation of peptide nitrogens in presence of terminal amino group or other anchors in the side chain (imidazole of His, thiolate of Cys etc.), but stabilizing role of asparagine has not been observed for nickel(II) or other metal ions, yet. We have concludet the studies of nickel(II) and zinc(II) complexes of rat amylin fragments that nickel(II) complexes have higher stability than those of simple oligopeptides. NMR measurements on the N-terminally free peptide SSNA-NH_2 clearly demonstrated that an equilibrium between the common (NH_2,$3N^-$(peptide)) and (NH_2,$2N^-$(peptide),N^-(asparagine)) coordination modes can exist in basic solutions. These observations support that a single asparaginyl residue cannot be an anchor for nickel(II) binding but with an amino group in chelating position can contribute to the nickel(II) binding of peptide molecules. Studies on the corresponding zinc(II) complexes ruled out the anchoring and even the stabilizing role of the internal asparagine moieties and only low stability complexes were detected with the exclusive binding of the amino terminus. From the comparison of the results obtained for the copper(II), nickel(II) and zinc(II) ions, it can be unambiguously stated that although

the -SSNN- sequence binds nickel ion more effective than the simple oligopeptides, the 19-22 peptide fragments of rat amylin have an outstanding affinity for copper(II) binding. This metal binding site, however, is different from that of the human amylin which may also play a role in their different aggregation behaviour.

2.3.2. Side chain effect on metal binding ability of modell peptides of amyloid β protein [12,13]

Alzheimer's disease characterized by the deposition of extracellular aggregates of amyloid-β peptides. Different metal ions accumulate within amyloid deposits, which suggests an involvement of metal ions in AD pathogenesis. Cu(II)-induced Aβ neurotoxicity might result from changes in the coordination of the metal ion during Aβ oligomerization or from different peptide/metal ratios. We studied the Cu(II) coordination modes with a synthetic Aβ(1–16) dimer. The formation of macrochelate complex species with the involvement of imidazole nitrogen donor atoms of His13 and His14, during copper(II) binding at physiological pH and low metal to peptide ratios, is observed. The results provide further support for the copper(II) binding ability of the (8–16) domain of amyloid-β and support the previous assumptions that via the bis(ligand) complex formation copper(II) ions may promote the formation of the oligomers of amyloid-β.

2.3.3. The effect of different environment of histidine on the metal binding affinity and selectivity of tau protein fragments [14-17]

Many recent studies suggest that in addition to amyloid-β, tau protein may also play a significant role in the development of Alzheimer's disease and/or other neurodegenerative disorders. The biological role of tau protein is associated with its interaction with tubulins to promote their assembly into microtubules and to stabilize the microtubules. This protein is an intrinsically disordered large protein consisting of 441 amino acids including 12 histidyl residues as potential metal binding sites. The neuropathological hallmarks of these diseases are related to the accumulation of amyloid-β into senile plaques and of hyperphosphorylated tau into neurofibrillary tangles. Moreover, huge number of recent publications support that metal ions also play a crucial role in neurodegeneration.

The association of metal ions with tau protein and its various peptide fragments are much less studied and the results are partly contradictory. It is widely accepted that the aggregation and toxic deposition of tau protein is triggered by hyperphosphorylation of the peptide and this process is significantly influenced by various metal ions, but the exact characterization of the metal binding sites of tau protein has not been satisfactorily clarified. Based on this knowledges we studied numerous histidine peptide modelling the His14, His32 and His329,330 binding site of tau proteins: the investigated peptides include the HAVAHHH-NH_2 peptide, two N-terminal fragments and their mutants (tau(9-16) Ac-EVMEDHAG-NH_2 and tau(26-33) Ac-QGGYTMHQ-NH_2) and fragment from R3 region and their mutants (tau(326-333) Ac-GNIHHKPG-NH_2). The studies of peptides was expanded to several metal ions (copper(II), nickel(II), zinc(II) and in the first case to organometallic ruthenium(II) ([(η^6-*p*-cym)Ru(H_2O)$_3$]$^{2+}$) to study the selectivity of the peptide.

The results obtained on the metal complexes of the NH_2-HAVAHHH-NH_2, with the well preserved HAV sequence revealed some interesting features of the metal binding ability and selectivity of this ligand. Both potentiometric and spectroscopic data unambiguously prove that the amino terminus of the peptide with the histamine-like (NH_2,N_{im}) coordination mode is the primary metal binding site in slightly acidic samples for all the four studied metal ions. For the [(η^6-*p*-cym)Ru(H_2O)$_3$]$^{2+}$ the histamine-like coordinated, rather inert species predominates in the whole pH range, and the coordination of amide nitrogens and/or internal histidine side chain cannot be observed in the

mononuclear species. The internal histidyl residues, however, can contribute to the thermodynamic stability via the formation of macrochelates in case of copper(II), nickel(II) and zinc(II) complexes. This multidentate binding mode enhances the overall stability of the complexes but cannot prevent the copper(II) and/or nickel(II) induced amide deprotonation of amide functions around physiological pH range. This process is accompanied by a change in the coordination modes and spectroscopic data support the co-existence of coordination isomers with (NH_2,$3N^-$) and (N_{im},$3N^-$) binding sites. Interestingly, the ratio of these isomers depends on the metal ions: a slight preference of the coordination to the internal histidines is preferred for copper(II), while it is just the opposite for nickel(II). The relatively good separation of the binding sites mentioned above also makes possible the formation of dinuclear complexes with copper(II) and nickel(II) ions including the formation of mixed metal complexes. In addition, this peptide is able to bind two ruthenium(II) ions as well, and the internal histidyl residue is the secondary binding site and ruthenium(II) induced deprotonation of peptide nitrogen can be supposed.

The histidyl residue is the primary metal binding site for metal ion in all the studied peptide models of tau proteins. In the case of Tau(9-16) the side chain carboxylate functions enhance the stability of the M-N_{im} coordinated complexes compared to Tau(26-33). These species are present only in slightly acidic solutions. Increase of pH results in the deprotonation and metal ion coordination of the amide groups preceding the histidyl sites. This process results in the (N_{im},N^-,N^-) coordination mode and this is the predominating species in the physiological pH range. Deprotonation and copper(II) coordination of the third amide group occurs under slightly alkaline conditions. As a consequence, the donor atoms of the major species in basic solutions are (N_{im},N^-,N^-,N^-). The change of the binding mode from the simple monodentate imidazole binding to the amide bonded complexes changes the metal ion preference of the two histidyl sites, as well. For the latter binding mode His32 site predominates over His14 around physiological pH (90%-10%) and in alkaline samples (96%-4%) (Figure 6a). To compare the binding ability of H14 and H32 in a single molecule the decapeptide Ac-EDHAGTMHQD-NH_2 (Tau(12-16)(30-34)) has also been synthesized and studied. This decapeptide contains histidines in the same environments as in the native fragments. The CD spectra give an unambiguous proof for the binding of copper(II) to the second histidyl residue in alkaline samples similar to the one-histidine counterpart in agreement with the results obtained on the two tau fragments.

We continued the studies of tau fragments: the copper(II) complexes of a peptide fragment of the R3 domain of tau protein (tau(326-333) Ac-GNIHHKPG-NH_2) and its mutants have been studied. The native peptide represents the possible metal binding sites of the R3 domain of tau protein. The big variety of the complex formation processes comes from the presence of two adjacent histidyl residues in the sequence (H329, H330) and in equimolar samples this results in the formation of mononuclear 1:1 complexes. However, it is also clear from this study that bis(ligand) and dinuclear species can also be formed in the presence of ligand and metal ion excess, respectively. The two histidines are independent copper(II) binding sites in the dinuclear complexes and their coordination modes are described by the binding of imidazole-N and deprotonated amide nitrogen donor atoms. This binding mode allows the formation of coordination isomers because any of the two histidine moieties can be the primary ligating site. On the other hand it is quite surprising that copper binding affinity of an independent histidyl residue (H32) in the N-terminal region of the protein surpasses that of the histidines in the R3 domain (Figure 6b), which may be explained by the stabilizing role of the thioether side chain of methionine in the N-terminal fragment.

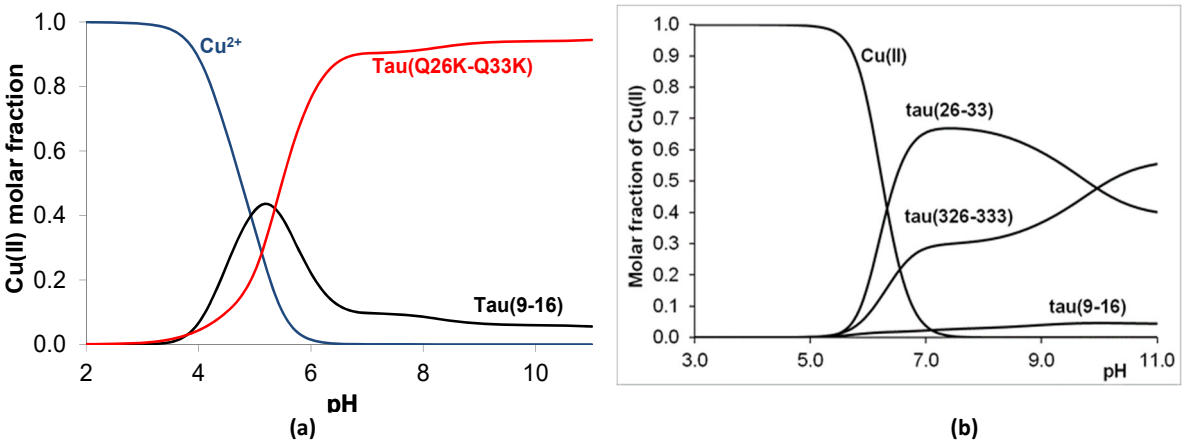


Figure 6*. Concentration distribution of copper(II) ion in a model system containing copper(II) and the peptides tau(9-16) and tau(Q26K-Q33K) mutant* ***(a)*** *and the peptides tau(326-333), tau(9-16) and tau(26-33)* ***(b)*** *in equimolar concentration (c = 1 mM)*

The studies of nickel(II) and zinc(II) complexes of various peptide fragments of tau protein reveal that the histidyl residues of the N-terminal and R3 regions of tau protein can effectively bind nickel(II) and zinc(II) ions. In case of nickel(II) and zinc(II) the M-N_{im} coordinated complexes are the major species in the physiological pH range and their stability is significantly enhanced by the presence of Glu and Asp residues in the neighbourhood of His14 site. For all studied peptides, nickel(II) ions are able to promote the deprotonation and coordination of amide groups preceding histidine resulting in the exclusive formation of square planar (N_{im},$3N^{-}$) complexes in alkaline samples. The native fragment of the R3 region and its mutants containing two adjacent histidine moieties also bind only one nickel(II) ion with the His330 residue being the primary metal binding site. Exclusive binding of the independent imidazole side chains (His14 and His32 sites) cannot prevent the hydrolysis of zinc(II) in slightly basic solution but the adjacent histidines of the R3 domain can promote the formation of amide coordinated zinc(II) complexes.

As a conclusion we can stated that although copper(II) and nickel(II) form higher stability complexes with all fragments of tau protein than zinc(II) but His32 is the preferred binding site for copper(II) and nickel(II) and His329-His330 for zinc(II). Thus, if both binding domains are present zinc(II) ions are forced to accumulate at His329-His330. This can be well presented by a model calculation where the distribution of metal ions between the two binding sites is compared in equimolar solution containing Cu(II)-Zn(II)-tau(26-33) (Gln/Lys)-tau(326-333) or Ni(II)-Zn(II)-tau(26-33) (Gln/Lys)-tau(326-333) (Figure 7). These figures clearly show that almost all of the copper(II) or nickel(II) is coordinated by His32, while the His329-His330 provides a binding site for zinc(II). These observations are in agreement with those assumptions that the

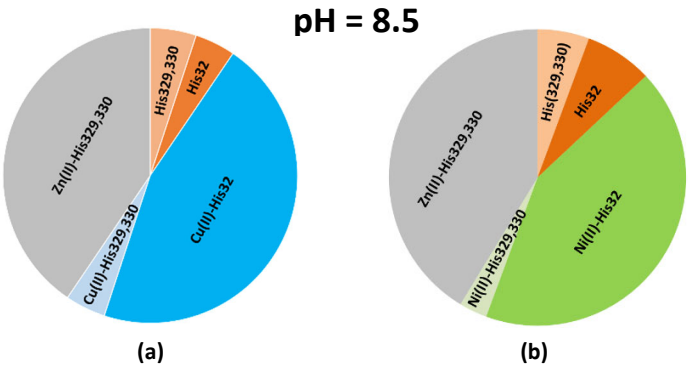


Figure 7. *Distribution of metal ions between the His32 and His329-His330 in equimolar solution containing Cu(II)-Zn(II)-tau(26-33) (Gln/Lys)-tau(326-333)* ***(a)*** *and Ni(II)-Zn(II)-tau(26-33) (Gln/Lys)-tau(326-333)* ***(b)*** *(c(M) = c(L) = 1 mM)*

zinc binding to residues of R3/R4 regions contributes the aggregation and at least partially enhances tau-R3 toxicities. However, in the case of zinc (II), one or both cysteine residues in the R3 region are also important binding sites, so we continue our research by studying tau model peptides containing both histidine and cysteine.

3. The effect of the change of redox properties of metal-protein adducts

A series of literature studies suggest the oxidative role of metal ions in the development of neurodegeneration. The coordination of the studied metal ions (first of all copper(II) and iron(II)/(III) in some cases) to the proteins can induce redox processes (oxidation, reduction) as well changing electrochemical properties of molecules. Accordingly the previous research will be continued with the studies of the redox properties of different copper(II) (and iron(II)/(III)) complexes of protein fragments and mutants.

The previous investigations of large polypeptides, which serve as the model of different metalloproteins, have shown that the the coordination of side chain donor atoms comes to the front. The formed complexes contain macrochelates, which results in not regular structures and easier reducibility of copper(II) complexes. The electrochemical studies of the peptide complexes, in which the systematic change of the amino acid sequence is carried out, give possibility to find the relation between coordination mode and reducibility, and contrary, the structural information can be concluded from the redox parameters of complexes. On the other hand, the redox parameters of the different model peptides can contribute to understand the role of copper(II) complexes in redox processes connecting to the oxidative stress.

The other direction of the redox processes is the copper(II) and iron(III) catalysed oxidation of peptides. The amino acid residues of histidine and methionine have been proposed to play important roles in metal mediated oxidative stress. Histidine oxidation predominantly forms oxo-histidine, methionine oxidation forms methionine sulfoxide and, under extreme conditions, sulfone. For study the role of copper(II)-histidine and -methionine interaction in the oxidation and hydrolysis of peptides, the systematic studies of methionine and histidine containing peptides are planned, where the oxidation and fragmentation processes are followed by HPLC-ESI-MS and MS/MS techniques.

3.1. Equilibrium and electrochemical studies of iron(II)/iron(III) complexes

3.1.1. Iron(II)/iron(III) complexes Asp_2 and Asp_3 peptides [2]

For iron(III) complexes the carboxylate groups of molecules are the primary binding sites and complex formation processes take place parallel with hydrolysis resulting in mixed hydroxido complexes. In the case of iron(II)-Asp_2 system, the interaction between the metal center and carboxylate functions could be supposed, however, the coordination does not result in pH effect. In this case the terminal amino group probably can coordinate to the iron(II) through the formation of 6-membered β-alanine like coordination supporting with macrochelation of distant β-carboxylate group. Nevertheless, this coordination mode is not able to hinder the formation of precipitation around pH 8. The stability constant of FeL in Asp_3 containing system is lower than that was described in the case of Asp_2. Similar tendencies have been reported in the nickel(II):Asp_2 and Asp_3 systems. This effect can be explained by the regular octahedral geometry of Fe(II), that is not preferred for the binding of the three β-carboxylate group.

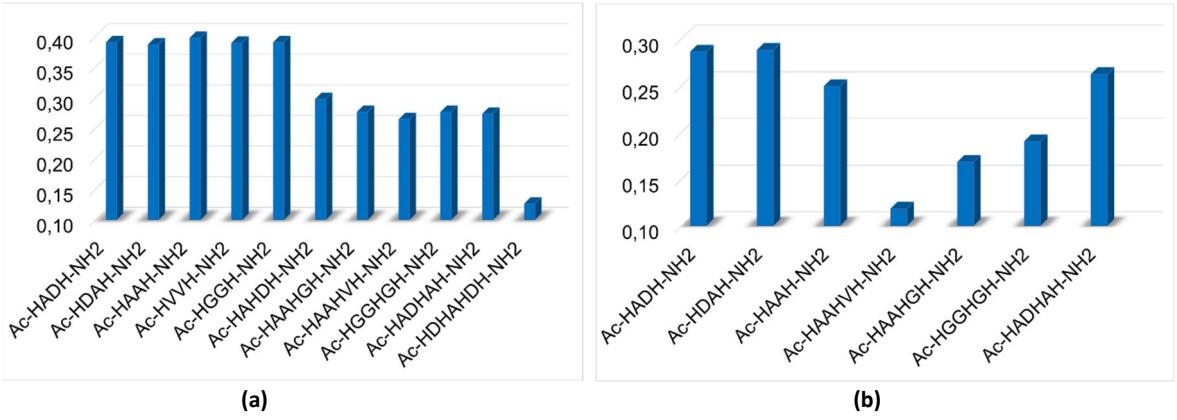
3.1.2. Iron(III) complexes of tridentate (O,N,O) ligands [18]

Iron(III) complexes of three ligands containing phenolic and azo- or triazol-nitrogen groups have been studied. All three ligands are able to bind iron(III) in strong acidic pH via (O^-,N,O^-) donor set. The coordination of two ligands in the bis(ligand)complexes saturates the coordination sphere of iron(III) and the meridonial arrangement was supported by DFT methods. The ligands with (O^-,N,O^-) donor set are able to bind iron(III) selectively at physiological pH. Moreover, the electrochemical parameters of complexes rule out that iron(III) complexes can initialize the formation of reactive oxygen species. These results clearly show that this type of ligands with (O^-,N,O^-) donor set are suitable for the designing of new chelator molecules with possible application to iron overload.

3.2. Redox properties of copper(II) complexes

3.2.1. The effect of side chain on the redox properties and SOD activity of Cu(II) complexes of multihistidine peptides [3]

The imidazole coordinated Cu(II) complexes of multihistidine peptides have similar coordination environments to that of copper(II) center of CuZnSOD. Based on the cyclic voltammetric studies we concluded that the more imidazole nitrogens are present in the coordinations sphere, the lower formal reduction potential values belong to the imidazole nitrogen coordinated complexes. However, redox parameters of $CuLH_{-1}$ and $CuLH_{-2}$ complexes containing amide nitrogen coordination can be determined as well (Figure 8). The measured SOD activity of these complexes are similar to those previously determined for other Cu(II) complexes of multihistidine peptides. Taking into account the species distribution curves of Cu(II) complexes, the SOD activity data reveal, that not only the imidazole coordinated CuL, but the $CuLH_{-1}$ and/or $CuLH_{-2}$ species have SOD activity due to their distorted geometry. As a conclusion we can say that such copper(II) complexes can be good models of the SOD enzymes, that have slightly distorted geometry and less rigid structure: the copper(II) complexes of those peptides which contain large number of amino acids, more histidine residues at different position in their sequences and both their exclusively imidazole coordinated complexes and the amide nitrogen bounded species have distorted geometry. The aspartic acid side chain with negative charge, lysine side chain with positive charge or the hydrophobic side chain of phenylalanine, however, have not significant effect on the redox parameters and SOD activity of copper(II) complexes.



(a) **(b)**

Figure 8. *The formal potential of the CuL (a) and CuH–2L (b) complexes of different multihistidine peptides*

3.2.2. Copper(II) catalyzed oxidation of the human prion 103-112 fragment and its mutants [19-22]

Metal catalyzed oxidation (MCO) is a hallmark of oxidative stress and accompanies biological aging and neurodegenerative diseases such as Alzheimer's and Parkinson's disease. MCO of proteins is mainly a site-specific process in which only one or a few amino acids at the metal-binding sites of the protein are preferentially oxidized. The amino acid residues of histidine and methionine have been proposed to play important roles in metal mediated oxidative stress.

Misfolded prion protein (PrPSc) is known for its role in fatal neurodegenerative conditions, such as Creutzfeldt–Jakob disease. PrP fragments and their mutants represent important tools in the investigation of the neurotoxic mechanisms and in the evaluation of new compounds that can interfere with the processes involved in neuronal death. Metal-catalyzed oxidation of PrP has been implicated as a trigger for the conformational changes in protein structure, which, in turn, lead to misfolding. Targeting redox-active biometals copper and iron is relevant in the context of protection against the oxidation of biomolecules and the generation of oxidative stress, observed in several conditions and considered an event that might promote sporadic prion diseases as well as other neurodegenerative disorders. In this context, ortho-pyridine aroylhydrazones are of interest, as they can act as moderate tridentate ligands towards divalent metal ions such as copper(II).

We studied the copper(II) catalysed oxidation of the HuPrP (103-112) fragment and its mutants, in which methionine residues are systematically replaced or displaced. The studied HuPrP fragment contains the −MKHM− sequence which has determent significance in the Cu(II) ion binding, and the oxidation of histidine and methionine residues can be followed simultaneously in it. On the other hand, the replacement of methionine residues help us to clarify the role of methionine in these processes.

The histidine residue behaves as an anchor site, and the (N_{im},N^-,N^-/N_{im},N^-,N^-,N^-) coordination modes prevent the oxidation of this side chain. The mutant peptide which does not contain methionine did not undergo oxidation, only the fragmentation of the peptide chain is perceived. The cleavage of the peptide bonds occurred far away from the histidine residue. However, in the case of methionine containing peptides, the peptide chain was not cleaved; the presence of methionine residues protects the peptides from fragmentation. In these cases copper(II) ions catalyze the oxidation of methionine to methionine sulfoxide. This process occurs randomly, it does not depend on the position of the methionine; methionine residues undergo oxidation either involved in the coordination of the copper(II) ions, or near or far the binding sites. Our results revealed that methionine residues of prion protein can play a role as ROS scavenger.

In the next step our studies have focused to explore the potentiality of this chemical class as peptide protecting agents against the deleterious metal-catalyzed oxidation in the M112A mutant fragment of human PrP. The effect of two pyridine-2-carboxaldehyde-derived aroyl-hydrazones, namely, HPCIH and HPCFur, on the copper-catalyzed oxidation of the M112A PrP103–112 mutant fragment was studied. The compounds inhere studied, especially HPCFur, showed an improved stability in aqueous solution compared to the classical, 8-hydroxyquinoline based metal-protein attenuating compounds, displaying a very interesting protective effect toward the oxidation of methionine and histidine, processes that are related to both physiological and pathological aging.

Copper(II) interacts with Ac-SKPKTNMKHA-NH_2 at pH 7.4 primarily through the anchoring site constituted by the side chain of histidine, in a coordination pattern completed by two or three deprotonated amide nitrogens from the main-chain peptide bonds involving H111, K110 and, possibly, M109. The thioether sulfur atom from methionine should be axially coordinated. Additionally, a

contact with the oxygen atom from the T107 side chain cannot be completely ruled out, especially if considering the putative formation of linkage isomers in this kind of systems. This redox-active metal is able to catalyze the oxidation of the peptide by hydrogen peroxide through the generation of ROS. When only the peptide, copper(II) and H_2O_2 are present, one major product is formed, corresponding to the oxidation of the methionine residue to methionine sulfoxide. On the other hand, the presence of compounds such as ascorbic acid may decreas the extent of peptide oxidation. However, its effect is not manifestly beneficial since hindering the reversible oxidation of methionine can be accompanied by the irreversible oxidation of the histidine side chain (Figure 9). Iron(III) ions are also catalyze the oxidation of the peptide, but they are less effective.

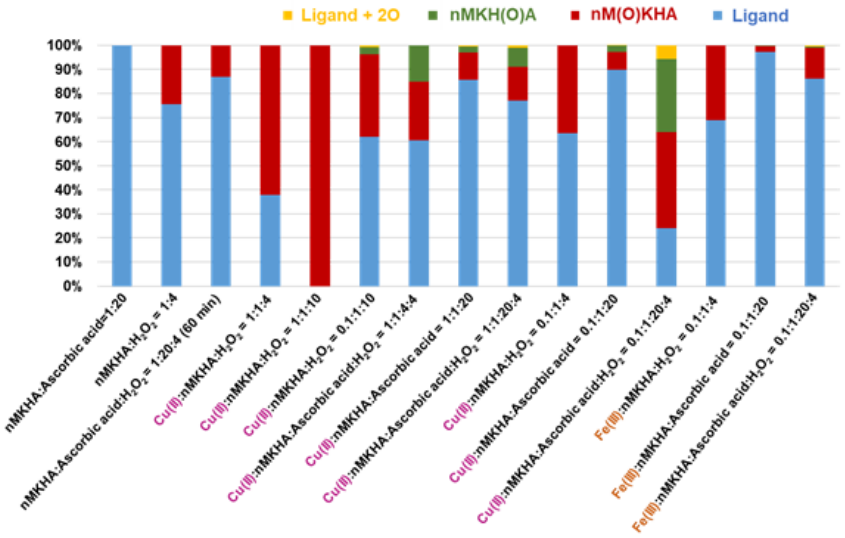


Figure 9. The ratio of products formed after 30 minutes oxidation

Concerning the impact of the synthesized aroylhydrazones in the oxidation of Ac-SKPKTNMKHA-NH_2, peptid one could state that HPCIH possesses a similar effect to that of ascorbic acid, protecting methionine from oxidation and, as an undesirable side effect, promoting, although to a lesser extent, histidine oxidation. Only a small amount of doubly oxidized product is generated, while the single oxidized product in which the additional oxygen atom is on the histidine residues is negligible. The compound HPCFur, on the other hand, not only prevented the irreversible oxidation of histidine, but it also protected 85% of the peptide from undergoing methionine oxidation. In the presence of ascorbic acid, the protective effect of HPCFur is still observed. Although aroylhydrazones are able to compete for the binding of copper with some amyloidogenic proteins, experimental evidences seem to point to a different mechanism in the present case, through the formation of a Ac-SKPKTNMKHA-NH_2–Cu(II)–HPCFur ternary complex. Our proposition is that steric- or kinetic-related factors associated with this species may impair the production of a coordinated hydroxyl radical, thus partially protecting the peptide from oxidative damage.

Similar studies was performed with the rationally designed and synthesized copper chelating scaffold, Salpyran (HL). This ligand is a tetradentate ligand, which offers a 3N,O coordination environment and possesses good drug-likeness. Salpyran exhibits an extremely high affinity for Cu and excellent Cu(II) selectivity over Zn(II). Under physiological pH values and anaerobic conditions, the $[Cu(II)L]^+$ complex remains intact for at least 2 days, while in the presence of H_2O_2, an oxidation procedure occurs. The Salpyran slows the ascorbate consumption, thus preventing ROS production. Its action as an antioxidant was tested in human prion protein (HuPrP(103– 112) assays, which reveal that Salpyran prevents the formation of reactive oxygen species from the binary Cu(II)/H_2O_2 system, demonstrating its potential use as a therapeutic small molecule metal chelator.

3.2.3. The study of oxidation reactions of prion protein fragment containing His85, His96 and His111 moiety [23]

It is widely accepted that at least six histidyl residues of human prion protein can take part in copper binding. These include four histidines of the octarepeat (His61, His69, His77 and His85) and two histidines (His96 and His111) outside the octarepeat domain. Our systematic studies on the peptide fragments of the protein revealed that the order of copper(II) binding affinity of the various histidyl sites is His111 > His96 > His(octarepeat). In addition to the complex formation, copper ions play an important role in various oxidative reactions of peptides. It has an importance also in the case of prion protein. Copper-bound prion protein undergoes redox cycling in the presence of electron donors, such as superoxide ions, dopamine or ascorbate. The mechanism of oxidative damage to proteins involves catalysis by transition metals. This process consists of reduction of Fe(III) or Cu(II) by electron donors, such as O_2^-, H_2O_2, ascorbate or thiols, and generation of the hydroxyl radical through reduction of H_2O_2 by the reduced metals. This highly reactive free radical immediately oxidizes neighbouring amino acid residues. Histidine and methionine are the most important targets of protein oxidation.

The studied N- and C-terminally protected tridecapeptide (Ac-PHAAAGTHSMKHM-NH_2) contains the main binding site of the octarepeat domain in addition to the aforementioned His96 and His111 moieties. The equiblibrium studies have shown, that this peptide is able to bind three equivalent of copper(II) ions, since the histidine residues behave as independent metal binding sites. Nevertheless the metal binding ability of histidine residue mimicking the octarepeat domain (His85) is decreased, while the other parts of the peptide mimicking the histidine residues outside the octarepeat domain binds the copper(II) ions in comparable concentration. Copper(II) catalyzed oxidation of the peptide proved that the order of tendency for oxidation is His96 > His85 ≫ His111. However in the case of the singly oxidized products, only the oxidation of His96 residue is proved. The difference in the copper(II) ion oxidation susceptibility may be due to the steric arrangement of the peptide. The copper(II) ion is easily available for the hydrogen peroxide in the case of the -GTHS- domain, where the side chains are relatively small. However, owing to the axial interactions of the larger side chains in the -MKHM- domain the copper(II) ion is locked and therefore the oxidation at that position occurs to a smaller extent. The oxidation of the two methionine residues cannot be avoided. The metal binding ability of the histidine residues outside the octarepeat region is almost comparable, but the susceptibility for oxidation differs. The first order can be explained by the fact that the coordination mode at the -GTHS- and -MKHM- domain is the same, (Nim,N^-,N^-) in the form of (6,5,5)-membered fused chelate rings, which is preferred over the (7,5,5)-membered one.

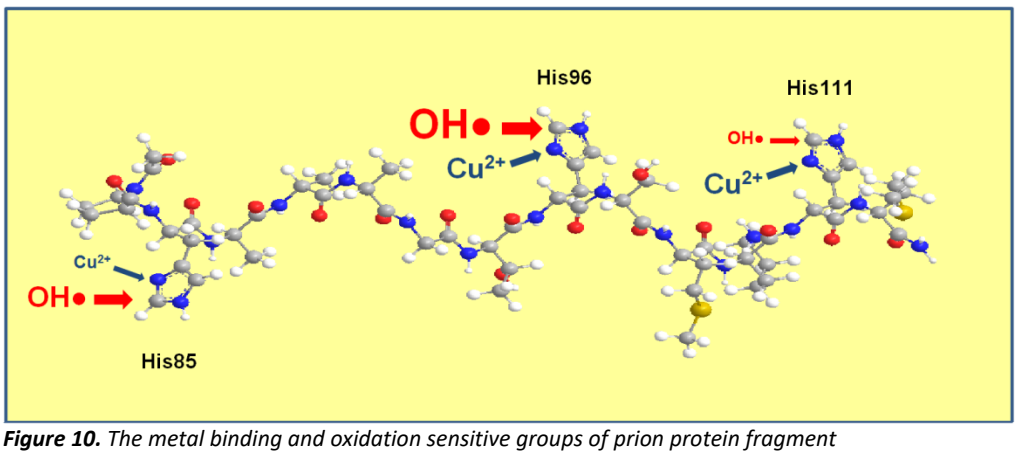


Figure 10. *The metal binding and oxidation sensitive groups of prion protein fragment*

4. The potential role of the binding of toxic elements (cadmium(II) and lead(II)) to the modell peptides related to the neurodegenaration

Normally cadmium(II) and lead(II) ions are not present in the biological systems, have no known biological functions in humans, but theirs toxicity is highly based on the fact that these ions are able to replace the essential metal ions

In addition to trace metal ions assumed participation in the neurodegenerative diseases the potential role of toxic metal ion should have in sight. For example cadmium(II) and lead(II) are widely used metals causing increased accumulation in the biological systems. This means, that these metal ions give rise to potential risk in health disorders. Cadmium has been implicated as a possible ethological factor of neurodegenerative diseases such as PD, AD and amyotropic lateral sclerosis (ALS). The earlier studies and literature data obviously show, that the most effective binding site for cadmium(II) ion is the thiolate group of cysteine. To clarify the possible role of these two metal ions in these diseases, the peptides containing histidine, aspartic acid, glutamic acid and/or cysteine are planned to be synthesized and studied. Literature data are scarcely available in this field and the reactions are more complicated than for the histidine containing counterparts. In the first step peptides containing both histidine / aspartic acid / glutamic acid and cysteine residues will be synthesized and studied. The last part of the project some multicysteine peptides with 2 (or max 3) cysteine residues will be included. To get information about the metal binding selectivity of these molecules the characterization of other metal (e.g. zinc(II) and nickel(II)) complexes are planned as well.

4.1. The effect of Asp side chain on the cadmium(II) and lead complexes of small peptides [2]

The effect of more polar side chain donor groups on the coordination ability of peptides was studied for Cd((II) and Pb(II) ion complexes of di- and tripeptides containing two or more aspartic and/or glutamic acids in the sequence. The stability of the cadmium(II) and lead(II) complexes increases in the AspAla < Asp_2 < AspGlu < Asp_3 < Asp_4 order. The increase of the number of carboxylate groups results in the increase of stability, which refers to the fact that the deprotonated carboxylate groups take part in the metal ion binding, and the increasing negative charge of the complexes do not hinder the formation of them. The contribution of carboxylate groups in the metal binding – similarly to zinc(II) complexes – results only in slight effect and cannot prevent the hydrolysis of metal ion above pH 7.

4.2. The effect of cysteinyl residue on the selectivity of cadmium(II) and lead complexes of peptides

4.2.1. Cadmium(II) and lead(II) complexes of multicysteine peptides [9]

Toxic metal ions, such as Cd(II) or Pb(II) can replace essential Zn(II) or other transition metal ions causing a failure in the function of enzymes. Removal of these harmful ions can be achieved by the development of peptides having enhanced metal ion selectivity towards these elements. To achieve the goal to synthesize peptides with high selectivity we performed the systematic studies of small terminally protected peptides containing CXXX, XXXC, XCCX, CX_nC (*n* = 1-3) sequences The cysteine thiolate group is the primary binding site for both cadmium(II) and lead(II) metal ions, but the presence of a histidyl or aspartyl side chain in the molecule contributes to the stability of the

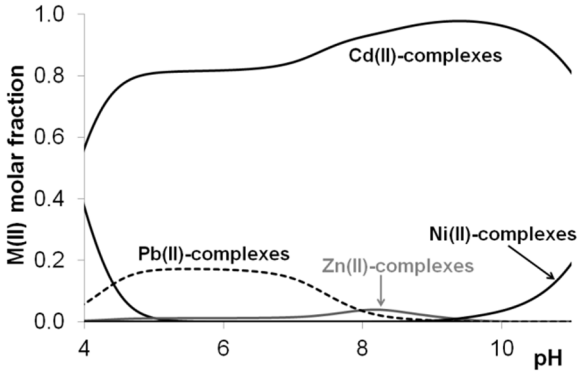


Figure 11. *Concentration distribution curves of model system containing Cd(II):Pb(II):Zn(II):Ni(II):Ac-CSSC-NH_2 in 1:1:1:1:1 ratio (c_L = 1 mM)*

complexes. For two-cysteine containing peptides the (S^-,S^-) coordinated species are formed in the physiological pH range and the stability increases in the Ni(II) < Zn(II) < Pb(II) < Cd(II) order (Figure 11). As a conclusion, the inserting of –CXXC– sequence into the peptide makes the synthesis of peptides with high selectivity to toxic Cd(II) or Pb(II) ion possible. In addition, the spectroscopic characterization of these complexes can contribute to the recognition of the exact binding site and binding mode of longer peptides mimicking the biologically important proteins

4.2.2. Lead(II) complexes of oligopeptides containing two cysteine residues [24]

The hexa- and heptapeptides containing two cysteine residues in separated positions (CSSACS-NH_2, ACSSACS-NH_2) have outstanding zinc(II) and cadmium(II) binding affinity and the selectivity in the cadmium(II) binding was proved. Since lead(II) ion has similar soft character as cadmium(II) ion it seemed to be worth the continuation of these studies on one hand with characterization of lead(II) complexes of both oligopeptides, on the other hand with synthesis and studies of octapeptides containing two cysteine residues. The studied three peptides are CSSACS-NH_2 hexapeptide, ACSSACS-NH_2 heptapeptide and SSCSSACS-NH_2 octapeptide, in which the longer is the peptide the farther is the –CSSAC– sequence from N terminus. It means that in these ligands one cysteine is in the first, second and third N-terminal position, respectively, together with a distant cysteine residue on the C-termini.

The data obtained in complex formation processes of above three oligopeptides reveal that for all three ligands the stability of complexes follows the Cd(II) > Pb(II) > Zn(II) order.

The stability of ML complex of ligand containing N-terminal cysteine group is different from that of other two peptides due to the stable tridentate coordination of the ligand. If the –CSSAC– sequence is farther from the N-terminal part of the molecule the (S^-,S^-) sequence is the main binding site and the contribution of N-terminal amino group in the metal binding significantly decreases or it is negligible. The formation of bis(ligand) complexes can be observed only for the ACSSACS-NH_2 and SSCSSACS-NH_2. The main difference is between these bis(ligand) complexes is that Pb(II) is bounded through $3S^-$ donor atoms, while the $2\times(S^-,S^-)$ coordination mode is characteristic for CdH_xL_2 and ZnH_xL_2 (x=0-2) complexes, which can be explained the different coordination geometry of metal ions. It was concluded that these ligands have high affinity to bind zinc(II), cadmium(II) and lead(II) ion. The higher thermodynamic stability of lead(II) and cadmium(II) complexes, however, results in a good selectivity for Pb(II) and/or Cd(II) over Zn(II) and this selectivity increases with the increase of distance between the terminal amino group and –CSSAC– sequences.

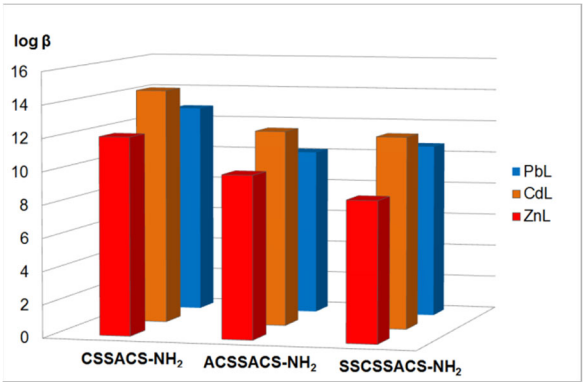


Figure 12. The stability constants of [ML] complexes of three studied oligopeptides

These results were published in 24 papers in international Q1 and Q2 journals (14 Q1 (including two D1 journals), sum of IF: 91.452, independent references: 97. There were submitted in 5 PhD thesis and presented in international conferences in 23 posters and 6 lectures and in domestic conference in 11 lectures.

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