Proposition of PhD theses

THE EFFECT OF THE THIOLATE GROUP ON THE COMPLEX FORMATION PROCESSES OF PEPTIDES CONTAINING DIFFERENT DONOR GROUPS IN SIDE CHAINS

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I. INTRODUCTION AND THE AIM OF THE WORK

Several metal ions play important role in the regulation of catalytic processes in the biological systems, namely, performing on redox- and acid-base processes. It has been known that metal ions also play active roles in protein structure as well to create the active site of the enzymes.

These interactions usually are coordinative between the metal ion and proteins. The amino acids containing weakly and strong coordinating donor groups in the side chains are taking part in the coordination to the metal ion. As a representative example, carboxylate group of aspartic acid contains weakly coordinating side chain, in contrast, imidazole nitrogen of histidine contains a strong coordinating side chain. The coordination behavior of latter amino acid is frequently investigated because of the involvement of multihistidine peptides in neurodegenerative disorders (ALS, Alzheimer or prion disease).

However, in addition to multihistidine and multicysteine proteins there are several examples in the nature where the same protein contains different metal binding sites (for example zinc finger proteins). From coordination chemistry point of view, the existence of different binding sites may enhance the structural diversity of the complexes. In biological systems, however, proteins have specific binding modes for playing role in enzymatic functions. For giving deeper insight into this specific interactions, the investigation of model peptides containing different binding sites in separated positions is indispensable.

Nevertheless, the interactions between the peptides and metal ions do not exclusively occur with the coordination of the peptide side chains because some of metal ions are able to increase the acidity of the peptide amide nitrogen and the deprotonation and coordination of the peptide nitrogen could be observed in the measurable pH range. For example, in the presence of palladium(II) this effect falls into the pH range between 2-3, while copper(II) ions are able to induce this deprotonation and coordination around pH 4-5.

Beside the quality of metal ion the above mentioned effect is depending on several factors; the number and quality of donor atoms being in position to form chelate ring with the metal ion, and the enhanced stability effect of the formation of macrochelate.

Obviously, complex formation processes of a small oligopeptide containing separate donor functions are relative complicated because of the structural diversity and the preference to form coordination isomers.

Based on the above mentioned details, our goals were to investigate how the metal binding affinity of the peptides containing cysteine on C-terminal part

can be modified if the N-terminal part of peptides contains strong metal binding domain. Thus, a systematic study has been performed to synthesize N-terminally free but C-terminally amidated peptides containing cysteine on the C-terminus and to investigate the complex formation processes of these peptides with transition metal ions (copper(II), nickel(II), zinc(II), cadmium(II), palladium(II)).

Our goal was also to investigate the effect of the thiolate group on the complex formation processes of the N-terminal part of the peptide and to discover the effects these are necessary for the C-terminal part to be primary metal binding site.

Furthermore, we were interested in how the DFT method can be used for calculating UV-vis parameters of nickel(II)-peptide complexes. Based on this fact, systematic studies have been performed using DFT approach with different functionals for predicting the electronic absorption spectra of the optimized complexes in aqueous solution. These results may contribute for better understanding the spectroscopic behavior of complicated metal-peptide systems.

II. EXPERIMENTAL METHODS

The investigated ligands were synthesized by **solid phase peptide synthesis** using a microwave-assisted Liberty 1 Peptide Synthesizer (CEM, Matthews, NC) that is available in our lab. Fmoc-protected amino acid derivatives were introduced according to the Fmoc/tBu technique and the TBTU/HOBT/DIPEA strategy and Rink Amide AM resins was used as a solid phase. DMF was also used as a solvent. Schematic structure of the synthesized ligands is shown in Figure 1.

The purity of the peptides was checked by using **high-performance liquid chromatography (HPLC)**. These studies were performed on Teknokroma Europa Peptide C18 reversed-phase column (250 x 4.6 mm; 120 Å pore size; 5 μm particle size) using a Jasco MD-2010 plus multiwavelength detector on 222 nm that is characteristic of peptide bond. Gradient elution was carried out using solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) at flow rate of 1 mL/min. From 1 min to 15 min 0 to 25 % of B from 15 min to 20 min 25 % of B was applied that was decreased to 0 % of B.

pH-potentiometric titrations were used to check the purity of the peptides and to determine the protonation constants. For calculating these values, a general computational program –SUPERQUAD– was used while PSEQUAD program was applied to determine the stoichiometry and stability of the metal complexes. The pH potentiometric measurements were carried out at 25 °C at ~3 mM ligand concentration. The metal to ligand ratio was selected between 1:2 and 2:1. The ionic strength was adjusted to 0.2 M with KCl or KNO_3. Based on the calculated protonation and stability constants the concentration distribution curves for the corresponding systems were plotted with MEDUSA program.

UV-vis spectroscopy measurements were also carried out to investigate the complex formation processes in the nickel(II)-, palladium(II)- and copper(II)-containing systems. The spectra were registered on a Perkin Elmer Lambda 25 double beam spectrophotometer in the wavelength range between 250 – 800 nm using 1,000 cm cuvettes. **Circular dichroism (CD) spectroscopy** is a widely-used technique to investigate the structure of optically active metal complexes. CD spectra usually contain more information comparing to UV-vis spectra because the line broadening of the electronic transitions are rather strait. The CD spectra were recorded on a Jasco-810 spectropolarimeter available at the *Department of Organic Chemistry at the University of Debrecen*. The spectra were registered in the 220 – 800 nm wavelength range using 0,100 cm and 1,000 cm cuvettes. Experimental conditions

(concentration, ionic strength) were the same to those were used in the case of pH-metry.

In order to check the purity of the ligands and to gain a deeper understanding of the complex formation processes **NMR spectroscopy** was used in the zinc(II)-, cadmium(II)-, palladium(II)- and nickel(II)-containing systems. In the case of nickel(II) only the square-planar diamagnetic complexes could be measured. One-dimensional ^{1}H and ^{113}Cd spectra were registered, moreover, diffusion NMR and several two-dimensional homonuclear techniques were used that was combined in some cases with water suppression. The spectra were recorded on a Bruker AM 360 MHz FT-NMR or on a Bruker Avance 400 MHz FT-NMR instrument. Topspin 3.2, Mestrenova 8.1 and OriginPro 9.0 softwares were used for evaluating the spectra.

Electron spin resonance spectroscopy was used to determine the structure of paramagnetic copper(II) complexes. The spectra were recorded at *Istituto CNR di Chimica Biomolecolare (Sassari) in Italy*. Anisotropic EPR spectra were registered on a Bruker EMX spectrometer equipped with a HP 53150A microwave frequency counter. The samples contained $^{63}Cu(II)$ in 1 mM concentration to achieve higher resolution of spectra.

Electrospray ionization mass spectrometry (ESI-MS) was used to identify the synthesized ligands and their zinc(II)- and cadmium(II)-complexes. The measurements were performed on a Bruker micrOTOF-Q 9 spectrometer *at the Department of Applied Chemistry at the University of Debrecen*.

DFT calculations have been used to determine the relative free energies of coordination isomers and TD-DFT studies were also used to predict the UV-vis and ECD spectra of the complexes optimized in aqueous media. PCM solvent model was used to account for the effect of water. The UV-vis and ECD spectra were generated as a sum of Gaussian curves with a 0.6 eV half-height width. All calculations were performed using Gaussian 09 (revision C.01) software package that is available on the computer system of *National Information Infrastructure Development Institute (NIIF).*

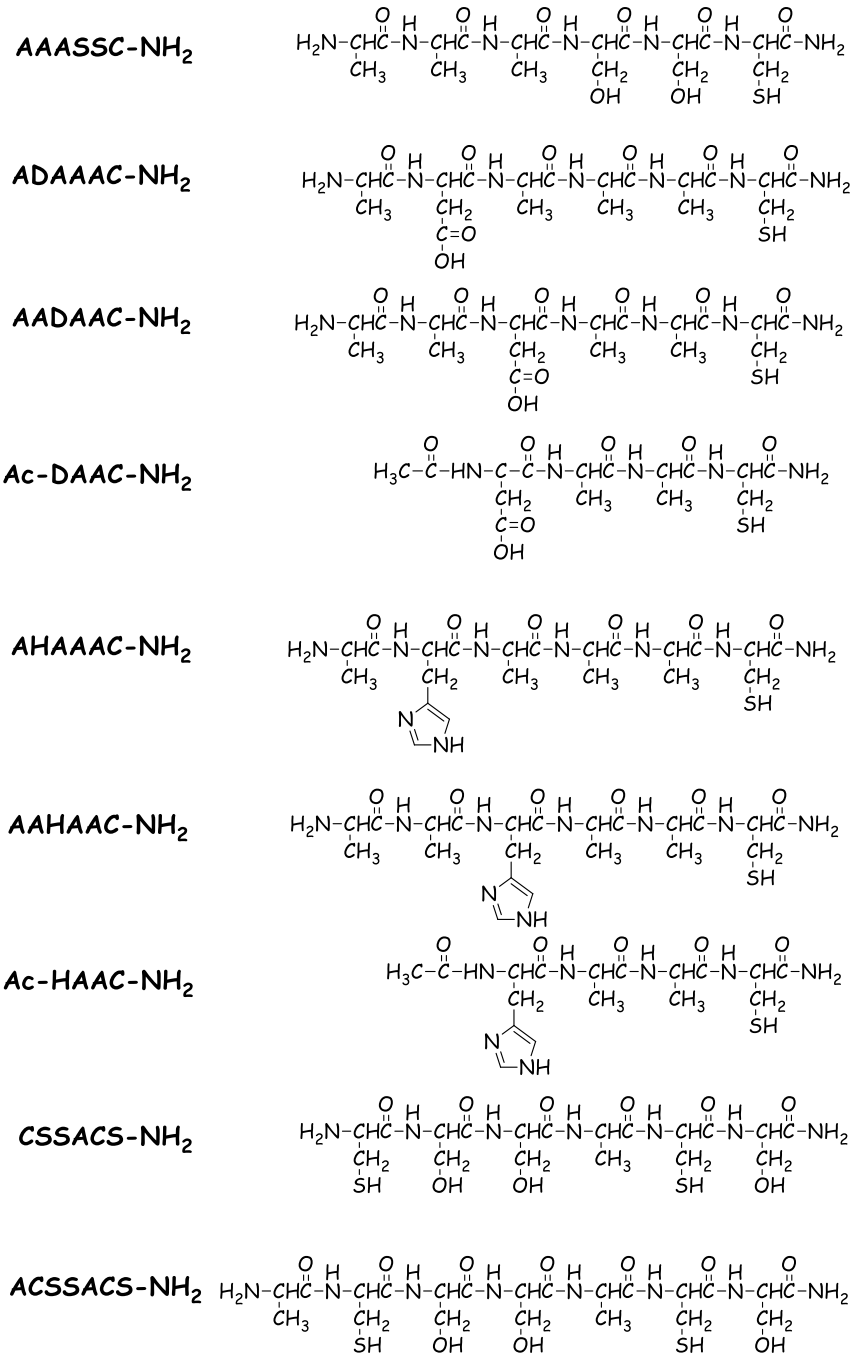

Figure 1. Structural formulae of the investigated ligands.

III. NEW SCIENTIFIC ACHIEVEMENTS

3.1 New method was suggested for calculating UV-vis spectra of nickel(II)-peptide complexes using DFT method.

- Geometry optimizations have been performed in the case of four nickel(II) complexes with simple oligopeptides (glycyl-glycyl-glycine (GGG), glycyl-glycyl-glycyl-glycine (GGGG), glycyl-glycyl-histidine (GGH) and glycyl-glycyl-cysteine (GGC)) using different functionals and def2-TZVP basic set with PCM model. These complexes have square-planar (NH_2,N^-,N^-,COO^-), (NH_2,N^-,N^-,N^-), (NH_2,N^-,N^-,N_{im}) and (NH_2,N^-,N^-,S^-) coordination environment. In order to predict electronic absorption spectra of the optimized complexes TD-DFT calculations were performed. It was shown that mPW1PW91 and PBE0 functionals are able to well predict the electronic spectra because the calculated relative error that was determined as the difference between the measured and calculated absorption maxima is small.

3.2 Acid-base properties of the synthesized ligands were characterized from equilibrium and spectroscopic points of view. Thus, the deprotonation macro- and micro-processes could be determined.

- The investigated ligands except of AAASSC-NH_2, Ac-DAAC-NH_2 and Ac-HAAC-NH_2 contain three dissociable protons. Based on equilibrium studies, the deprotonation macro-processes could be determined, while using ^{1}H NMR the characterization of micro-processes was possible.
- Our results indicated, that in the case of hexapeptides containing aspartic acid, the HL form contains protonated thiolate functions in 80 %, while amino group is protonated in 20 %.
- In contrast, the determined macro-constants of the hexapeptides containing histidyl residues practically correspond to the micro-constants, while in the case of two cysteine containing peptides (CSSACS-NH_2 and ACSSACS-NH_2) the overlap of ^{1}H NMR signals hindered the characterization of micro-processes.

Coordinative properties of peptides containing both aspartyl and cysteinyl residues (AADAAC-NH_2 and ADAAAC-NH_2) and their model ligands (AAASSC-NH_2 and Ac-DAAC-NH_2)

3.3 Complex formation processes of AADAAC-NH_2 with nickel(II) were investigated and based on our results, the formation of (NH_2,N^-,N^-,β-COO^-) coordinated complex is the most preferred. However, the C-terminal part of the peptide behaves as an independent metal binding site in alkaline media, thus, the existence of coordination isomers were observed. Our results were confirmed by the investigation of model ligands, AAASSC-NH_2 and Ac-DAAC-NH_2, respectively.

- Based on equilibrium and spectroscopic results the (NH_2,N^-,N^-,β-COO^-) coordinated complex is the major species in the slightly alkaline pH range. Above pH 9, circular dichroism spectra indicated the rearrangement of the coordination sphere and the existence of coordination isomers. The ratio of the different coordination isomers could be calculated as the metal ion is binding to the N-terminal part of the peptide in 75 % with (NH_2,N^-,N^-,N^-) donor set, while the remained 25 % of metal ion has (N^-,N^-,N^-,S^-) coordination environment.
- Both N-terminal amino group and C-terminal thiolate group are primary metal binding site for model peptide of AAASSC-NH_2. In alkaline conditions the formation of both tetraalanine like coordinated complex and C-terminal coordinated species were observed.
- In the case of nickel(II) complexes of model peptide of N-terminally protected Ac-DAAC-NH_2, ML is coordinated *via* (β-COO^-,S^-) donor set, that was followed by the cooperative deprotonation and coordination of peptide nitrogens. In alkaline pH range, the substitution of β-COO^- was occurred resulting in MLH_{-3} complex with (N^-,N^-,N^-,S^-) donor groups.

3.4 Thiolate group is the primary nickel(II) binding site for the peptide containing aspartyl residue in the secondary position. It was demonstrated by CD spectroscopy. Based on our results it was shown, that the binding of the metal ion to the N- or C-terminal part of the peptides may be finely tuned with the replacement of the aspartyl residue in the peptide.

- For nickel(II) complex of ADAAAC-NH_2 existing at physiological pH the thiolate group behaves as an anchoring group promoting the metal induced cooperative deprotonation and coordination of peptide

nitrogens. Similarly to nickel(II) complexes of AADAAC-NH_2, the formation of coordination isomers was observed at alkaline pH range. Using CD spectroscopy the percentage distribution of nickel(II) could be calculated between the (NH_2,N^-,N^-,N^-) and (N^-,N^-,N^-,S^-) coordinated forms, that was 60% and 40%, respectively.

3.5 For zinc(II) and cadmium(II) complexes of the ligands (AAASSC-NH_2, ADAAAC-NH_2, AADAAC-NH_2 and Ac-DAAC-NH_2) thiolate group is the primary metal binding site, however, the effect of stability enhancement of the complexes was observed in the case of aspartyl containing peptides.

- In the case of MLH complexes of AAASSC-NH_2 the N-terminal amino group is protonated resulting in the binding of the thiolate group.
- Bidentate (NH_2,S^-) coordination was observed for ML complex, however, the excess of ligand is not able to form bis(ligand) complexes. The above mentioned coordination mode is not able to hinder the hydrolytic processes of the coordinated water molecules and the formation of mixed hydroxido species was occurred in alkaline pH range.
- ML complex of Ac-DAAC-NH_2 has (β-COO^-,S^-) coordination environment. The excess of ligand results in the formation of bis(ligand) species.
- The binding of the carboxylate group enhanced the stability of the complexes formed in the ADAAAC-NH_2 and AADAAC-NH_2 containing systems. The calculated stability constants were higher than those of zinc(II) and cadmium(II) complexes of AAASSC-NH_2. Based on this fact, tridentate coordination was supposed in the aspartic acid containing systems. The coordination of the amino group was indirectly proved with the formation constants of ZnL and CdL complexes (where L = Ac-DAAC-NH_2) because these values are smaller than those of ML complexes of ADAAAC-NH_2 and AADAAC-NH_2.
- The formation of bis(ligand) species was observed in the case of cadmium(II) containing systems that could be explained by the octahedral coordination sphere.

Complex formation processes of peptides containing both histidyl and cysteinyl residues (AAHAAC-NH_2 and AHAAAC-NH_2) and their model ligand (Ac-HAAC-NH_2)

3.6 The ligand containing histidyl residue in the third position (AAHAAC-NH_2) easily forms albumin-like coordinated complex with nickel(II), however, in the case of palladium(II) the formation of this fused-chelate system is hindered because of the coordination of thiolate group.

- Nickel(II) complexes of the ligand have outstanding stability due to the formation of (NH_2,N^-,N^-,N_{im}) fused-chelate system. *(Figure 2.)*

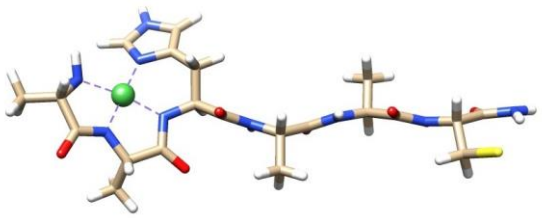

Figure 2. Optimized structure of MLH_{-2} complex of Ni(II):AAHAAC-NH_2**.**

- This binding mode provides in an independent metal binding site for the C-terminal part of the peptide and in the excess of nickel(II) the formation of dinuclear complexes were observed.
- In the presence of palladium(II) the binding of the thiolate group results in only one amide nitrogen in the coordination sphere. The tridentate binding mode is able to hinder the deprotonation and coordination of this peptide nitrogen below pH ~ 7. This effect is unusual because this deprotonation process occurs around pH 2-3 in the case of palladium(II) complexes of simple oligopeptides.

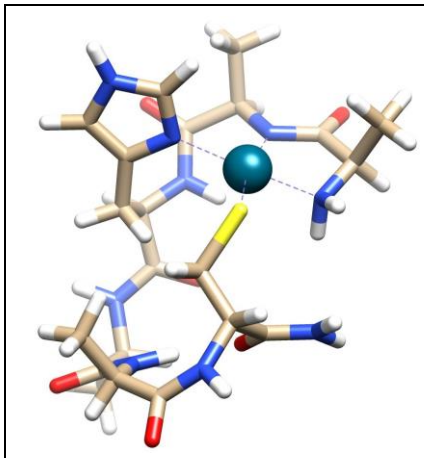

Figure 3. Optimized structure of MLH_{-1} complex in the Pd(II):AAHAAC-NH_2 system.

- By using DFT, it was possible to determine the relative energy of the possible coordination isomers. Our calculations indicated that the first amide nitrogen coordinated form is energetically preferred *(Figure 3.)* and the calculated and experimental ECD curves were in good agreement with this observation.

- It is well-known the high affinity of thiolate containing ligands to form polymer structures. Based on this fact, the hydrodynamic radii of the palladium(II) complex was measured using diffusion NMR. Two different concentration of the complex was investigated by DOSY and the calculated hydrodynamic radii were in good agreement with each other. This results indicated that the formation of polymer species was not occurred.
- Both imidazole nitrogen of histidine and thiolate sulphur of cysteine are coordinated in the nickel(II) complex of Ac-HAAC-NH_2 resulting in ML and in the presence of excess of ligand ML_2 complexes. By increasing pH, the deprotonation and coordination of two amide nitrogens were observed with the formation of (N_{im},N^-,N^-,S^-) coordinated complex. This complex has outstanding stability and able to shift the deprotonation and coordination of the adjacent peptide nitrogen into the strongly alkaline pH range (pH > 10).

3.7 The copper(II) ion with AAHAAC-NH_2 is able to form (NH_2,N^-,N^-,N_{im}) coordinated complex. This binding mode hinders the redox reaction between the thiolate group and metal ion.

- Based on the equilibrium and spectroscopic results, the albumin-like coordination is able to protect the copper(II) ion from the reduction of thiolate group due to the change of Cu(II)/Cu(I) reduction potential.

3.8 In the case of zinc(II)- and cadmium(II)-complexes of AAHAAC-NH_2, the thiolate group is the primary metal binding site, however, the cadmium(II) ions form complexes with higher thermodynamic stability that can be explained by the high affinity of cadmium(II) for binding thiolate.

- The binding of thiolate and imidazole nitrogen of histidine was observed in the case of zinc(II)- and cadmium(II)-complexes in slightly acid pH-range. This bidentate coordination is able to form bis(ligand) complexes in the presence of ligand excess. However, the ligand is coordinated tridentate in ML monocomplex *via* the coordination of terminal amino group.
- Derivative stability constant of ML complex of Ac-HAAC-NH_2 indirectly proves the coordination of imidazole nitrogen and thiolate sulphur. Nevertheless, this bidentate coordination mode is not able to hinder the deprotonation of coordinated water molecules resulting in mixed hydroxido species.

3.9 Nickel(II)- and zinc(II)-complexes of peptide containing histidyl residue in the secondary position (AHAAAC-NH_2) was investigated and our results indicated that the complex formation processes could be influenced by the replacement of the position of histidyl residue.

- For ML complexes of nickel(II) and zinc(II), the ligand is bounded tridentate *via* the terminal amino group and the other donor groups of side chain. In this case, the zinc(II) complex has higher stability resulting in different stability order from Irving-Williams series.
- The tridentate coordination of the ligand is able to stabilize the ML complex and shifts the deprotonation and coordination of peptide nitrogen into the more alkaline pH range than in the case of similar complexes with peptides of XaaHis motif.
- The coordination of distant cysteinyl residue *(Figure 4.)* hinders the formation of binuclear species, in contrary to AAHAAC-NH_2, this ligand is not able to bind two equivalents of nickel(II).

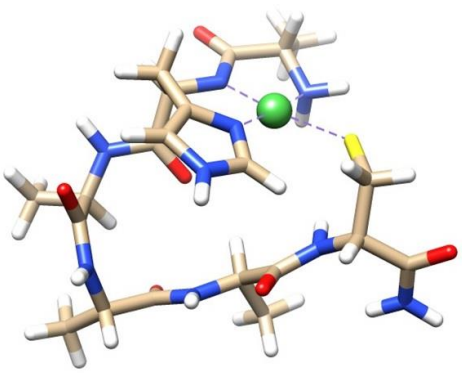

Figure 4. Optimized structure of MLH_{-1} complex in the Ni(II):AHAAAC-NH_2 system.

3.10 The formation of complexes with high thermodynamic stability was observed in the palladium(II):AHAAAC-NH_2 system similar to those of the AAHAAC-NH_2 containing system. The binding of the first amide nitrogen was also hindered because of the tridentate coordination and shifted into the slightly acidic pH range.

- Coordination isomers are present in acidic pH range. Both isomers contain N-terminal amino group and the other donor group is the imidazole-N or thiolate-S.
- By increasing pH ML complex is formed *via* the tridentate coordination that hinders the deprotonation and coordination of first peptide nitrogen. This effect was observed in unusual pH range similarly to Pd(II):AAHAAC-NH_2 system.

3.11 The saturation of coordination sphere is different in the copper(II):AHAAAC-NH_2 system than in the nickel(II)- and zinc(II) containing systems. It can be explained by the high affinity of copper to form (NH_2,N^-,N_{im}) fused chelate system.

- At slightly acidic pH, copper(II) ions form (NH_2,N^-,N_{im}) coordinated species. This coordination sphere is unsaturated that can be completed by macrochelation of distant thiolate group. The copper(II)-thiolate interaction was proved by UV-vis spectroscopy.
- This coordination mode was modeled using equilibrium and spectroscopic methods in the Cu(II):NAc-Pen:AlaHis 1:1:1 ternary system. The formation of mixed complex was observed where the copper(II) ion is bounded through the (NH_2,N^-,N_{im}) donor set of alanyl-histidine, while this unsaturated coordination sphere was completed by binding of thiolate from N-acetyl-penicillamine.

3.12 We showed that the speciation of cadmium(II) ions with AHAAAC-NH_2 is similar to those of AAAHAAC-NH_2 containing system. It means that the replacement of histidyl residue into the secondary position does not have significant effect on the complex formation processes.

- The complex formation starts at slightly acidic pH range with the binding of thiolate-S and imidazole-N that is followed by the deprotonation and coordination of terminal amino group.
- In the case of excess of ligand, the formation of bis(ligand) species is favorable, however, the coordination of amino group could not be supposed or only a weak interaction can be detected.

Complex formation behavior of peptides containing two cysteinyl residues (CSSACS-NH_2 and ACSSACS-NH_2) with zinc(II)- and cadmium(II)-ions

3.13 Our equilibrium studies showed that both zinc(II) and cadmium(II) ions form high stability complexes with the peptide of CSSACS-NH_2. This coordination mode is able to shift the formation of mixed hydroxido species into the strongly alkaline pH range.

- The terminal amino group is able to form 5-membered chelate with the cysteinyl residue on the N-terminal part of the peptide. Based on this fact, this part of the peptide behaves as a primary metal binding site. This coordination mode was supported by macrochelation of the distant thiolate in the complex with ML stoichiometry and the (NH_2,S^-,S^-) species is dominant in the whole pH range.
- The existence of the above mentioned coordination mode was proved by ^{113}Cd NMR spectroscopy.
- ESI-MS measurements have also been performed for giving evidence about the zinc(II)- and cadmium(II)-complexes. The mass spectra also reinforced the exclusive existence of this complex.

3.14 Zinc(II)- and cadmium(II)-complexes of ACSSACS-NH_2 were widely studied and it was pointed out that the thiolate groups are the primary metal binding sites that was followed by the coordination of terminal amino group.

- The stability of ML complex of this ligand is smaller than that of CSSACS-NH_2. It can be explained by the lack of the formation of 5-membered chelate on N-terminus.
- Therefore, the complex formation processes start with the binding of thiolate groups at slightly acidic pH range. This coordination mode was proved by NMR spectroscopy.
- The bidentate coordination results in the formation of bis(ligand) species, contrary to CSSACS-NH_2 peptide.
- The formation of bis(ligand) complexes was proved by ESI-MS measurements.

3.15 An exclusive coordination behavior was observed in the case of MLH_{-1} complex of ACSSACS-NH_2. Namely, it was the cadmium(II)- and zinc(II)-induced deprotonation and coordination of peptide nitrogen.

- MLH_{-1} complex of CSSACS-NH_2 was a mixed hydroxido species, while in the case of ACSSACS-NH_2, the formation of complex with (NH_2,N^-,S^-,S^-) donor set was observed *(Figure 5.)* that was confirmed by NMR and ESI-MS measurements.
- Therefore, it was the first example for the formation of Cd-N(peptide nitrogen) bond, moreover, the zinc(II) induced deprotonation and coordination of amide groups have described only in varios peptides of histidine.

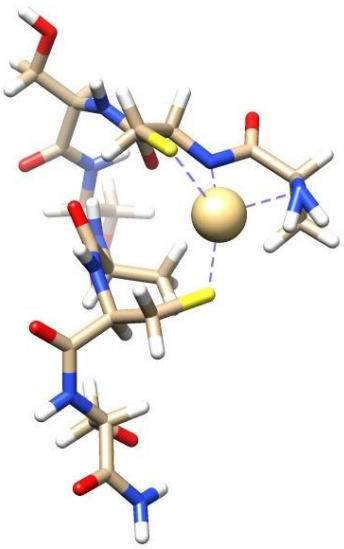

Figure 5. Optimized structure of MLH_{-1} complex in the Cd(II):ACSSACS-NH_2 system.

3.16 Based on our results, a donor atom preference could be suggested for ML complexes (M=Zn(II) and Cd(II)) and the peptides containing two cysteinyl residues are able to form complexes with the highest thermodynamic stability.

- The stability enhancement of the cysteine is already described in the case of zinc(II)- and cadmium(II)-ions. Our thermodynamic data indicated that the stability enhancement increases in the order of $COO^- < N_{im} << S^-$, however, the replacement of the donor group in the N-terminal region does not have significant effect on the stability of the complexes *(Figure 6.)*.

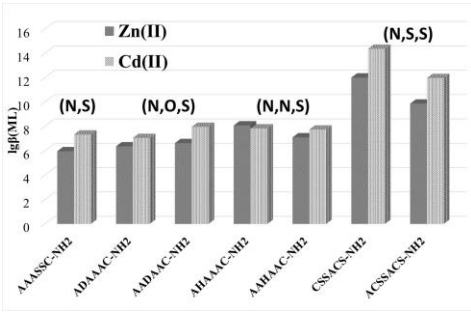

Figure 6. Stability constants of ML complexes in the case of zinc(II)- and cadmium(II)-ions.

Based on the above mentioned result the following statements could be summarized about the effect of the thiolate group.

Nickel(II)ions:

(i) For the peptide containing only N-terminal amino group, the thiolate group is the primary metal binding site and the coordination of amino group results in macrochelate structure.

(ii) The existence of the aspartyl residue on the second or third position is able to promote the binding of the metal ion to the N-terminal part of the peptide. However, this effect is comparable with the metal binding ability of the C-termini. Based on this fact, the formation of coordination isomers was observed.

(iii) The replacement of aspartic acid to histidine results in that the N-terminal region of the peptide is the primary metal binding site. The binding of the thiolate was observed in the case of unsaturated coordination sphere or in the presence of metal excess.

(iv) Based on the above mentioned details it is evident that the change of the quality of amino acid is able to regulate selectively the binding of nickel(II).

Copper(II)ions:

(i) It is well-known that the redox reaction between the copper(II) and thiolate groups results in copper(I) species. However, the albumin-like coordination is able to protect the metal ion from the reduction.

(ii) Nevertheless, the formation of Cu(II)-thiolate bond is also possible if the coordination sphere of copper(II) is unsaturated. The investigated ligand (AHAAAC-NH_2) easily forms (NH_2,N^-,N_{im}) coordinated species. This coordination mode is able to modify the reduction potential of Cu(II)/Cu(I) and hinders the redox activity of thiolate group resulting in stable Cu(II)-thiolate coordinative interaction.

(iii) The above mentioned effect is not possible in the case of peptides containing aspartyl residue and the reduction of copper(II) was observed.

Palladium(II)ions:

(i) Thiolate group is the primary metal binding site for palladium(II).

(ii) The tridentate coordination via (NH_2,$N_{im,}S^-$) donor set is able to shift the deprotonation and coordination of peptide nitrogen into the slightly acidic pH range.

Zinc(II)ions:

(i) Thiolate group is the most preferred metal binding site for zinc(II).

(ii) Based on this behavior, the donor groups placed on the N-terminal domain increase the stability of the complexes in the following order: β-COO^- < N_{im} < S^-.

(iii) The terminal amino group and thiolate group are not able to form bis(ligand) complexes and similar conclusion could be stated in the case of peptides containing aspartyl residue. Nevertheless, the existence of histidine results in the formation of bis(ligand) species. It can be explained by the lack of the preferred geometry of zinc(II) because of d^{10} electron configuration that does not provide crystal field stabilization energy. In the case of zinc(II) complexes of aspartic acid containing peptides, zinc(II) has an tetrahedral coordination geometry that hinders the formation of bis(ligand) complexes. In contrast, octahedral environment could be supposed in the presence of histidine resulting in the formation of bis(ligand) species.

(iv) The specific AlaHisXaa motif allows the deprotonation and coordination of peptide nitrogen and the N-terminal part of the peptide is the primary metal binding site that was supported by macrochelation of distant thiolate.

(v) Similar effect has been observed in the case of AlaCysXaa…..YaaCys motif as well.

Cadmium(II)ions:

(i) Similarly to zinc(II), thiolate group is the primary metal binding site for cadmium(II), however, the stability of the cadmium(II) complexes approximately 1-2 order of magnitude higher that can be explained by the high affinity of cadmium(II) for binding thiolate.

(ii) The existence of multiple binding sites in the coordination sphere increases the stability of the complexes in the following order: β-COO^- < N_{im} < S^-.

(iii) The formation of bis(ligand) complexes was already observed in the case of aspartic acid containing peptides, unlike zinc(II).

(iv) The presence of amino group and thiolate groups in the coordination sphere results in the formation of complexes with outstanding stability.

(v) The specific coordination with AlaCysXaa sequence supported with macrochelation of distant cysteinyl residue results in the deprotonation and coordination of first amide nitrogen of N-termini.

IV. POSSIBLE APPLICATIONS OF THE RESULTS

The metal ions are usually bonded to the side chains of protein in biological systems and this type of binding mode is coordinative. The metal-protein interactions could be modeled with small oligopeptides containing the active site of the origin enzyme separately. This knowledge is able to give deeper insight into the stability of complexes and help for understanding the selective binding modes existing in metalloproteins and metalloenzymes.
The detailed results of the above chapter are fundamental research in the place of bioinorganic and biocoordination chemistry, however, our results clearly indicate that a small oligopeptides is able to show a well-manifold coordination chemistry depending on the position and quality of the donor side chain. These information may give help to design new peptides where the binding to the metal ion can be fine-tuned. It could be designed by the so-called *chimera* type peptides where peptides combine two or more functions of different enzymes. Based on the knowledge of selective binding modes it is possible to design peptides with redox functions. In this case two different binding sites are bonded to the redox active metal ions to perform multiple redox reactions. Moreover, *de novo protein* design that is the using of peptide sequences that do not exist but inspired by nature, allows for building of new functions.

V. TUDOMÁNYOS PUBLIKÁCIÓK (PUBLICATIONS)

Az értekezés alapját képező közlemények (Articles related to the dissertation)

Tudományos folyóiratban megjelent közlemények

1. N. Lihi, Á. Grenács, S. Timári, I. Turi, I. Bányai, I. Sóvágó, K. Várnagy, **Zinc(II) and cadmium(II) complexes of N-terminally free peptides containing two separate cysteinyl binding sites,** *New Journal of Chemistry,* 39 (2015) 8364-8372.
   Impact factor: 3,277 (2015)
2. M. Raics, N. Lihi, A. Laskai, Cs. Kállay, K. Várnagy, I. Sóvágó, **Nickel(II), zinc(II) and cadmium(II) complexes of peptides containing separate histidyl and cysteinyl binding sites,** *New Journal of Chemistry,* 40 (2016) 5420-5427.
   Impact factor: 3,277 (2015)
3. I. Sóvágó, K. Várnagy, N. Lihi, Á. Grenács, **Coordinating properties of peptides containing histidyl residues,** *Coordination Chemistry Reviews,* 327-328 (2016) 43-54.
   Impact factor: 12,944 (2015)
4. N. Lihi, D. Sanna, I. Bányai, K. Várnagy, I. Sóvágó, **Unusual binding modes in the copper(II) and palladium(II) complexes of peptides containing both histidyl and cysteinyl residues,** *New Journal of Chemistry,* 41 (2017) 1372-1379.
   Impact factor: 3,277 (2015)
5. N. Lihi, M. Lukács, D. Szűcs, K. Várnagy, I. Sóvágó, **Nickel(II), zinc(II) and cadmium(II) complexes of peptides containing separate aspartyl and cysteinyl residues.** *Polyhedron,* 133 (2017), 364-373.
   Impact factor: 2,107 (2015)

Tudományos folyóiratban még meg nem jelent közlemények

1. N. Lihi, T. Czine, G. Sciortino, E. Garribba, **DFT and TD-DFT studies on nickel(II) complexes containing peptides and Schiff base ligands.** (ready for publication)

Az értekezés anyagához szorosan nem kapcsolódó közlemények (Articles not detailed in the dissertation)

1. D. Sanna, K. Várnagy, N. Lihi, G. Micera, E. Garribba, **Formation of new non-oxido vanadium(IV) species in aqueous solution and in the solid state by tridentate (O, N, O) ligands and rationalization of their EPR behavior.** *Inorganic Chemistry* 52(14) (2013) 8202-8213.
   Impact factor: 4,820

2. Á. Dávid, Cs. Kállay, D. Sanna, N. Lihi, I. Sóvágó, K. Várnagy, **Potentiometric and spectroscopic studies on the copper(II) complexes of rat amylin fragments. The anchoring ability of specific non-coordinating side chains.** *Dalton Transactions,* 44(39) (2015) 17091-17099.
   Impact factor: 4,177
3. Zs. Bihari, V. Ugone, E. Garribba, N. Lihi, P. Buglyó, **Complex formation between $[(\eta^6\text{-p-cym})Ru(H_2O)_3]^{2+}$ and oligopeptides containing three histidyl moieties.** *Journal of Organometallic Chemistry* 823 (2016) 116-125.
   Impact factor: 2,336
4. N. Lihi, A. J. Godó, G. Sciortino, E. Garribba, K. Várnagy, **Tridentate (O, N, O) ligands as potential chelator compounds for iron overload.** *Polyhedron,* 123 (2017) 192-205.
   Impact factor: 2,107
5. N. Lihi, M. Lukács, M. Raics, Gy. Szunyog, K. Várnagy, Cs. Kállay, **The effect of carboxylate groups on the complexation of metal ion with oligopeptides.** *Inorganica Chimica Acta,* (2017) submitted manuscript.

Az értekezés anyagához kapcsolódó előadások (lectures)

1. Várnagy Katalin, Lihi Norbert, Raics Mária, Grenács Ágnes, Szunyog Györgyi, Timári Sarolta, Turi Ildikó: **A cisztein tartalmú peptidek fémionmegkötő képességének szelektivitása.** *49. Komplexkémiai Kollokvium,* Siófok, 2015. május 26-28.
2. Várnagy Katalin, Lihi Norbert, Raics Mária, Grenács Ágnes, Szunyog Györgyi, Timári Sarolta, Turi Ildikó: **Az oldalláncok hatása a cisztein tartalmú peptidek fémionmegkötő képességének szabályozására.** *MKE 2. Nemzetközi Konferencia,* Hajdúszoboszló, 2015. augusztus 31-szeptember 2.
3. Lihi Norbert, Raics Mária, Daniele Sanna, Várnagy Katalin, Sóvágó Imre: **A cisztein hatása hisztidint is tartalmazó peptidek átmenetifém-komplexeiben.** *50. Komplexkémiai Kollokvium*, Balatonvilágos, 2016. május 30-június 1.
4. Norbert Lihi, Mária Raics, Daniele Sanna, Katalin Várnagy, Imre Sóvágó: **Transition metal complexes of peptides with cysteine and histidine binding sites: a thermodynamic and structural study.** *13th European Biological Inorganic Chemistry Conference*, Budapest, 2016. augusztus 28-szeptember 1.
5. Lihi Norbert: **A tiolátcsoport hatása peptidek fémionmegkötő sajátságaira.** *MTA Fizikai-Kémiai Bizottság kihelyezett ülése*, Debrecen, 2016. november 25.

Az értekezés anyagához kapcsolódó poszterek (posters)

1. Norbert Lihi, Ágnes Grenács, Sarolta Timári, Ildikó Turi, Györgyi Szunyog, Katalin Várnagy and Imre Sóvágó: **Metal ion selectivity of cysteine containing peptides**, *13th International Symposium on Applied Bioinorganic Chemistry (ISABC13)*, Galway, Írország, 2015. június 12-15.
2. Katalin Várnagy, Mária Raics, Norbert Lihi, Daniele Sanna, Csilla Kállay and Imre Sóvágó: **The effect of side chain donor groups on the metal binding selectivity of cysteine containing peptide**, *13th International Symposium on Applied Bioinorganic Chemistry (ISABC13)*, Galway, Írország, 2015. június 12-15.
3. Ágnes Grenács, Norbert Lihi, Sarolta Timári, Ildikó Turi, Katalin Várnagy and Imre Sóvágó: **Transition metal complexes of peptides containing thiolate functions**, *13th International Symposium on Inorganic Biochemistry,* Karpacz, Lengyelország, 2015. szeptember 1-7.
4. Györgyi Szunyog, Márton Lukács, Norbert Lihi, Ágnes Grenács and Katalin Várnagy: **Metal complexes of cysteine containing peptides**, *International Symposium on Metal Complexes 2016 (ISMEC 2016),* Barcelona, Spanyolország, 2016. június 7-10.
5. Norbert Lihi, Mária Raics, Csilla Kállay, Katalin Várnagy and Imre Sóvágó: **Thermodynamic and structural characterization of transition metal complexes of peptides with thiolate and other binding sites**, *International Symposium on Metal Complexes 2016 (ISMEC 2016),* Barcelona, Spanyolország, 2016. június 7-10.
6. Norbert Lihi, Ágnes Grenács, Katalin Várnagy, Imre Sóvágó: **The effect of distant cysteinyl residues on the complex formation processes of peptides conatining other binding sites,** *14th International Symposium on Applied Bioinorganic Chemistry (ISABC 14),* Tolouse, Franciaország, 2017. június 7-10.

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List of publications related to the dissertation

Foreign language scientific articles in international journals (5)

1. **Lihi, N.**, Lukács, M., Szűcs, D., Várnagy, K., Sóvágó, I.: Nickel(II), zinc(II) and cadmium(II) complexes of peptides containing separate aspartyl and cysteinyl residues.
   Polyhedron. "Accepted by Publisher", [31], 2017. ISSN: 0277-5387.
   DOI: http://dx.doi.org/10.1016/j.poly.2017.05.044
   IF: 2.108 (2015)
2. **Lihi, N.**, Sanna, D., Bányai, I., Várnagy, K., Sóvágó, I.: Unusual binding modes in the copper(II) and palladium(II) complexes of peptides containing both histidyl and cysteinyl residues.
   New J. Chem. 41 (3), 1372-1379, 2017. ISSN: 1144-0546.
   DOI: http://dx.doi.org/10.1039/C6NJ03735F
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3. Sóvágó, I., Várnagy, K., **Lihi, N.**, Grenács, Á.: Coordinating properties of peptides containing histidyl residues.
   Coord. Chem. Rev. 327-328, 43-54, 2016. ISSN: 0010-8545.
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4. Hadháziné Raics, M., **Lihi, N.**, Laskai, A., Kállay, C., Várnagy, K., Sóvágó, I.: Nickel(II), zinc(II) and cadmium(II) complexes of hexapeptides containing separate histidyl and cysteinyl binding sites.
   New J. Chem. 40 (6), 5420-5427, 2016. ISSN: 1144-0546.
   DOI: http://dx.doi.org/10.1039/C6NJ00081A
   IF: 3.277 (2015)

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5. **Lihi, N.**, Grenács, Á., Timári, S., Turi, I., Bányai, I., Sóvágó, I., Várnagy, K.: Zinc(II) and cadmium(II) complexes of N-terminally free peptides containing two separate cysteinyl binding sites.
New J. Chem. 39 (11), 8364-8372, 2015. ISSN: 1144-0546.
DOI: http://dx.doi.org/10.1039/C5NJ01677K
IF: 3.277

List of other publications

Foreign language scientific articles in international journals (4)

6. **Lihi, N.**, Godó, A. J., Sciortino, G., Garribba, E., Várnagy, K.: Tridentate (O,N,O) ligands as potential chelator compounds for iron overload.
Polyhedron. 123, 192-205, 2017. ISSN: 0277-5387.
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7. Bihari, Z., Ugone, V., Garribba, E., **Lihi, N.**, Buglyó, P.: Complex formation between [[eta]6-p-cym)Ru(H2O)3]2+ and oligopeptides containing three histidyl moieties.
J. Organomet. Chem. 823, 116-125, 2016. ISSN: 0022-328X.
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8. Dávid, Á., Kállay, C., Sanna, D., **Lihi, N.**, Sóvágó, I., Várnagy, K.: Potentiometric and spectroscopic studies on the copper(II) complexes of rat amylin fragments. The anchoring ability of specific non-coordinating side chains.
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9. Sanna, D., Várnagy, K., **Lihi, N.**, Micera, G., Garribba, E.: Formation of New Non-oxido Vanadium(IV) Species in Aqueous Solution and in the Solid State by Tridentate (O, N, O) Ligands and Rationalization of Their EPR Behavior.
Inorg. Chem. 52 (14), 8202-8213, 2013. ISSN: 0020-1669.
DOI: http://dx.doi.org/10.1021/ic401080q
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