COMPLEX FORMATION REACTIONS OF HISTIDINE CONTAINING PEPTIDES. METAL BINDING SELECTIVITY OF PEPTIDE FRAGMENTS OF PRION PROTEINS.

PhD thesis abstract

Jószai Viktória

Supervisor: Dr. Sóvágó Imre

University of Debrecen
Debrecen, 2008

1. Introduction and objectives

Histidine residues bound in the proteins are the primary binding sites for metal ions. There are a great number of enzymes which contain the metal ion coordinated to the histidine side chain. Examples include carbonic anhydrase, haemoglobin, SOD enzymes, prion proteins. Therefore new impetus has been given to studying complexation of histidine containing peptides with transition metals. These metal complexes could be good models of the active sites of the enzymes or proteins.

The main aim of our work was the investigation of the possible role of side chain imidazole nitrogen of different histidine containing peptides as an anchor for metal ions.

First, we studied the complex formation reactions of peptides containing two or three histidine residues. The latter ones could be the model of SOD enzyme. In the active site of this enzyme, there are three imidazole rings coordinated to the copper ion through pyridine nitrogens (except the bridging imidazole ring).

However, several metal ions can induce the deprotonation of backbone amide nitrogens previously coordinated to the anchor. The anchor can be the histidine side chain. Only a few examples (prion protein, amyloid precursor protein and albumin) are known for enzymes in which the metal ion is coordinated not only to the histidine side chain, but also to the backbone of the molecule.

We have studied the anchoring ability of histidine residues of the peptide models of prion proteins.

The prion protein is a cell surface glycoprotein, a considerable amount of which is expressed in the neurons of mammals. In the last few years, significant attention has been given to this protein. The cause is the possibility of transferring the prion disease from bovine to man most likely through the infected beef.

The prion disease of the bovines is known as mad cow disease, while the human form of the disease is the variant Creutzfeldt–Jakob syndrome. The cause of conformational change from normal to diseased form is unknown at present. However, researchers accept that the prion protein ordinarily binds copper. While the amount of some metal ions change in the tissue after the structural change.

In the beginning of our work, the octarepeat region of the prion protein was already well studied. That is why we have investigated the prion models outside the octarepeat, namely the peptide models of His(96) and His(111). In addition, the 31-mer peptides have also been investigated, including His(85), His(96), His(111) and also their mutants (His→Ala). We have studied the complexation with the following metal ions:

- Cu(II) (controversial literature data)
- Zn(II), Mn(II) (essential metal ions)

- Ni(II), Co(II) (potential model ions for Cu(II) and Zn(II) complexation)
- Cd(II), Pd(II) (toxic metals, moreover, in the case of palladium the affinity toward peptides is high).

2. Applied methods and ligands

The pH potentiometric titrations were used to identify the protonation and stability constants of ligands and metal complexes, respectively. The calculation of these constants were made by computational programs SUPERQUAD and PSEQUAD. The primary supposed model was confirmed by UV-Vis spectrophotometry, CD, ^{1}H NMR and ESR spectroscopic measurements.

During our work, the complex formation reactions of following ligands have been studied: Ac-HisGlyHis-OH, Ac-HisGlyHis-NHMe, Ac-HisHisGlyHis-OH, Ac-HisHisGlyHis-NHMe (for these four ligands we have studied the complexation only with copper ion); HuPrP(Ac94-97NH_2): Ac-GlyThrHisSer-NH_2, HuPrP(Ac109-112NH_2): Ac-MetLysHisMet-NH_2, ChPrP(Ac122-125NH_2): Ac-PheLysHisVal-NH_2, HuPrP(91-114), HuPrP(Ac84-114NH_2), HuPrP(Ac84-114NH_2)His(85)Ala, HuPrP(Ac84-114NH_2)His(96)Ala, HuPrP(Ac84-114NH_2)His(111)Ala (fig. 1), Ac-His-NHMe, Ac-HisGlyGlyGly-NH_2, Z-HisGly-OH, Acetyl-histamine, 1-methyl-1H-imidazole, 4-methyl-1H-imidazole.

Fig. 1. shows the list of investigated metal ions as well.

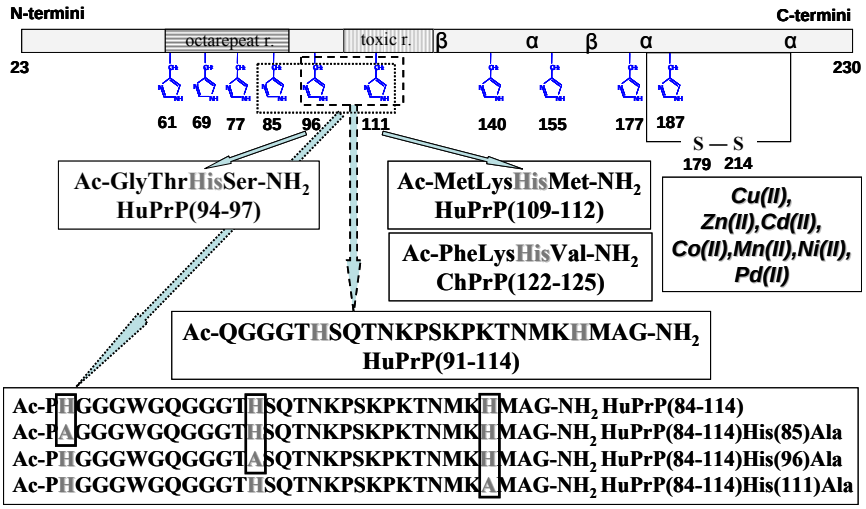


Figure 1. The studied ligands (prion models) and metal ions

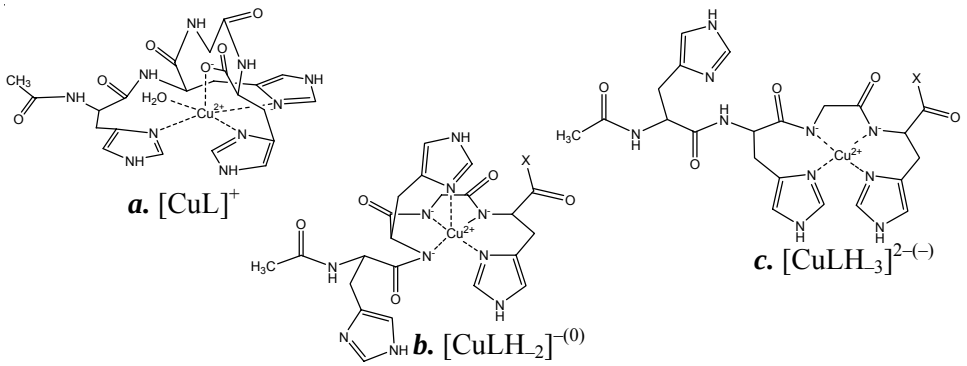
3. New scientific results

During my Ph.D. work, I have studied complex formation reactions of histidine containing peptides. The results obtained can be divided into two parts. The *first part,* included a study of the role of histidine side chain in the complex formation reactions of N-terminally protected tri- and tetra-peptides containing two or three histidyl residues.

3.1. Copper(II) complexes of N-terminally protected peptides containing two and three histidines

The interaction of Ac-HisGlyHis-OH, Ac-HisGlyHis-NHMe, Ac-HisHisGlyHis-OH and Ac-HisHisGlyHis-NHMe with copper(II)ion have been investigated by potenciometric, UV-Vis spectrophotometric, CD, ^{1}H NMR and ESR spectroscopic methods. The major conclusions obtained from the results are as follows:

- the macrochelates could form with the simultaneous coordination of two and three histidine side chains for tri- and tetrapeptides, respectively;
- the imidazole nitrogen donor atom is an efficient anchor and is able to induce deprotonation and coordination of amide nitrogens in all cases;
- the deprotonation of the first two amide nitrogens occurs almost in a cooperative manner for tripeptides, while this process takes place in a stepwise deprotonation in the case of tetrapeptides;
- the domination of [N_{im}, N^-, N_{im}] and [N_{im}, N^-, N^- N_{im}] coordinated species was observed in the physiological pH-range. It is interesting that the latter contains an unusual (7,5,6)-membered joined chelate. The deprotonation of the third amide function was detected at higher pH;
- the presence of the third imidazole side chain in tetrapeptides results in higher copper binding ability as compared with the two histidine residues of tripeptides;
- the presence of the carboxylate group gives higher metal binding ability as well.



Scheme 1. Structure of complexes formed in the Cu(II)–Ac-HisHisGlyHis-OH and Cu(II)–Ac-HisHisGlyHis-NHMe systems (X = O^- or NHMe)

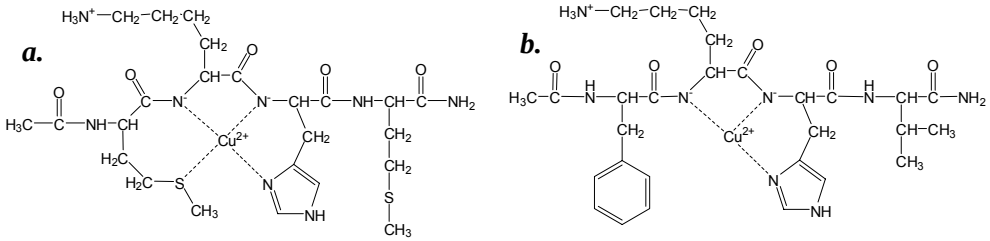
3.2. Transition metal complexes of peptide fragments of prion proteins

The *second part* of the thesis deals with the histidine containing peptide fragments of prion proteins (especially His(96) and His(111)) and their transition metal complexes. The main findings are best discussed by forming three groups of metal ions.

3.2.1. With ***copper(II)*** *ion*:

- the complex formation reaction starts with the monodentate coordination of imidazole moiety as an anchor in all of these cases;
- the deprotonation of the first two amide functions takes place in a cooperative manner and consequently, small changes in pH can result in significant changes in the ratio of free and complexed copper(II) ions;
- we have confirmed the weak coordination of Met(109) side chain at physiological pH. In biological systems, this may have possible protective role as an endogenous antioxidant toward copper-catalysed oxidation;
- the ε-amino groups of Lys residues are not metal-binding sites at any pH values;
- at physiological pH, we have found the [N_{im}, N^-, N^-] coordination mode, while the binding sites can be given as [N_{im}, N^-, N^-, S] in the case of Met containing peptide (scheme 2.);
- in contrast with the octarepeat region, the deprotonation and coordination of amide nitrogens goes toward the N-termini;
- at physiological pH, the His(111) containing peptide has higher copper binding ability than the His(96) containing peptide and all of them have higher binding ability than the octarepeat monomer;

- small tetrapeptide fragments are reliable models of metal binding sites. The complex formation processes of the tetra- and nona-peptide fragments are almost the same.



Scheme 2. Structure of $[CuLH_{-1}]$ complexes formed in the ***a.***:Cu(II) – HuPrP(Ac109-112NH_2) (L: Ac-MetLysHisMet-NH_2) and ***b.***:Cu(II) – ChPrP(Ac122-125NH_2) (L: Ac-PheLysHisVal-NH_2) systems

3.2.2. The complexes of ***palladium(II)*** ion are the most stable among the studied metal ions. The investigation of small model ligand Ac-His-NHMe shows that complex formation reactions with palladium(II) depends on the metal to ligand ratio to a great extent. In equimolar solution of palladium(II) and Ac-FKHV-NH_2:

- the complex formation reaction starts even under strongly acidic conditions;
- the imidazole nitrogens and also the amide nitrogens coordinate to the palladium(II) ion;
- the coordination mode is very similar to that found for the copper(II) complexes, but the formation of analogous complexes is shifted toward more acidic pH in the case of palladium;
- the deprotonation and coordination of amide nitrogens is successive in contrast with the cooperativity observed in the case of copper(II) complexes.

The presence of soft donor functions in the side chains of peptides significantly influences the complexation with palladium. In equimolar solution of palladium(II) and Ac-MKHM-NH_2:

- the complexation starts even under strongly acidic pH, but via the primary coordination of thioether sulfur atom (Met(109)) in contrast with the previous system;
- the increase of pH results in the formation of Pd – N_{im} and Pd – N^-_{amide} bonded species because these species have high thermodynamic stability;
- the structural rearrangement of palladium(II) complexes is a very slow process. This can be explained by the different formation kinetics of Pd – S and Pd – N bonded species, the latter one formed in much slower reactions;

- the formation of precipitate at alkaline pH (pH > 9) can be explained by the formation of polynuclear species in which the imidazole moieties can act as bridging residues between coordinatively unsaturated species.

3.2.3. For the ***other metal ions (Ni(II), Zn(II), Co(II), Cd(II), Mn(II))*** the major conclusions can be drawn as follows:

- they have only low affinity to bind to the short peptide fragments of prion proteins;
- after the monodentate imidazole coordination of the ligand, it cannot prevent the hydrolysis of the metal ions in slightly alkaline solutions;
- amide coordination can occur only with nickel(II) at alkaline pH values;
- the weakest interaction with the peptide fragments of prion protein among the studied metal ions have been observed in the case of manganese. This suggest that histidines are not sufficient anchors for manganese and the pivotal role in the structural conformation caused by Mn(II) is not the coordination to histidine residue.

3.2.4. Finally, we studied the ***zinc(II)*** binding ability of large 31-mer peptides which contain two or three histidine residues (PrP(84-114)). It has been found that the affinity toward zinc(II) ion was also low despite the higher number of histidines, the imidazole moieties are the exclusive binding sites. On the other hand, peptides have high affinity for copper(II) binding even in the presence of high excess of zinc(II) ions.

The stabilities of studied complexes show different affinity towards the binding of peptide fragment of prion proteins, this affinity follows the order:

$$Pd(II) >> Cu(II) >> Ni(II) \geq Zn(II) > Cd(II) \sim Co(II) > Mn(II)$$

Taking into account that palladium(II) ions have no biological role, we can conclude that peptide fragments of prion protein have an outstanding selectivity to bind copper among the essential transition elements.

4. Possible application of the results

The results presented in the dissertation are of basic research. The studied ligands could be divided into two groups.

The first group consists of ligands with two or three histidines. The results of copper complexation with these ligands can be useful in the selection of other models of the SOD enzyme active site. The molecules mimicking the natural enzymatic function can be applied as possible medicines.

The second group is the short prion models. The aim of the work was the investigation of solution equilibrium of prion peptide fragments with Pd(II)-, Cu(II)-, Ni(II)-, Zn(II)-, Cd(II)-, Co(II)-, and Mn(II), and also the investigation of the structure of formed complexes. The results obtained may be valuable in biological research concerning the cause of the prion disease.

5. List of publication

Papers involved in the dissertation:

1. Daniele Sanna, Giovanni Micera, Csilla Kállay, Viktória Rigó and Imre Sóvágó
Copper(II)Complexes of N-Terminal Protected Tri- and Tetrapeptides Containing Histidine Residues
Dalton Transactions, 2702-2707 (2004)

2. Imre Sóvágó, Katalin Ősz, Zoltán Nagy, Viktória Rigó, Daniele Sanna, Diego La Mendola, Giuseppe Di Natale, Giuseppe Pappalardo and Enrico Rizzarelli
Transition Metal Complexes of Peptide Fragments of Prion Proteins
Advances in Coordination, Bioinorganic and Inorganic Chemistry, 363-376 (2005)

3. Giuseppe Di Natale, Giulia Grasso, Giuseppe Impellizzeri, Diego La Mendola, Giovanni Micera, Nikoletta Mihala, Zoltán Nagy, Katalin Ősz, Giuseppe Pappalardo, Viktória Rigó, Enrico Rizzarelli, Daniele Sanna, Imre Sóvágó
Copper(II) Interaction with Unstructured Prion Domain Outside the Octarepeat Region: Speciation, Stability and Binding Details of Copper(II) Complexes with PrP106-126 Peptides
Inorganic Chemistry, **44**, 7214-7225 (2005)

4. Viktória Jószai, Zoltán Nagy, Katalin Ősz, Daniele Sanna, Giuseppe Di Natale, Diego La Mendola, Giuseppe Pappalardo, Enrico Rizzarelli and Imre Sóvágó
Transition Metal Complexes of Terminally Protected Peptides Containing Histidyl Residues
Journal of Inorganic Biochemistry, **100**, 1399-1409 (2006)

5. Viktória Jószai, Zoltán Nagy, Katalin Ősz, Giuseppe Di Natale, Giuseppe Pappalardo, Enrico Rizzarelli and Imre Sóvágó
Copper(II) and zinc(II) mixed metal complexes of large peptide fragments of prion protein
(under preparation)

Lectures and posters connected to the dissertation:

1. Sóvágó I., Ősz K., Nagy Z., Rigó V.
A prion protein peptidfragmenseinek komplexképződési folyamatai (lecture in Hungarian: Complexation reactions of peptide segments of prion protein)
XXXIX. Komplexkémiai Kollokvium (Colloqium on Coordination Chemistry), May 26-28, 2004, Gárdony, Hungary

2. Rigó V., Kállay Cs., Sóvágó I.
A Cu,Zn-SOD enzim aktív centrumának modellezésére alkalmas hisztidintartalmú peptidek Cu(II)komplexeinek oldategyensúlyi vizsgálata (lecture in Hungarian: Solution equilibria of copper(II) complexes of histidine containing peptides for modelling the active sites of Cu,Zn-SOD enzymes *Fiatal kárpátaljai magyar kutatók a természettudományi kutatásban (Young hungarian scientists of trnscarpathia in scientific research),* 2004. October 30, Beregszász, Ukraine

3. Rigó V., Kállay Cs., Sóvágó I.
Solution equilibria of copper(II)complexes of peptides containing histidine residues for modelling the active sites of Cu,Zn-SOD enzymes (lecture)
10th International Conference of Chemistry, November 12-14, 2004, Kolozsvár, Roumania

4. Viktória Rigó, Katalin Ősz, Zoltán Nagy, Imre Sóvágó
A prion protein peptidfragmenseinek átmenetifém-komplexei (lecture in Hungarian: Transition metal complexes of peptide fragments of prion protein)
XL. Komplexkémiai Kollokvium (Colloqium on Coordination Chemistry), May 18-20, 2005, Dobogókő, Hungary

5. Imre Sóvágó, Katalin Ősz, Zoltán Nagy, Viktória Rigó, Daniele Sanna, Diego La Mendola, Giuseppe Di Natale,Giuseppe Pappalardo, Enrico Rizzarelli
Transition Metal Complexe of Peptide Fragments of Prion Proteins (lecture)
20th International Conference on Coordination and Bioinorganic Chemistry, June 5-10, 2005, Smolenice, Slovakia

6. Viktória Jószai, Zoltán Nagy, Katalin Ősz, Imre Sóvágó, Daniele Sanna, Giovanni Micera, Giuseppe Pappalardo, Diego La Mendola, Giuseppe Di Natale, Enrico Rizzarelli
Transition metal complexes of peptide fragments of prion protein outside the octarepeat region (poster)
20th International Conference on Solution Chemistry, August 21-25, 2005, Portoroz, Slovenia

7. Viktória Jószai, Zoltán Nagy, Katalin Ősz, Imre Sóvágó, Daniele Sanna, Giovanni Micera, Giuseppe Pappalardo, Diego La Mendola, Giuseppe Di Natale, Enrico Rizzarelli
Transition metal complexes of peptide fragments of prion protein outside the octarepeat region (poster)
X International Symposium on Bioinorganic Chemistry – Challenge for new generation, September 20-25, 2005, Szklarska Poreba, Poland

8. Imre Sóvágó, Katalin Ősz, Zoltán Nagy, Viktória Jószai, Daniele Sanna, Giovanni Micera, Diego La Mendola, Giuseppe Di Natale, Giuseppe Pappalardo, Enrico Rizzarelli
Copper(II) Complexes of the (84-114) Peptide Fragment of Human Propn Protein (lecture)
X International Symposium on Bioinorganic Chemistry – Challenge for new generation, September 20-25, 2005, Szklarska Poreba, Poland

Lectures and posters do not closely connected to the dissertation:

1. I. Sóvágó, K. Ősz, Z. Nagy, Cs. Kállay, V. Rigó, D. Sanna, G. Micera, G. Pappalardo and E. Rizzarelli
Copper(II)complexes of peptides of histidine. Models of the binding sites of the enzyme CuZn-SOD and prion proteins (lecture)
EUROBIC 7, August 29- September 2, 2004, Garmisch-Partenkirchen, Germany

2. Katalin Ősz, Zoltán Nagy, Viktória Rigó, Imre Sóvágó, Daniele Sanna, Govanni Micera, Diego La Mendola, Giuseppe Di Natale, Giuseppe Pappalardo, Enrico Rizzarelli
A possible mechanism for formation of prion deseases: copper(II) coordination to prion protein fragments containing histidines (poster)
Gordon Research Conferences in Inorganic Reaction Mechanisms, February 13-18, 2005, Ventura, CA, USA

3. Katalin Ősz, Zoltán Nagy, Viktória Rigó, Imre Sóvágó
A HuPrP(84-114) protonálódási és réz(II)ionnal való komplexképződési makro- és mikrofolyamatai (lecture in Hungarian: Protonation and copper(II) complex formation macro- and microscopic processes of HuPrP(84-114))
XL. Komplexkémiai Kollokvium (Colloqium on Coordination Chemistry), May 18-20, 2005, Dobogókő, Hungary

4. Katalin Ősz, Zoltán Nagy, Viktória Jószai, Imre Sóvágó, Daniele Sanna, Giovanni Micera, Diego La Mendola, Giuseppe Di Natale, Giuseppe Pappalardo, Enrico Rizzarelli

Protonation and coordination macro- and microscopic equilibria in the copper(II) – Human Prion Protein (84-114) system (poster)

20th International Conference on Solution Chemistry, August 21-25, 2005, Portoroz, Slovenia

5. Katalin Ősz, Zoltán Nagy, Viktória Jószai, Imre Sóvágó, Daniele Sanna, Giovanni Micera, Diego La Mendola, Giuseppe Di Natale, Giuseppe Pappalardo, Enrico Rizzarelli

Protonation and coordination equilibria in the copper(II) – Human Prion Protein (84-114) system (poster)

X International Symposium on Bioinorganic Chemistry – Challenge for new generation, September 20-25, 2005, Szklarska Poreba, Poland