Propositions of PhD theses

COMPLEX FORMATION PROCESSES OF DERIVATIVES OF AMYLOID-β PEPTIDE WITH COPPER(II), NICKEL(II) AND ZINC(II) IONS

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I. INTRODUCTION AND THE AIM OF THE WORK

Increase of the concentration of some metal ions and formation of amyloid plaques can be observed in certain regions of brain of patients with Alzheimer's disease. The disorder of the homeostasis of these metal ions can be either the ***cause*** or the ***result*** of the development of the disease. This question still remains unanswered and in the focus of research in the last decades.

Study on the interaction of copper(II), zinc(II) and iron(II/III) with β-amyloid playing an important role in Alzheimer's disease is of unambiguous importance. The accumulation and aggregation of the pathologic form of the peptide is accompanied with the increase of the amount of the above mentioned metal ions. The amyloid-β peptide consists of 40-43 amino acids including metal binding and polar side chains confirming the role of Cu(II) and Zn(II) ions in the development of amyloidosis.

Many research groups including the *Bioinorganic Chemistry Research Group of the Inorganic and Analytical Chemistry Department of the University of Debrecen* have studied the metal binding ability of the peptide. It is proved that the N-terminal fragment of the peptide is responsible for metal binding while the C-terminal part of the molecule containing nonpolar side chains represents hydrophobic character and therefore the chance for aggregation.

The solution equilibrium studies were hindered by solubility problems but the pegylation of the peptide gave the possibility for determining the stability of metal complexes and the affinity of metal ions towards the different binding sites. The measurements involved Cu(II), Zn(II) and Ni(II) ions, too, latter being a model of copper(II) because of the stable and similarly coordinated complexes with the peptide. The results stated that the 1-16 fragment of the peptide containing the terminal amino group and His6, 13 and 14 as anchoring side chains, plays a role in metal binding. In the case of Aβ(1-16) the coordination of the mentioned transition metal ions are summarized in *Table 1*.

Table 1: Distribution of transition metal ions among the binding sites of the Aβ(1-16) fragment

	–NH_2 (terminal)	*His6*	*His13*	*His14*	*Number of bound metal ions*
Cu(II)	1	1	1	1	4
Ni(II)	1	–	1		2
Zn(II)	1		2		3

The distribution of metal ions between the binding sites were described before, however, the exact set of donor atoms involved in the formed species remained still unknown. The studied peptide sequence contains various and similar coordination sites, too, but their affinity towards the metal ions may differ.

As the first part of our work we planned to model coordination modes including histidine(s) and/or terminal amino group as anchoring sites in the same molecule by using ligands containing isolated chelating environments in order to compare the stability of their complexes. The study of fragments and/or mutants of the Aβ(1-16) region represented the other section of work. These peptides included the native fragments or derivatives of Aβ(1-9) and Aβ(8-16). In order to describe the exact coordination modes we synthesized model ligands containing the binding sites of the native peptides in the same or separate molecules in the research group.

In the past few years new insights were found about the development of aggregation and plaque formation attached to AD in which the key step is referred to the dimerization of the amyloid-β peptide. These findings gave the idea to synthesize the amyloid dimer derivative built up of two Aβ(1-16) metal binding fragments connected at the C-termini and pegylated to enhance the solubility of the molecule.

The formation of copper(II), nickel(II) and zinc(II) complexes of the mentioned ligands were studied using pH-potentiometric, UV-Vis, CD, ^{1}H NMR, ESR spectroscopic and ESI-MS techniques. Due to the biological relevance the simultaneous presence of these metal ions are also important to investigate if mixed metal complexes are possible to be formed.

II. EXPERIMENTAL METHODS

1. Solid phase peptide synthesis (SPPS):

The oligopeptides were synthesized using a microwave-assisted Liberty1 peptide synthesizer (CEM, Matthews, NC) in our research group. The C-terminally free or protected molecules were prepared using Fmoc-Gly-Wang or Rink Amide resins as solid phase containing glycine amino acid or amino groups on their surfaces. Introducing of the amino acids followed the Fmoc/*t*Bu technique while the activation of carboxyl and amino groups were carried out according to the TBTU/HOBt/DIEA strategy in *N*,*N*-dimethylformamide as solvent. The structure of the peptides and the peptide derivative prepared at the *University of Catania*, Italy are collected in *Figures 1.a* and *1.b*.

2. High pressure liquid chromatography (HPLC)

The purity of the ligands was checked by using HPLC. 2-3% of eluent A (0.1% TFA in water) and 97-98% of eluent B (0.1% TFA in acetonitrile) and flow rate of 0.8-1.0 ml/min was used to gain information about the number of compounds in the crude product. The experiments were carried out on a Vydac C18 reversed phase column (250 mm × 4.6 mm, pore size: 300 Å, particle size: 5μm) using a Jasco MD-2010 plus detector on 222 nm wavelength.

3. pH-potentiometric titration was used to check the purity of the peptides and to determine the protonation constants of the ligands and the stability constants of copper(II), nickel(II), zinc(II) and mixed metal complexes. The experiments were carried out in aqueous solution at different metal to ligand ratios ($0 \leq M{:}L < 2$) at 25 °C and constant ionic strength of 0,20 mol/dm^3 KCl. The concentration of the ligands was set between 1×10^{-3} and 2×10^{-3} mol/dm^3. Protonation constants of the ligands and stability constants of the metal complexes were calculated by using the computational programs PSEQUAD and SUPERQUAD based on the experimental data. According to the determined values complex distribution curves of the systems were plotted using the MEDUSA program.

4. UV-visible spectrophotometric studies were carried out in the copper(II) and nickel(II) containing systems at room temperature in the wavelength range 250-800 nm. Absorption spectra were recorded in the 3-12 pH range using a Perkin Elmer Lambda 25 double beam scanning spectrophotometer and quartz cuvettes with 1,000 cm path length. The concentrations and the metal to ligand ratio were similar to those of the pH-potentiometric studies.

5. Circular dichroism (CD) spectroscopic measurements were performed on a JASCO-810 spectropolarimeter. This technique is appropriate to determine the structure of metal complexes having optical activity, in our case the species containing Cu(II) and/or Ni(II). The ratio of the metal to ligand, the temperature, pH range and wavelength range were similar to those of the spectrophotometric measurements, the path length was 0,100 and 1,000 cm.

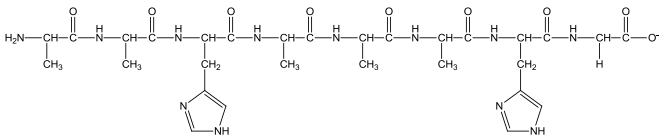
6. ^{1}H NMR spectroscopic studies were carried out on a BRUKER AM 360 MHz FT-NMR spectrometer and the spectra were evaluated by using 1D WIN NMR Bruker software. This method gives information about the purity of the peptide and the coordination modes of some Zn(II) complexes and square planar Ni(II) containing species by comparing them with the spectra of the free ligand.

7. Electrospray ionization mass spectrometry (ESI-MS) was used to identify the synthesized ligands and some dinuclear and mixed metal complexes. The measurements were carried out on a micrOTOF-Q 9 ESI-TOF spectrometer in samples with 1×10^{-4} mol/dm^3 ligand concentration.

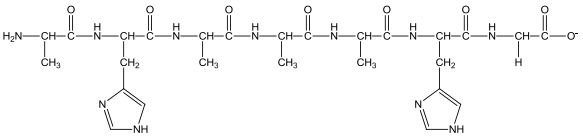
8. Electron spin resonance spectroscopy (EPR) was carried out at the *University of Sassari* on a Bruker EMX spectrometer using ^{63}Cu enriched $CuSO_4 \cdot H_2O$ and 10% ethylene glycol in the samples at T = 120 K.

III. STRUCTURE OF THE STUDIED LIGANDS

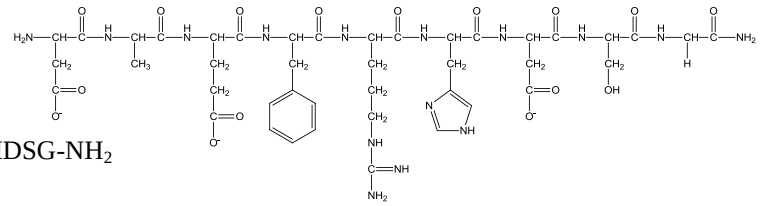
I.A, H_2N-AAHAAAHG-OH



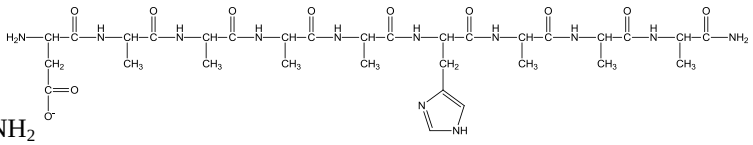
I.B, H_2N-AHAAAHG-OH



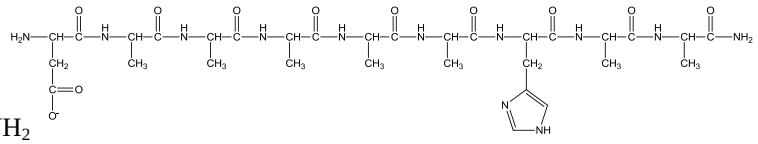
II.A, H_2N-DAEFRHDSG-NH_2



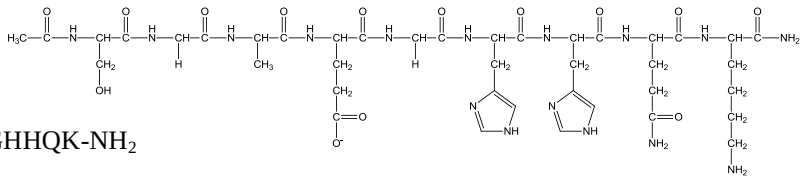
II.B, H_2N-DA_4HA_3-NH_2



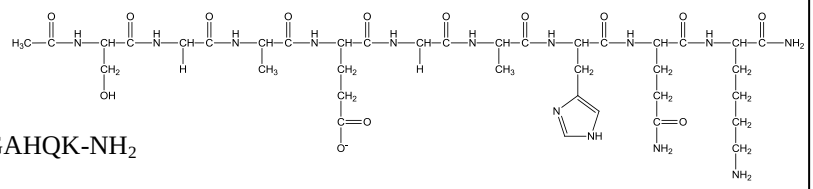
II.C, H_2N-DA_5HA_2-NH_2



III.A, Ac-SGAEGHHQK-NH_2



III.B, Ac-SGAEGAHQK-NH_2



III.C, Ac-SGAEGHAQK-NH_2

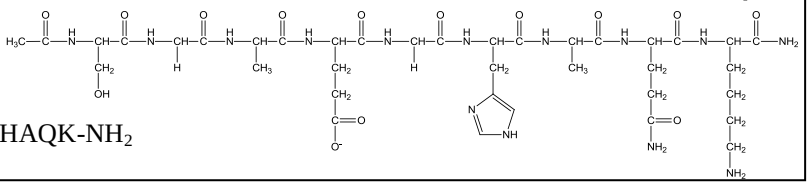


Figure 1.a: Structure of the oligopeptides synthesized at the Bioinorganic Chemistry Research Group

IV.A, (H_2N-DAEFRHDSGYEVHHQK)$_2$-*N,N*-bis(3-aminopropyl)glycine-NH-PEG

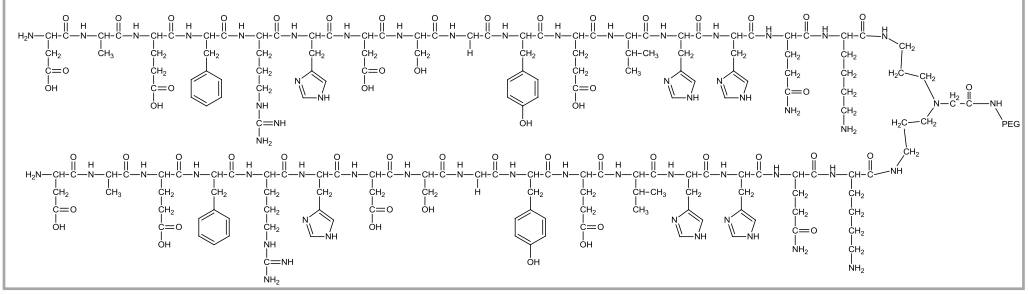


Figure 1.b: Structure of the synthesized amyloid dimer derivative

IV. NEW SCIENTIFIC ACHIEVEMENTS

The interaction of copper(II), nickel(II) and zinc(II) ions with fragments, mutants and model peptides of amyloid-β peptide was described in my PhD theses. The deprotonation and Cu(II), Ni(II) and Zn(II) complex formation processes were studied by pH-potentiometry and according to the calculated stability constants species distribution curves were plotted. Spectroscopic parameters of the complexes formed in the copper(II) and/or nickel(II) containing systems were determined by means of UV-visible and CD spectroscopic studies. The structure and the ratio of the formed complexes were given on the comparison of CD spectra recorded for small ligands mimicking the binding sites. The presence of dinuclear and bisligand species was also confirmed by ESR and MS measurements.

A *hepta-* and an *octa-peptide* were studied each containing two binding sites including terminal amino group and/or histidine which can also form mixed metal complexes. Further parts of the work involves the native 1-9 fragment of the amyloid-β peptide and its two mutants, three derivatives of the 8-16 fragment and the amyloid dimer form built up of two Aβ(1-16) chains. Latter ligand was studied only in the presence of copper(II) and zinc(II) ions.

1. Ligands containing isolated chelating donor functions

Comparison of metal binding ability to different coordination modes involving similar donor atoms is possible by using ligands containing separated anchoring groups. The *octapeptide* H_2N-AAHAAAHG-OH and the *heptapeptide* H_2N-AHAAAHG-OH can be used as models for this study with structures represented in *Figure 1.a*, group I. On the amino terminus of the *octapeptide* the (H_2N,N^-,N^-,N_{Im}) coordination mode can form stable complexes with copper(II) and nickel(II) ions mimicking the ATCUN motif. Both ligands contain an internal histidine being the starting point of amide coordination towards the N-terminus with the donor set (N^-,N^-,N^-,N_{Im}). The amino terminus of the *heptapeptide* offers an unsaturated coordination sphere to metal ions including the donor atoms (H_2N,N^-,N_{Im}) accompanied with the macrochelation of His6.

a, H_2N-AAHAAAHG-OH *(octapeptide)*

- The N-terminal part of both ligands is the primary ligating site in the case of Cu(II) and Ni(II) ions in the major species $MH_{-1}L$ ($MH_{-2}LH$), however, dinuclear complexes can be also formed with these metal ions.
- Zn(II) ions are coordinated to the amino group and the histidine side chains forming a macrochelate structure in slightly acidic medium. It results in higher stability of the complexes compared with the olygoglycines, but the hydroxide precipitation appears at pH 7. Deprotonation of amide nitrogens cannot be observed, the stability of zinc(II) complexes are lower than those of copper(II) or nickel(II) ions.
- Cu(II)/Ni(II) mixed metal complexes can be also detected and supported by mass spectrum, the coordination isomer with copper(II) on the N-terminal part and nickel(II) at the His7 is present as the major species according to the CD-spectra.

b, H_2N-AHAAAHG-OH *(heptapeptide)*

- On the contrary in case of the *heptapeptide* the preference of the amino terminus can be observed not only for copper(II) and nickel(II) but also for zinc(II) ions.
- Metal ion coordination starts in slightly acidic pH-range on the N-terminus, the equatorial binding of the imidazole nitrogen of His6 enhances the stability of the complex $MH_{-1}L$.
- At high pH the structure of mononuclear copper(II) and nickel(II) complexes alters: either the His2 leaves the coordination sphere with further amide binding or the rearrangement of the metal ion occurs into the environment of His6.
- Dinuclear species are also formed except with zinc(II), however, they appear unambiguously at higher pH than detected for the *octapeptide*. The explanation for this phenomenon is the presence of the highly stable macrochelate structure of the mononuclear species.
- Mixed metal complexes are formed in the Cu(II)/Ni(II) system, furthermore, nickel(II) ions behave remarkably different in the presence of zinc(II).
- Distribution of metal ions between the three binding modes represented by the two model peptides shows an interesting picture. The well-known albumin-like sequence on the N-terminus of the *octapeptide* has lower metal binding ability compared with the (H_2N,N^-,N_{Im},N_{Im}) coordination mode stabilized by the macrochelate-type binding represented by the *heptapeptide*. The enhanced stability of the latter binding site is indicated by the different pH values of the appearance of the species $CuH_{-1}L$ ($CuH_{-2}LH$) and CuL ($CuH_{-1}LH$) for the *octa-* and *hepta-peptide*. In the speciation curves of the ternary system of

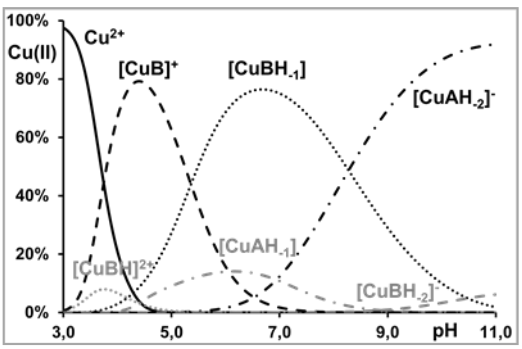


Figure 2: Complex distribution diagram of the Cu(II): H_2N-AAHAAAHG-OH(A): H_2N-AHAAAHG-OH(B) equimolar system

the two ligands and copper(II) shows high ratio of metal ions bound to the *hepta-peptide* in contrast with the albumin-like coordination highly preferred by Cu(II) and Ni(II) ions. *(Figure 2)*

- Zn(II) ions have significantly higher affinity to the (H_2N,N^-,N_{Im},N_{Im}) coordination mode which is supported by the formation of mixed zinc(II) complexes. In the presence of zinc(II) distribution of copper(II) or nickel(II) alters between the binding sites resulting in a higher ratio of latter ions bound at the His6.

2. Aβ(1-9) fragment and its model peptides

The native amyloid-β peptide fragment contains terminal amino group and histidine at the position 6 as anchoring side chains with the coordination modes (H_2N,N^-,N^-,N^-) and (N^-,N^-,N^-,N_{Im}) which were described before using the Aβ(1-6) fragment. In the full-length $A\beta_{40-42}$ peptide, however, 9 amino acids on the N-terminus can be involved in metal ion coordination with the binding mode (N_{Im},N^-,N^-,N^-), furthermore, coordination isomers can be formed. According to the results of the study of the *Aβ(1-9)* fragment the ratio of the isomer complexes can be determined. *(Figure 3)* In order to give the exact donor sets playing role in metal binding we also studied two model peptides with the sequences H_2N-DAAAAHAAA-NH_2 *(DA_4HA_3)* and H_2N-DAAAAAHAA-NH_2 *(DA_5HA_2)*.

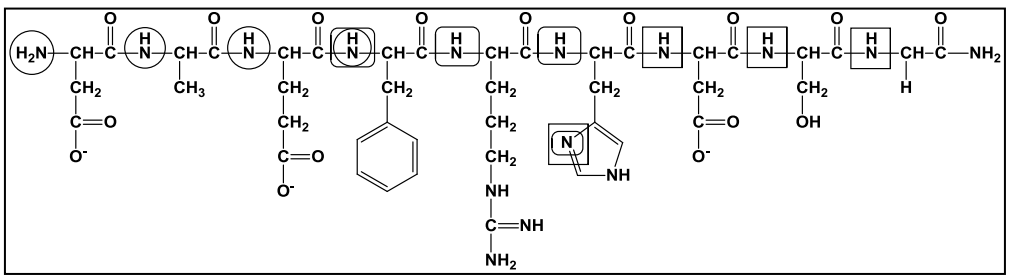


Figure 3: Donor atoms involved in the possible coordination modes of the Aβ(1-9) native fragment.

a, H_2N-DAAAAHAAA-NH_2 *(DA_4HA_3)*

- The first model peptide was used to examine the absence of side chains with the replacement of the amino acid residues to alanine except Asp1 (Ala2) and His6. The complex distribution curves are similar to those of the native Aβ(1-9) fragment in the studied

pH range, however, species with the same structure formed with lower stability.

- The primary ligating site was the amino terminal part again, at higher amount for copper(II), too (~90%). As a consequence, absence of polar and weakly coordinating side chains decreases the metal binding ability at His6.
- Only weak interaction can be observed in the case of Zn(II) ions with the involvement of the terminal amino group, side chains of Asp1 and His6 resulting in the hydrolysis of the metal ion above pH ~ 8.
- Dinuclear complexes form exclusively with copper(II) ions only at high pH after the dissolution of the hydroxide precipitation. Amide coordination towards the C-terminus (N_{Im},N^-,N^-,N^-) can be observed only in strongly alkaline medium.

b, H_2N-DAAAAAHAA-NH_2 *(DA_5HA_2)*

- Compared with the *DA_4HA_3* sequence His6 was shifted toward the C-terminus into position 7 for the saturation of the coordination spheres with the appropriate number of amide nitrogens. It means that in spite of the *DA_4HA_3* and *Aβ(1-9)* ligands in dinuclear copper(II) complexes (H_2N,N^-,N^-,N^-) and (N^-,N^-,N^-,N_{Im}) coordination can be present simultaneously.
- The metal binding ability of the *DA_5HA_2* model ligand is similar to that of the above mentioned peptide, the position of histidine does not result in the change of complex formation processes. Coordination modes present in the two model peptides are alike to the binding modes in the Aβ(1-9) fragment.

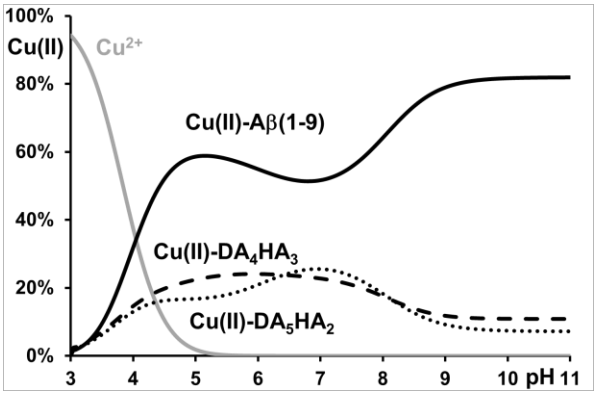


Figure 4: Distribution of Cu(II) among the Aβ(1-9) fragment and its mutants Cu(II):Aβ(1-9):DA_4HA_3:DA_5HA_2=1:10:10:10

- The sequence of the native fragment is especially suitable for copper(II) binding as it is shown by the comparison with the DA_4HA_3 and DA_5HA_2 ligands on *Figure 4*.

c, H_2N-DAEFRHDSG-NH_2 *(Aβ(1-9))*

- All the three metal ions are able to form mononuclear complexes with the coordination mode (NH_2,β-COO^-,N_{Im}) accompanied with aspartyl and glutamyl side chains.
- Deprotonation and coordination of amide nitrogens occur only in the case of Cu(II) and Ni(II) ions.
- Both metal ions prefer the amino terminal part of the ligand, the amount of nickel(II) bound at the N-terminus is higher (~100%) than the ratio of copper(II) (~75%).
- Dinuclear species is formed only with copper(II) ions in which 4N coordination takes place rather at the N-terminus.

3. Derivatives of the amyloid-β (8-16) fragment

The metal ion affinity of histidine 13 and 14 in the native fragment was studied using the Val12Gly point mutated former examined Aβ(8-16)Tyr10Ala derivative. Inserting the optically inactive glycine residue is necessary to determine the structure based on the CD-spectra. Therefore the amide-coordination starting at the His13 or His 14 can be differentiated according to their spectra. In order to characterize these two binding modes in addition to the mentioned mutations the His13Ala or His14Ala replacements were also needed.

a, Ac-SGAEGAHQK-NH_2 *(Aβ(8-16)AH)*

- The His13Ala derivative containing one histidine residue is able to bind one equivalent of copper(II) or nickel(II) with the donor set (N^-,N^-,N^-,N_{Im}).
- Zinc(II) ions form low stability complexes resulting hydroxide precipitation above pH ~ 6.

b, Ac-SGAEGHAQK-NH_2 *(Aβ(8-16)HA)*

- Mononuclear complexes are formed in the presence of the His14Ala mutated peptide, too, similarly to the *Aβ(8-16)AH* derivative.
- Hydrolysis of Zn(II) occurs, precipitation appears above pH ~ 6.
- According to these results two histidine residues in the peptide sequence are necessary to form stable zinc(II) complexes.
- The complex formation processes differ for the two derivatives containing one histidine in the stability of the species $CuH_{-1}L$ ($CuH_{-2}LH$). The enhanced stability of this complex with the His14Ala mutant can be explained by the axial binding of Glu(COO^-) resulting in the coordination of the third amide nitrogen shifted to higher pH values.

c, Ac-SGAEGHHQK-NH_2 *(Aβ(8-16)HH)*

- The possible coordination of metal ions can start either at His13 or His14 towards the amino terminus with the donor set (N^-,N^-,N^-,N_{Im}) and from His14 toward the C-terminus with (N_{Im},N^-,N^-,N^-) coordination.
- The amount of the isomer mononuclear complexes is approximately equal with (N^-,N^-,N^-,N_{Im}) binding mode towards the N-terminus and the additional axial coordination of the other histidine.
- Formation of amide-coordinated dinuclear species is hindered by the structural change resulting in the rearrangement of the first equivalent of copper(II) to the His13 and amide coordination towards the C-terminus from His14.
- In the presence of peptide excess the formation of bisligand complex CuL_2H_2 is proved by ESR spectroscopy.
- The stoichiometry of nickel(II) complexes is exactly Ni(II):L = 1:1 and the His14 is the preferred binding site (~100%)
- High Zn(II) binding ability can be observed similarly to the former studied Aβ(8-16)Tyr10Ala derivative. Bisligand complexes can form in the presence of ligand excess while at higher metal to ligand ratio dinuclear species can be calculated according to the pH-potentiometric titration data. Latter complex is supported by ESI-MS spectra.

4. The amyloid dimer derivative

The dimeric behaviour of the β-amyloid peptide can be strengthened by the study of the $A\beta(1\text{-}16)_2$-$C_8ON_4H_{17}$-PEG oligopeptide derivative containing 8 binding sites. *(Figure 1.b)*

- Protonation constants of the 21 function groups of the ligand are in the $1.5 < pK < 11.5$ range.
- Although the pH-potentiometric studies gave only qualitative information about the interactions because of the low concentration of the ligand (7×10^{-4} mol/dm^3), the UV-visible and CD spectroscopic measurements on the Cu(II):L = 1:1 system alludes to the present coordination modes.
- The synthesized ligand is able to bind 8 equivalent of copper(II) ions, only higher amount of the metal ion results in precipitation.
- Based on the pH-dependence of CD spectra in the presence of equivalent amount of copper(II) coordination of amide nitrogens is hindered compared to the monomer form in the physiologic pH range. This observation underlines the importance of the formation of Aβ dimers during the aggregation process.
- Adding zinc(II) ions to the Cu(II):L = 1:1 system causes, surprisingly, the increase of CD-bands, indicating the modification of coordination modes of copper(II). As a conclusion, zinc ions cannot remove copper(II) from the complex but alters its coordination in the species.

V. POSSIBLE APPLICATION OF THE RESULTS

Histidyl side chains are important and frequent binding sites in proteins. As it is proved identical coordination modes can have different affinities toward transition metal ions. Binding sites including histidine were compared in two model peptides based on the stability of their copper(II), nickel(II) and zinc(II) complexes to give information about the ability of metal binding in the case of histidine containing peptides. It was found that the (NH_2,N^-,N_{Im},N_{Im}) coordination mode is significantly stable in acidic, neutral and also in slightly basic media compared with the ATCUN motif, which forms highly stable Cu(II) and Ni(II) complexes. Furthermore, the *heptapeptide*, H_2N-AHAAAHG-OH is preferred by Zn(II) ions, too. The amyloid-β peptide which is important because of its basic role in Alzheimer's disease contains these anchoring groups, too, and their fragments were studied in the presence of the mentioned three metal ions. According to the structural study of the formed complexes, donor atoms, preference of metal ions towards the binding sites and ratio of the isomer species were determined. In accordance the high affinity of Cu(II) and Zn(II) ions toward the amyloid-β peptide can be unambiguously stated. The preference of the two metal ions are, however, different: while in the case of copper(II) ions the amino terminus is the primary ligating site, zinc ions are preferably bound at histidines 13 and 14. The addition of Zn(II) ions causes the redistribution of Cu(II) ions from the imidazole-coordinated complex into an amide-coordinated species on the amino terminus in the *amyloid dimer* ligand. In the case of Aβ(8-16) derivatives bisligand complexes were also detected which supports the ***metal ion induced oligomerization*** of amyloid-β in vivo. The amide coordination in the $A\beta(1\text{-}16)_2$ dimer derivative is shifted to higher pH value and to slightly basic medium, confirming also the dimerization process.

As a conclusion our results can contribute to the better understanding of the formation of amyloid plaques and the design of novel compounds which can be applied in the inhibition or therapy of Alzheimer's disease.

TUDOMÁNYOS PUBLIKÁCIÓK (PUBLICATIONS)

Az értekezés alapját képező közlemények (Articles connected to the thesis)

Tudományos folyóiratban megjelent közlemények

1. Ágnes Grenács, Anikó Kaluha, Csilla Kállay, Viktória Jószai, Daniele Sanna, Imre Sóvágó, **Binary and ternary mixed metal complexes of terminally free peptides containing two different histidyl binding sites.** *Journal of Inorganic Biochemistry* 128 (2013) 17-25
   Impakt faktor: 3,274 (2014)
2. Ágnes Grenács, Imre Sóvágó, **Copper(II), nickel(II) and zinc(II) complexes of the N-terminal nonapeptide fragment of amyloid-β and its derivatives.**
   Journal of Inorganic Biochemistry 139 (2014) 49-56
   Impakt faktor: 3,274 (2014)

Tudományos folyóiratban még meg nem jelent közlemények

1. Ágnes Grenács, Imre Sóvágó, Daniele Sanna, **Copper(II) and nickel(II) binding sites of peptides containing adjacent histidyl residues.**
   (közlésre benyújtva)
2. Ágnes Grenács, Imre Sóvágó, Giuseppe Di Natale, Giuseppe Pappalardo, Enrico Rizzarelli, **Dimeric behaviour of an $A\beta(1\text{-}16)_2$ derivative in the presence of Cu(II) and Zn(II) ions.**
   (közlésre előkészítve)

Konferenciakötetben megjelent tanulmány

Imre Sóvágó, Ildikó Turi, Ágnes Grenács: **Factors influencing the formation of mixed metal complexes of peptide fragments of prion protein and related ligands,** *11th European Biological Inorganic Chemistry Conference*, 12-16 September 2012, Granada, Spain, MEDIMOND International Proceedings, p. 31-36.

Az értekezés alapját képező előadások (Lectures connected to the thesis)

1. Grenács Ágnes, Kaluha Anikó, Jószai Viktória, Kállay Csilla, Sóvágó Imre, **Hisztidin kötőhelyeket tartalmazó peptidek fémion-szelektivitásának vizsgálata,** *46. Komplexkémiai Kollokvium*, Mátrafüred, 2012. május 21-23.
2. Grenács Ágnes, Sóvágó Imre, Giuseppe Di Natale, Giuseppe Pappalardo, **Az amyloid-β peptid N-terminális fragmensének – Aβ(1-9) – és dimer konjugátumának komplexképződési folyamatai,** *47. Komplexkémiai Kollokvium,* Mátraháza; 2013. május 29-31.

3. Grenács Ágnes, Sóvágó Imre, **Az amiloid-β peptid N-terminális fragmenseinek és mutánsainak kölcsönhatása Cu(II)-, Ni(II)- és Zn(II)-ionokkal,** *48. Komplexkémiai Kollokvium*, Siófok, 2014. május 28-30.

4. Imre Sóvágó, Ágnes Grenács, **Copper(II), nickel(II) and zinc(II) binding ability of the N-terminal fragment of amyloid-β peptide,** *12th European Biological Inorganic Chemistry Conference,* Zürich, Svájc, 2014. augusztus 24-28.

Az értekezés alapját képező poszterek (Posters connected to the thesis)

1. Grenács Ágnes, Kaluha Anikó, Jószai Viktória, Kállay Csilla, Sóvágó Imre, **Peptidek többmagvú és vegyes fémkomplexei képződését befolyásoló tényezők vizsgálata modellrendszerekben,** *MKE 1. Nemzeti Konferencia*, Sopron, 2011. május 22-25.
2. Ágnes Grenács, Csilla Kállay, Imre Sóvágó, **Mixed metal complexes of multihistidine peptides,** *11th European Biological Inorganic Chemistry Conference,* Granada, Spanyolország, 2012. szeptember 12-16.
3. Ágnes Grenács, Imre Sóvágó, **Copper(II), nickel(II) and zinc(II) complexes of Aβ(1-9) and its model peptides,** *XII. International Symposium on Inorganic Biochemistry,* Wrocław, Lengyelország, 2013. augusztus 28. - szeptember 1.
4. Ágnes Grenács, Imre Sóvágó, **Copper(II), nickel(II) and zinc(II) binding ability of the N-terminal fragments of amyloid-β peptide,** *International Symposium on Metal Complexes 2014*, Pavia, Olaszország, 2014. június 8-12.

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Candidate: Ágnes Grenács
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List of publications related to the dissertation

Foreign language scientific article(s) in international journal(s) (2)

1. **Grenács, Á.**, Sóvágó, I.: Copper(II), nickel(II) and zinc(II) complexes of the N-terminal nonapeptide fragment of amyloid-[beta] and its derivatives.
   J. Inorg. Biochem. 139, 49-56, 2014. ISSN: 0162-0134.
   DOI: http://dx.doi.org/10.1016/j.jinorgbio.2014.06.001
   IF:3.274 (2013)

2. **Grenács, Á.**, Kaluha, A., Kállay, C., Jószai, V., Sanna, D., Sóvágó, I.: Binary and ternary mixed metal complexes of terminally free peptides containing two different histidyl binding sites.
   J. Inorg. Biochem. 128, 17-25, 2013. ISSN: 0162-0134.
   DOI: http://dx.doi.org/10.1016/j.jinorgbio.2013.07.008
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Foreign language conference proceeding(s) (1)

3. Sóvágó, I., Turi, I., **Grenács, Á.**: Fators influencing the formation of mixed metal complexes of peptide fragments of prion protein and related ligands.
   In: 11th European Biological Inorganic Chemistry Conference. Ed.: Josefa María González-Pérez, Antonio Matilla-Hernández, Juan Niclós Gutiérrez, MEDIMOND, Bologna, 31-36, cop. 2013. ISBN: 9788875876586

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