Summary of PhD thesis

EQUILIBRIUM INVESTIGATIONS OF METAL ION COMPLEXES OF AMYLIN HORMONE FRAGMENTS AND ITS MUTANTS

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1. INTRODUCTION

My research work performed in course of the PhD studies is linked to the chemical background of development type 2 diabetes.

Investigations in the Bioinorganic Chemistry Research Group of the University of Debrecen have been aimed for decades to determination the cause of neurodegenerative and hormone diseases corresponding with complexes of biogenic trace metal ions. These studies can help to understand the complex interactions between these metal ions and peptides engaged to neurodegenerative and hormone diseases, for example, Alzheimer's disease, prion morbidity and diabetes as well.

Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. Diabetes caused 1.5 million deaths in 2012.

Although the diabetes has been studied for a long time, the reasons for development have not been correctly explained. Amyloidosis is a common histological phenomena of the neurodegenerative and hormone (e.g. diabetes) diseases, therefore nowadays these morbidities are often connected in the literature.

The formation of amyloid aggregates is strongly associated with β-cell degeneration in type 2 diabetes, because more than 95% of patients exhibit hIAPP amyloid upon autopsy. Human islet amyloid polypeptide (hIAPP) is a peptide hormone consisting of 37 amino acid residues which are co-secreted with insulin by pancreatic islet β -cells.

Although the physiological role of hIAPP has not been well established yet, several functions have been associated with the soluble form of hIAPP including the control of hyperglycemia by regulating glucose homeostasis. At the same time reasons of aggregation of amylin remained an unanswered question.

Rat amylin (rIAPP) does not show a tendency for self-aggregation and is not toxic to islet cells. Rat amylin is highly homologous to human amylin but differs in six amino acids **(Table 2)** resulting in a nonamyloidogenic peptide.

It is well-known from earlier studies that conformational changes of amilin are responsible for the development of certain neurodegenerative disorders. Therefore it has been indicated that transition metals are ascribed having a potential role in this field. The aggregation and plaque formation is attached with the increased concentration of Cu(II), Zn(II) and Fe(II/III) in the Langerhans-islands.

In our research group investigations have been carried out for discovering of the interaction of copper(II)-ion and rIAPP peptides and models.

Despite the lack of any common strongly coordinating donor functions some of these fragments are able to bind copper(II) ions in the physiological pH range.

Besides this, the exact coordination mode and determining of coordinating donor atoms became an interesting and important question.

Table 2: Sequences of the human and rat amylin

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
Hu	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	**H**
Rat	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	**R**

19	*20*	*21*	*22*	***23***	*24*	***25***	***26***	*27*	***28***	***29***	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37*
S	S	N	N	**F**	G	**A**	**I**	L	**S**	**S**	T	N	V	G	S	N	T	Y
S	S	N	N	**L**	G	**P**	**V**	L	**P**	**P**	T	N	V	G	S	N	T	Y

2. AIMS

Based on preliminaries the different behavior of the human and rat amylin are caused by sequential differences, polar side chains (mainly the asparagine, serine, occasionally arginine). Polar side chains collectively may contribute to the metal binding ability of *r*IAPP (17-29).

For this reason in the course of our research work firstly we focused on the description of the special *binding sites* and the *binding donor atoms* in the case of *terminally protected rat amylin fragments and its mutants*. The surprisingly high affinity of this peptide towards complexation with copper(II) promoted a systematic study of the shorter fragments.

The second part of our work was the *studying of the earlier investigated sequences, but in form of N-terminally unprotected fragments and mutants*. The results of combined potentiometric and spectroscopic studies on the copper(II) complexes of *rat* amylin fragments and their mutants provide sufficient information for the understanding of the *possible role* of arginine-, serine - and asparagine side chains in copper(II) binding. We carried out this study in order to get more information and *further comparison* about the metal binding affinity of the polar side chains *in the presence of a strongly coordinating group* (amino group, albumine sequence).

Additionally, we were wondering the special binding site of molecules predominated in the presence of the Ni(II) and Zn(II).

The fourth studied subjects were the *terminally protected human amylin fragments*. Our aims were to characterize and compare the Cu(II) binding affinity of the central region of *r*IAPP and *h*IAPP.

In course of my work I measured in aqueous media in case of all peptide-metal ion systems, furthermore, I focused on the relevant pH-range (in the pancreas: pH 8-9).

3. APPLIED METHODS

In the case of all other peptides ***solid phase peptide synthesis*** was performed using a microwave-assisted Liberty Peptide Synthesizer (CEM, Matthews, NC), introducing the amino acid derivatives following the TBTU/HOBt/DIEA activation strategy on Rink Amide AM resin. Peptides were cleaved from their respective resins, with the simultaneous

removal of the side chain protective groups, by treatment with a mixture of TFA/TIS/H_2O/2,2'-(ethylenedioxy)diethanethiol (94/2.5/2.5/1 v/v%) for 90 minutes at room temperature. Each solution containing the free peptide was separated from the resin by filtration. The crude peptides were recovered from the pertinent solution by precipitation with cold diethyl ether. The precipitate was washed with cold diethyl ether and separated from it, then dried, re-dissolved in water, and finally lyophilized.

The purity of the peptides was checked by analytical *rp*-HPLC using a Jasco instrument, equipped with a Jasco MD-2010 plus multiwavelength detector. All the synthesized peptides showed the correct molecular mass, measured by mass spectrometry using the ESI-MS technique. Potentiometric measurements further confirmed the purity and the identity of the peptides.

The ***pH-potentiometric titrations*** were performed which is the most applied method for describing of complex formations in solutions. Protonation constants of the ligands and overall stability constants have been calculated by means of general computational programs (SUPERQUAD and PSEQUAD).

UV-Vis spectra were recorded on a Perkin-Elmer Lambda 25 double beam spectrophotometer. In the case of the metal complexes, the same concentration range was used as for pH-potentiometry.

The ***ESR continuous wave spectra*** were recorded at 120 K, using a Bruker EMX X-band spectrometer (9.46 GHz) equipped with a HP53150A frequency counter. Copper(II) stock solution was prepared from $CuSO_4 \cdot 5H_2O$ enriched with ^{63}Cu to get better resolution of EPR spectra. Metallic copper (99.3% ^{63}Cu and 0.7% ^{65}Cu) was purchased from JV Isoflex, Moscow, Russia for this purpose and converted into the sulfate.

CD spectra of metal complexes were recorded on a JASCO J-810 spectropolarimeter using 1 or 10 mm cells in the 200-800 nm range in the same concentration range as used for potentiometry.

The UV-Vis and CD spectra of the copper(II)ion containing systems were analyzed by the ***Convex Constraint Algorithm (CCA+) program***. The advantage of this method is that it uses only "natural constraints". It can successfully be used for quantitative analysis of CD and UV-Vis measurements. The spectra of the individual species were determined by this method. The UV-Vis spectra of the individual complexes were also calculated by means of the PSEQUAD program, using the original spectra and the total concentrations of metal ion, ligand and hydrogen ion. By this program, we obtained the stability constants of the species in addition to the spectra of the individual species.

5. NEW SCIENTIFIC RESULTS

I. MODELING OF METAL BINDING SITE OF RAT AMYLIN (17-29)

Terminally protected rat amylin fragments and its mutants

1. *As the fragments are protected at both termini and do not contain any dissociable proton in the measurable pH range, we had determined the concentration of our ligands by optimization of a literature-method. After that, we have calculated the stability constants of Cu(II)–amylin (17-29) complexes and its concentration distribution as a function of pH. Additionally, we have identified the individual UV-Vis spectra of formed Cu(II)-complexes.*

- Coordination behavior of five, new amylin fragment (Ac-VRSSNN–NH_2, Ac-VRSS–NH_2, Ac-SSNN–NH_2) and its mutants (Ac-VRAA–NH_2, Ac-VASS–NH_2) were described by the same methods. We have calculated the individual UV-Vis spectra of Cu(II)-complexes, as well.

/In what follows, the protected C-terminus will not be denoted, e.g.: Ac-VRSSNN/.

2. *We have proved that the role of the arginine side chain is negligible, while the serine and asparagine side chains may play a role in the metal binding of rat amylin.*

- For the tetrapeptides containing seryl residues (Ac-VRSS and Ac–VASS) the copper(II)-hidroxide dissolves only above pH 10, while in the case of Ac–VRAA, which contains only one amino acid with a polar side chain (arginine) the precipitate does not dissolve completely even above pH 11.

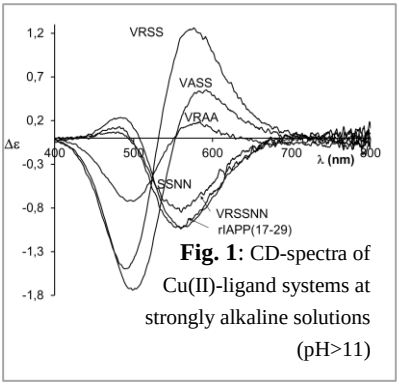

Fig. 1: CD-spectra of Cu(II)-ligand systems at strongly alkaline solutions (pH>11)

- The presence of electron withdrawing seryl-OH group could promote the dissolution of precipitate and the partial binding of the Cu(II)-ion in alkaline solutions.

3. *The Ac-SSNN tetrapeptide fragment was identified as the shortest sequence, which is responsible for metal binding.*

If we compare the CD spectra of the six studied peptides it can be seen that there are two types of spectra. Ac–VRSS, Ac–VRAA and Ac–VASS are in the first group, Ac–SSNN and Ac–VRSSNN and rIAPP(17-29) are in the second one **(Fig 1.).** Since all types of spectra of *r*IAPP(17–29) show a high similarity to those of the hexa- and tetrapeptides with the sequences VRSSNN and SSNN respectively, the role of the asparaginyl and seryl residues can be supposed.

4. *Our findings strongly support the participation of the deprotonated amide nitrogen of asparagine side chain in the metal binding of Ac-SSNN and the former discussed longer ligands (rIAPP 17-29, Ac–VRSSNN) even in the physiological and weakly alkaline pH range.*

Based on DFT-calculations and ESR-parameters the presence of many polar side chains in the SSNN fragment may protect the metal ion from hydrolysis and this interaction can induce the deprotonation of the side chain amide nitrogen of an Asn residue, followed by the metal binding of the preceding amide functions of the peptide backbone **(Fig. 2.).**

- A characteristic positive Cotton effect around 480–500 nm in the CD spectra can be assigned to the interaction of the asparaginyl side chains.

Moreover, the results of this study reveal that (in addition to the well-known anchoring capability of the side chains of His and/or Cys residues) *the amide group of asparagine can also be a primary metal binding site.* However, in the latter case, the presence of a single Asn moiety is not satisfactory for effective metal binding, which means that it requires the involvement of a specific sequence containing polar amino acids.

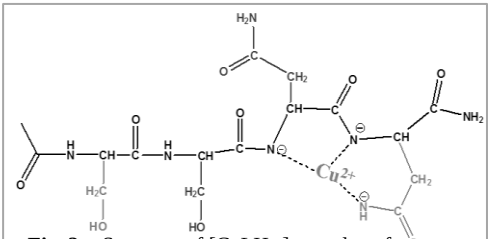

Fig. 2. : Structure of [CuLH$_{-3}$] complex of Ac-SSNN (pH~8,5)

II. Relative Role of polar side chains (Cu(II)-complexes)

Terminally unprotected rat amylin fragments and its mutants

1. *Copper(II) complexes of peptides modeling the sequence of the 17–22 residues of rat amylin have been synthesized and studied by potentiometric, UV-Vis, CD and ESR spectroscopic methods.*

The peptides were prepared in N-terminally free forms (NH_2–VRSSNN, NH_2–VRSSAA, NH_2–VRAANN, NH_2–VRSS, NH_2–SSNN, NH_2–AANN, NH_2–SSNA, NH_2–GGHSSNN) providing a possibility for the comparison of the metal binding abilities of the amino terminus and the –SSNN– domain.

2. *We have confirmed that the comparison of the data obtained for the N-terminally free peptides unambiguously demonstrates the dominating role of the terminal amino group over the asparaginyl side chains in copper(II) binding.*

The coordination behaviors of the tetra- and hexapeptides are, however, slightly different.

- In the case of tetrapeptides, when the side chains of asparagine are in the close vicinity of the coordinated peptide backbone a significant enhancement of thermodynamic stability can be observed, although the major binding modes are basically the same. On the contrary, this stability enhancement was not observed for the corresponding hexapeptides when the free rotation of the potential coordinating side chains can occur.
- An asparagine in the third position can enhance the stability of (NH_2, 2 N^-) coordinated complexes, as it can be seen for NH_2–SSNN, NH_2–SSNA and NH_2–AANN ligand, but not for NH_2–VRSSNN and NH_2–VRAANN peptides.
- The primary ligating sites of these molecules are, however, always the amino terminus, but a structure-influencing role of asparagine side chains is suggested on the base of pH-potentiometric and stabilitiy data.

3. *Based on our results from CD-, UV- and ESR-spectroscopic measurements we can state, that the asparagine side chains and the SSNN-domain even in presence of strongly coordinating groups (free amino group) take part in the binding of the Cu(II)-ion* **(Fig. 3).**

In the cases of NH_2-xxNy (peptides containing asparagine in the third position) ligands, the deprotonation and coordination of asparagine amide nitrogen cannot be excluded. Similar coordination sphere around copper(II) ion was supported in the case of $[CuH_{-4}L]$ of Ac-SSNN.

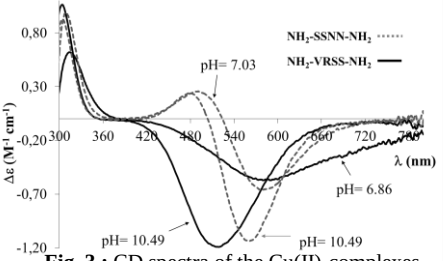

Fig. 3.: CD spectra of the Cu(II)-complexes of NH_2-VRSS and NH_2-SSNN measured in equimolar samples

4. *The presence of asparagine in the third position – even in the lack of serine side chains - results a stability enhancement of Cu(II)-complexes and the earlier mentioned characteristic CD-band could be detected. However, if serines are present in the sequence, the Cotton-effect is more expressed.*

5. *The primary ligating sites of these molecules are, however, always the amino terminus, but the SSNN sequence can provide a secondary metal binding site.*

- The formation of dinuclear complexes in the copper(II)–NH_2-VRSSNN system, however, gives an unambiguous proof for the anchoring capacity of the free asparagine side chains. Both ESR spectra and ESI-MS measurements provide a clear-cut evidence for the existence of dinuclear complexes in the 2:1 copper(II):peptide systems.
- Careful evaluation of the ESR spectra recorded for the dinuclear species seems to support the presence of two metal ions in a similar 3N coordination mode in the species $[Cu_2H_{-5}L]$ **(Fig. 4.).**

6. *These results demonstrated that all the serines and asparagines are necessary for the binding of the second equivalent Cu(II) at the C-terminus.*

We have found that the formation of dinuclear complexes is characteristic only for the copper(II)–NH_2-VRSSNN system in the physiological pH range.

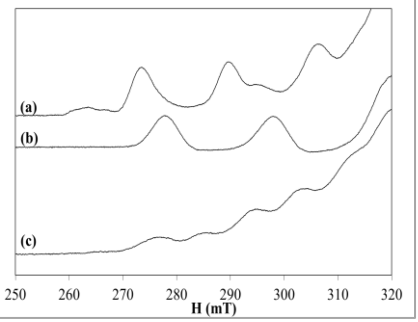

Figure 4:
ESR spectra recorded in the copper(II)–NH_2-VRSSNN system
(a) pH = 6.86 (Cu:L=1:1),
(b) pH=10.08 (Cu:L=1:1),
(c) pH=9.17 (Cu:L=2:1)

7. *The heptapeptide NH_2-GGHSSNN was synthesized, which heptapeptide provided a good chance to compare the binding affinities of the GGH and SSNN sequences.*

- We have proved that (in agreement with the expectations) in equimolar samples of copper(II) and NH_2-GGHSSNN the amino terminus is the exclusive copper(II) binding site and the polar side chains of Ser and Asn residues do not have any contribution to the stability of copper(II) complexes.
- We have shown that the heptapeptide can bind two equivalents of copper(II) ions, stable dinuclear complexes are formed in the presence of excess of copper(II) ions indicating that the SSNN domain can be an independent binding site.
- These findings strongly support the participation of the deprotonated amide nitrogen of asparagine side chain in the metal binding of this ligand and the formerly discussed ligands (NH_2-SSNN, NH_2-AANN, NH_2-VRSSNN) as well.

III. RELATIVE ROLE OF POLAR SIDE CHAINS (NI(II)- AND ZN(II)-COMPLEXES)

Ni(II)-complexes

1. *The comparison of Ni(II)-complexes of common oligopeptides with Ni(II)-complexes of these ligands reveals a significant impact of the polar side chains. These observations suggest that the presence of two adjacent asparaginyl and/or seryl residues may enhance the stability of their Ni(II)-complexes,* but we have to notice that the anchoring group is the amino group in case of the unprotected ligands.

- We stated that the Ni(II)-complexes formed at lower pH in the presence of asparagine residues, so the unusual impact of asparagine side chain(s) predominate even in the case of Ni(II).

2. *We detected the significant involvement of the asparagine side chain in the Ni(II)-binding assigning that with a characteristic CD-band around 400 nm.*

We have compared more relevant CD-spectra of Ni(II)-ligand systems at strongly alkaline solutions **(Fig. 5.)** It is apparent; if positive Cotton-effect is found around 400 nm, the ligand has a strongly coordinating side chain (e.g. imidazolyl: NH_2-GGHSSNN, NH_2-GTHS[1], NH_2-MKHM[1]). In case of ligands not having coordinating groups, positive CD-band cannot be seen (NH_2-VRSSNN, NH_2-AAAA-OH, NH_2-DAEF[2]).

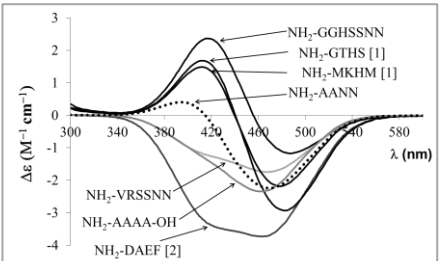

Fig. 5.: CD-spectra of Ni(II)-ligand systems at strongly alkaline solutions (pH>11)

Our ligands, which contains asparagine side chains in the third positions, possess this characteristic positive Cotton-effect.

[1] I. Turi; D. Sanna; E. Garribba; G. Pappalardo, I. Sóvágó; *Polyhedron,* 62, 7–17. (2013)
[2] Józsa É., „Az Alzheimer-kórért felelős amiloid-β(1-16) fragmenseinek Ni(II)-, Cd(II)- és Co(II)-komplexei" *Tudományos Diákköri dolgozat,* (2008)

3. *The investigation of mutants confirmed, the exclusive effect of asparagine side chains.*

In lack of serine side chains, the characteristic CD-band was also observed, suggesting only a slight effect of this side chain group.

- Four former mutants (NH_2–AANN, NH_2–SSNA, NH_2–VRSSNN, NH_2–SSS–OH) were studied for comparison and to obtain more information on the possible impacts of Ni(II) and asparagine side chains/SSNx-domain. The unusual influence of asparagines was observed (similarly to the Cu(II)-complexes).
- At the same time, this effect especially depends on the length of peptides and on the presence of other strongly coordinating groups. If the SSNN-type domain is found far away from the primary anchoring amino group (e.g. NH_2–GGHSSNN, NH_2–VRSSNN), the unusual influence cannot be detected (similarly to the Cu(II)-complexes).

4. *We obtained evidence for that the SSNN sequence cannot provide a secondary metal binding site in presence of strongly coordinating donor groups.*

- In contrast to the Cu(II)-complexes – the NH_2-VRSSNN and the NH_2-GGHSSNN peptides was not able to bind two equivalents of Ni(II)-ions.
- The clear-cut difference of the coordinating properties of Cu(II) and Ni(II) was obtained; the latter is not able to induce the deprotonation and coordination of the asparagine amide group in the side chain.
- In presence of other strongly coordinating group (e.g. terminal amino group), the SSNN-domain can enhance the stability of Ni(II)-complexes.

Studies of NH_2-GGHSSNN–Zn(II) and NH_2-SSNA–Zn(II) systems

Starting from the literature fact that imidazolyl group of histidine is able to take part in Zn(II)-ion binding, we wondered whether the asparagine side chains or the specific SSNN-motif could promote the deprotonation of the amide nitrogens in the peptide backbone or maybe the side chains.

5. *In the case of Zn(II)-ion, however, only weak interaction can be detected. This metal ion has no special affinity to bind to the SSNx-sequence. The polar side chains of serine and asparagine residues do not have any contribution to the stability of Zn(II)-complexes.*

- In the weakly acidic samples [ZnL] complexes are formed with (NH_2, CO) donor set. This coordination mode, however, cannot hinder the hydrolysis of metal ions by increasing pH.

6. *Zn(II)-ion is not able to induce the deprotonation and coordination of amide nitrogen.*

7. *Extraordinary effect of the SSNN-sequence did not prevail – not even in presence of albumin-binding site and SSNN-polar side chain set. These statements suggest that none of the aminogroup, histidine, or asparagine side chain(s) and the specific SSNN-domain are sufficient anchors for Zn(II) binding in the slightly alkaline conditions (for example pH 8-9).*

- Under pH 7 [ZnL] complexes could be detected with (NH_2, N_{im}) coordination mode. The monodentate imidazole coordination of the ligand cannot prevent the hydrolysis of metal ions in slightly alkaline solutions.

IV. MODELING OF METAL BINDING SITE OF HUMAN AMYLIN (15-22)

Terminally protected peptide models of the human amylin (15-22)

As the native sequence of *h*IAPP (15-22) does not contain terminally free amino group, therefore these studies provide a chance for the real comparison of possible binding sites and binding modes in the central region of human and rat amylin.

1. *Since the low solubility of the native sequence (hIAPP (15-22); Ac-ANFLVHSSNN) hinders the investigations, the Ac-AAAHSSNN mutant of hIAPP (15-22) was synthesized, which involves two independent binding sites for Cu(II)-ion; a histidine-binding site (AAAH) and the SSNN-domain. For comparison and to obtain more information about the possible binding mode, the Ac-AAAHAAAA mutant was also synthesized and studied, which can be the simplest model of the histidine-binding site.*

2. *We have shown that Cu(II)-ion is bound not only to the „histidine-binding site", but also the SSNN-domain takes part in metal binding. In other words, the SSNN domain of the molecule can be a new, independent binding site at pH~9.*

- As a result of our work, we detected isomer binding modes for the Cu(II)-ion. In agreement with the expectations, below pH 7 the peptide offered binding site at the histidine-containing part ($3N^-$, N_{im}). Surprisingly, in the pH 8-9 range a second isomer was also found. The stoichiometry of the formed complexes and also the concentration distribution of the major species as a function of pH suggested the existence of coordination isomers.
- The measured CD spectra of the Cu(II)-ligand solution at 1:1, 1:2 and 2:1 metal to ligand ratios can be well fitted with a simple superposition of the CD spectra of the [$CuH_{-3}L$] complex of Ac-FKHV and [$CuH_{-3}L$] of Ac-SSNN (at pH 8-9).

3. *Stable dinuclear complexes are formed in the presence of the excess of Cu(II)-ion representing that the SSNN domain can be a new, independent binding site, which was also confirmed by CD-, UV-Vis and ESR-spectroscopy.*

6. POSSIBLE UTILIZATION OF THE RESULTS

In the Bioinorganic Chemistry Research Group of the University of Debrecen we have been studying the coordinative behaviors of complexes of biologically important metal ions and peptides *in vitro*. These scientifically clarified results can be used in biological investigations *in vivo*.

During my work, I have studied and compared the metal ion binding ability of non-amyloidogenic rat amylin and amyloidogenic human amylin by different model peptides.

It is well-known that the amyloidogenicity and conformation changes are connected with the coordinated metal ions, therefore the coordination modes are crucial in these processes.

The importance of our results is that Cu(II)-ion coordinates not only to the „histidine-binding site", but also the SSNN-domain takes part in metal binding. In other words, the SSNN domain of the molecule can be a new, independent binding site at pH~9.

In the case of rat amylin, the Cu(II)-ion can be coordinated only by SSNN sequence, while the model peptide of sequence of *h*IAPP (15-22) (Ac-AAAHSSNN) offers more different binding sites. The complex formation processes in this Cu(II)-peptide system are more complicated ; at pH 8-9 range isomers and dinuclear complexes are also present. As the (pancreas) tissues are not homogeneous solutions, the metal ions are able to accumulate locally, so all the species previously mentioned are real possibilities.

In the cases of *rat* and *human* amylin fragments and mutants, we have found that the coordination modes and binding sites of Cu(II)-ions differ significantly. It means that the different coordination behaviour of *rat* and *human* amylin can cause different conformational changes.

Although we performed basic studies, our results may also provide information and useful models for the quantitative description of the interaction between biomolecules and metal ions.

7. TUDOMÁNYOS KÖZLEMÉNYEK (Publications)

Az értekezés alapját képező közlemények (Publications related to the thesis)

1. Ágnes Dávid, Csilla Kállay, Daniele Sanna, Norbert Lihi, Imre Sóvágó, Katalin Várnagy: *Potentiometric and spectroscopic studies on the copper(II) complexes of rat amylin fragments. The anchoring ability of specific non-coordinating side chains,*
Dalton Trans., **2015**, 44, 17091–17099.
IF: 4,197 DOI: 10.1039/c1dt10835b

2. Csilla Kállay, Ágnes Dávid, Sarolta Timári, Eszter Márta Nagy, Daniele Sanna, Eugenio Garribba, Giovanni Micera, Paolo De Bona, Giuseppe Pappalardo, Enrico Rizzarelli and Imre Sóvágó: *Copper(II) complexes of rat amylin fragments,*
Dalton Trans. **2011**, 40 (38), 9711 – 9721.
IF: 3,838 DOI: 10.1039/c5dt02445e

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

1. Katalin Várnagy Ágnes Dávid, Daniele Sanna, Csilla Kállay, Imre Sóvágó: *The anchoring ability of specific non-coordinating side chains in the rat amylin fragments,* 13th European Biological Inorganic Chemistry Conference (Eurobic 13), Budapest, Hungary, **2016**. 08. 28- 09. 01.

2. Dávid Ágnes, Kállay Csilla, Várnagy Katalin, Daniele Sanna, Hartman Éva, Sóvágó Imre: *Amilinfragmensek fémkötőhelyének vizsgálata,* 49. Komplexkémiai Kollokvium, Siófok, **2015**. 05. 26-28.

3. Katalin Várnagy, Gizella Csire, Sarolta Timári, Ágnes Dávid, Csilla Kállay: *The role of side chains in the fine tuning of metal binding ability of peptides,* 12th European Biological Inorganic Chemistry Conference (Eurobic 12), Zürich, Switzerland, **2014**. 08. 24-28.

4. Katalin Várnagy, Ágnes Dávid, Csilla Kállay, Daniele Sanna and Imre Sóvágó: *The role of the polar side chains in the metal binding ability of rat amylin fragments,* 12th International Symposium on Applied Bioinorganic Chemistry (ISABC12), Guangzhou, Kína, **2013**. 12. 03–06.

5. Dávid Ágnes, Várnagy Katalin, Kállay Csilla, Daniele Sanna, Sóvágó Imre: *Amilinfragmensek fémkötőhelyének vizsgálata,* 47. Komplexkémiai Kollokvium, Mátraháza, **2013**. 05. 30.

6. Dávid Ágnes: *Amilinfragmensek szintézise és oldategyensúlyi vizsgálata,* Kémiai Előadói Napok, Szeged, **2012.** 10. 29-31.

7. Dávid Ágnes: *Amilinfragmensek szintézise és oldategyensúlyi vizsgálata,* Hatvani István Szakkollégium hallgatói konferenciája, Debrecen, **2012**. 05. 03.

8. Dávid Ágnes: *Amilinfragmensek oldategyensúlyi vizsgálata,* XXX. Országos Tudományos Diákköri Konferencia, Pécs, **2011.** 04. 27–29.

9. Dávid Ágnes: *Amilinfragmensek oldategyensúlyi vizsgálata,* Hatvani István Szakkollégium hallgatói konferenciája, Debrecen, **2011**. 04. 21.

10. Dávid Ágnes: *Amilinfragmensek oldategyensúlyi vizsgálata,* Tudományos Diákköri Konferencia, Debrecen, **2010**. 11. 25.

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

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List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **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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Total IF of journals (publications related to the dissertation): 8,015

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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