Tioéter- és imidazolcsoportot tartalmazó peptidek fémkomplexeinek vizsgálata

Investigation of metal complexes of peptides containing thioether and imidazole groups

thesis of PhD dissertation

Beáta Bóka

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I. Introduction and the aim of the work

It is well known that some metal ions available in little amount in organisms have important role in control of biochemical processes. Information obtained from model studies can help to understand the complicate biological systems. Proteins are the strongest metal binding ligands in living organisms and these macromolecules can be modelled by peptides. Functional groups in side chains of proteins play the most important role in the binding of metal ions in the active site of metalloenzymes. Imidazole nitrogen and thioether sulfur atoms are among the most common binding sites in metalloproteins. In the frame of this work we studied the role of the above mentioned donor functions.

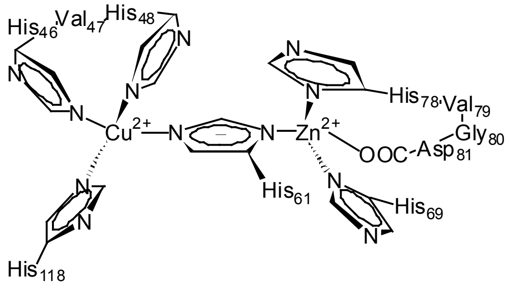
The effect of thioether functions in the enzymes and on the complex formation processes have not clarified. The thioether donor functions exhibit very high selectivity in metal binding preferring the coordination of soft metal ions. Their coordination depends on the location of other donor groups of the ligand, as well.

One of the main goal of this study was to investigate the coordination chemistry of the thioether sulfur donor atom with a wide variety of metal ions and ligands containing the donor group in different positions. In the biggest part of the work we studied the metal-sulfur interaction in the metal complexes of di- and tripeptides containing methionine in all possible locations (GlyMet, MetGly, GlyGlyMet, GlyMetGly, MetGlyGly, MetGlyMet, MetMetAla, and MetMetMet). The metal ions included divalent 3d (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}), 4d (Pd^{2+}, Cd^{2+}) and main group (Pb^{2+}) elements.

Although interactions with bioligands especially with proteins and peptides may have important role in the toxicity of lead, there is only a very limited number of data available in the literature concerning the lead(II)complexes of amino acids or peptides. Most of them are used at very different experimental conditions. In order to obtain reliable data for comparison we studied amino acids and peptides containing other possible coordinating donor atoms in the side chain besides thioether sulfur, namely hydroxyl and carboxylate oxygens, thiolate sulfur and imidazole nitrogen.

Due to the high number of enzymes containing imidazole nitrogen in the active site a lot of studies have been performed in the field of coordination chemistry of this function. One part of them covers enzymes and their modified forms, the other part deals with the investigation of the effect of the imidazole nitrogen in model systems. Studies on metal complexes of peptides containing histidine belong to the latter group.

Superoxide anion (O_2^-) and its drastic forms are damaging biomolecules, thereby causing inflammation, aging, carcinogenesis, amyotrophic lateral sclerosis, reumatoid arthritis and ischemia. Superoxide dismutases (SODs) comprise an integral part of the cellular protective mechanism against this oxidative stress. These enzymes catalyze the disproportionation of superoxide anion radical. The active centre of Cu,Zn-SOD (see figure) consists of two metal ions, namely Cu(II) and Zn(II) bridged by the imidazolato ring of a histidine residue of the protein backbone. Both metals are coordinated by two donor atoms from the side chains of amino acids located very near in the peptide sequences. This interaction can be modelled with the tripeptide HisValHis (copper binding site) and with the tetrapeptide HisValGlyAsp (zinc binding site). In order to obtain more realistic model the peptides with protected termini were synthesised. The copper(II) and zinc(II) complexes of these ligands were studied by potentiometric and spectroscopic methods (UV-Vis, EPR, CD). The mixed ligand complexes with N-acetyl-histamine gave information about the imidazolato bridge in the native enzyme.



II. Applied methods

The ligands except SOD model peptides were obtained from commercial sources. The protected peptides were synthesized in the solid state, using the modified form of the 2-chlorotrithyl chloride resin (H-Linker-CLTR). The Fmoc group was used for temporal protection of the α-amino group. After purification with HPLC the purity of the material prepared was checked by ^{1}H NMR and by potentiometric titration of the free ligand.

Protonation constants of the ligands and (the stoichiometry and) the stability constants of the metal complexes have been determined by potentiometric titration (T=278K, I=0.2 mol·dm^{-3} (KCl, and/or KNO_3)). The calculation of the stability constants were performed by using PSEQUAD computer program. For determination of the coordination mode in the complexes spectroscopic methods (UV-Vis, CD, EPR, ^{1}H NMR, ^{113}Cd NMR) were used.

III. New scientific results

1. Metal ion dependence of thioether coordination

There is significant metal ion dependence in the complex formation processes of the thioether ligands. Metal ions studied can be divided into three groups on the basis of the strength of metal-sulfur interaction.

1.1. The possibility of metal-thioether sulfur interaction was ruled out in the case of **zinc(II), cobalt(II), and cadmium(II) ions**. $[ML]^+$ type complexes can be formed with (NH_2,CO) coordination of the peptides.

1.2. Thioether has only secondary effect on the complex formation processes in **copper(II) and nickel(II) complexes**, the primary binding sites are the other donor functions of the peptide molecules.

All tripeptides form stable square-planar diamagnetic species with nickel(II) ion, and both the thermodynamic and the spectroscopic parameters of $[NiLH_{-2}]^-$ are very similar to those of triglycine. A weak axial coordination of thioether side-chain was concluded in the octahedral $[NiL]^+$ complexes of tripeptides with N-terminal methionyl residues (MetGlyGly, MetMetAla and MetMetMet). The metal ion is coordinated primarily via the terminal amino nitrogen and carbonyl oxygen donors and this species exists only in low concentration and in a narrow pH range, between pH 5 and 8. The species $[NiLH_2]^-$ predominates at higher pH values. In this complex the metal ion is coordinated by the terminal amino, two deprotonated amide nitrogens and the carboxylate oxygen, while the thioether moiety does not take part in metal binding.

In the case of copper(II) complexes the thioether sulfur has a small, but systematic influence on the complex formation processes. Namely, the coordination of the sulfur atoms depends on both the number and the position of methionyl residues in the peptide sequence. $[CuL]^+$, $[CuLH_{-1}]$ and $[CuLH_{-2}]^-$ are the main species in all cases and the bis complex, $[CuL_2H_{-1}]^-$, can also be formed in the presence of ligand excess. The terminal amino and the neighbouring carbonyl groups are the main binding sites in the $[CuL]^+$ complexes of all peptides with N-terminal glycyl residue. The carbonyl group, however, can be replaced by the thioether sulfur of N-terminal methionine in cases of MetGlyGly, MetGlyMet, MetMetAla and MetMetMet. This change in coordination has only a slight effect on the stability constants of the complexes, but existence of a CT band (UV-Vis, CD) around 320-330 nm is a good indication of the S (thioether) → Cu(II) interaction. In the case of tripeptides containing internal methionyl residues (GlyMetGly, MetMetAla and MetMetMet) a CT band (around 310 nm in UV-Vis and around 340-360 nm in CD) develops parallel with the formation of the species $[CuLH_{-1}]$, in which either axial or equatorial coordinations of sulfur atoms are possible. EPR spectra and the change in the sign of the Cotton effect in CD spectra strongly suggest the equatorial coordination of the thioether residues. The coordination of C-terminal methionyl residues was concluded in the $[CuLH_{-2}]^-$ complexes of GlyGlyMet, MetGlyMet and MetMetMet and the EPR spectra support an equilibrium between the isomeric forms involving carboxylate or sulfur in the equatorial positions.

1.3. On the contrary, thioether residues were detected as the primary metal binding sites of tripeptides in the **palladium(II) complexes**.

- Their coordination does not depend on pH, and results in an enhanced thermodynamic stability of the complexes and a significant difference in the binding modes of the peptides with and without methionyl residues.
- The thioether functions of the peptides containing methionine in C-terminal or internal positions act as an anchor and the palladium promoted amide deprotonation occurs in very acidic solution. This process results in the formation of (NH_2,N^-,N^-,S) or (NH_2,N^-,S) coordinated species.
- On the contrary, the thioether binding hinders the amide deprotonation in N-terminal position so it takes place at higher pH values in the case of MetGly and MetGlyGly. Stable metal ion coordination of the internal methionyl residue results in the dipeptide-like coordination of the tripeptide GlyMetGly. In contrast with common tripeptides the second amide group of GlyMetGly is not a metal binding site at any pH values.
- In the case of the latter peptide the formation of bis(ligand) complexes containing a tridentate and a monodentate ligand was detected. The pH dependence of the coordination of thioether/ amino groups and the NMR spectra made it possible to identify these linkage isomers.
- On the contrary, the coordination of the amino and thioether groups results in the formation of 6-membered chelate in the bis(ligand) complexes of peptides containing N-terminal methionine.

1.4. Metal ion selectivity of thioether donor atoms made it possible to prepare **mixed metal complexes via thioether bridging**.

Thioether sulfur is not a binding site in the $[CuLH_{-1}]$ complex of GlyMet or in the $[NiLH_{-2}]^-$ of GlyMetGly. Sulfur atoms of these metal complexes are, however, good candidates for binding to coordinatively unsaturated palladium(II) complexes ($[Pd(dien)]^{2+}$ or $[Pd(GlyMetH_{-1})]$). We studied the formation of these mixed species by spectroscopic methods (UV-Vis, NMR). The interaction of the complexes results in a blue shift of the spectra in the UV range, which is considered as an indication of the Pd-S interaction and provides a good base for stability constant determination. In the case of the square-planar diamagnetic nickel(II) complexes the formation of mixed metal complexes can be monitored by NMR measurements.

We concluded that the binding constant for the nickel(II) complex is higher than that of the corresponding copper(II) complex and the donor atoms in the coordination sphere of the parent palladium(II) complex also have an important effect on the stability.

2. Pb(II) complexes of amino acids and peptides

Pb(II) complexes formed with amino acids and peptides have rather low stability, the deprotonation and coordination of the amide group of peptides do not occur. The interaction of lead(II) with several donor atoms in the side chain, however, can enhance the termodynamic stability of the complexes formed.

- We established that the Pb(II) complexes of amino acids containing thioether, carboxylate or imidazole groups have higher thermodynamic stability than those of glycin. A weak interaction between thioether sulfur and the metal ion can be observed in complexes in which Pb(II) ion is already bound by two donor atoms in the form of a chelate and the coordination of the sulfur atom create another five membered chelate ring (e.g. Pb(II) complex of S-methyl cystein). The coordination of the side chain donor groups of histidine and S-methyl cystein was proved by NMR spectroscopy. Bis(ligand) complex formation was not detected.
- We observed a little increase in the stability of the complexes of peptides containing histidine or aspartic acid in the N-terminal position. NMR spectra of the former system supported the Pb-N(im) interaction.
- Peptides containing thiolate sulfur in the side chain (mercapto-propionil-glycine, CysGly, AlaCys) are much better complexing agents. The main binding sites are thiolate

groups. Other donor atoms in chelating position with thiolate sulfur coordinate as well, however, deprotonation of peptide nitrogens does not occur.

Lead(II) ions are able to compete with zinc(II) ions in binding of thiolates and can displace them in zinc fingers and in metalloenzymes containing thiolate group. This kind of interaction can be one of the reasons of lead poisoning.

3. Copper(II) and zinc(II) complexes of peptides related to the active site of enzyme Cu,Zn-SOD

Our results revealed that the peptide sequences in the enzyme exhibit excellent metal binding properties, both ligands (the tripeptide Ac-HisValHis-NH_2 and the tetrapeptide Ac-HisValGlyAsp-NH_2) are effective complexing agents for Cu(II) and Zn(II) ions.

- ML type complexes were found to be stabilized by the formation of a macrochelate similarly tothat can be found in the enzyme.
- In the case of zinc(II) complexes amide deprotonation does not occur. However, with copper(II) the donor groups in the side chains act as an anchor, their coordination promotes the deprotonation and coordination of amide nitrogens. In slightly basic media the species $[CuLH_{-2}]$ and $[CuLH_{-3}]^-$ containing 4N donor atoms are formed with Ac-HisValHis-NH_2. The former complex contains two deprotonated peptide nitrogens and two imidazole nitrogens, while in the latter species the imidazole group of the N-terminal histidine residue is replaced by the deprotonated acetamido group. In species $[CuLH_{-4}]^{3-}$ formed with Ac-HisValGlyAsp-NH_2 at high pH the coordination of four deprotonated amide nitrogen was supposed.
- Ternary complexes can be formed with Ac-histamine, but their stability constants are lower than the binding constant of imidazole to the free copper(II) ion. From this observation it can be concluded that the stabilization of the imidazolato bridge in the active center of Cu,Zn-SOD is not the result of a specific metal ion to ligand interaction, but governed by the conformational changes of the protein molecule.

IV. Some aspects of possible applications of the results

We studied the coordination chemistry of peptides, one of the major group of biologically important ligands. The metal ion – thioether sulfur interaction has important role in the blue copper proteins and in the transport and toxicity of various platinum anticancer agents. Platinum(II) complexes can be modelled by corresponding palladium complexes. Our results and conclusions can provide useful information for the better understanding of biological effects and design of new ligands. We proved that thioether sulfur is a major metal binding site for palladium(II) and consequently also for platinum(II), and its coordination together with nitrogen donors in a chelate can also be thermodynamically stable.

Mixed metal complexes can be obtained via thioether bridging and they are promising models of electron transfer systems and give useful information about this kind of process.

The design and application of synthetic low molecular mass metal complexes as SOD mimics have received considerable attention during the last decades. These synthetic enzymes can be potential drugs in the therapy. Complexes with coordination mode similar to the enzyme are promising models therefore the studies on the complexation of Cu(II) and Zn(II) with model ligands help in the selection of potential SOD mimics.

V. Publications

1. K. Várnagy, B. Bóka, I. Sóvágó, D. Sanna, P. Marras and G. Micera:
Potentiometric and spectroscopic studies on the copper(II) and nickel(II) complexes of tripeptides of methionine
Inorg. Chim. Acta, 275-276 (1998) 440-446.

2. B. Bóka, Z. Nagy, K. Várnagy and I. Sóvágó:
Solution equilibria and structural characterisation of the palladium(II) and mixed metal complexes of peptides containing methionyl residues
J. Inorg. Biochem., 83 (2001) 77-89.

3. K. Ősz, B. Bóka, K. Várnagy, I. Sóvágó, T. Kurtán and S. Antus:
The application of circular dicroism spectroscopy for the determination of metal ion speciation and coordination modes of peptide complexes
Polyhedron, 21, (2002) 2149-2159.

4. B. Bóka, I. Sóvágó, A. Myari, J.C. Plakatouras, N. Hadjiliadis:
Copper(II) and zinc(II) complexes of the peptides Ac-HisValHis-NH_2 and Ac-HisValGlyAsp- NH_2 related to the active site of the enzyme CuZnSOD
J. Inorg. Biochem., (manuscript)

VI. Lectures and posters

1. Bóka Beáta (lecture)

Metionintartalmú tripeptidek átmenetifém komplexei

XXIII. Országos Tudományos Diákköri Konferencia, 1997. április 2-4. Pécs, Hungary

2. Katalin Várnagy, Imre Sóvágó, Beáta Bóka, Csaba Gábor Ágoston, Giovanni Micera and Daniele Sanna (poster)

Equilibrium and Spectroscopic Studies on Copper(II) Complexes of Peptides Containing Sulfur Atoms

The 3rd Cost D1 Workshop on Biocoordination Chemistry, 11-13 September 1997, Bergen, Norway

3. Bóka Beáta (lecture)

Tioéterhidat tartalmazó vegyes fémkomplexek képződésének vizsgálata

XXI. Kémiai Előadói Napok, 1998. október 26-28. Szeged, Hungary

4. Imre Sóvágó, Zoltán Nagy and Beáta Bóka (lecture)

Thermodynamic, Kinetic and Structural Studies on Palladium(II) Complexes of Thioether Ligands

5th International Symposium on Applied Bioinorganic Chemistry, 13-17 April 1999, Ioannina, Greece

5. Bóka Beáta, Várnagy Katalin és Sóvágó Imre (lecture)

Tioétercsoportot tartalmazó peptidek átmenetifém komplexeinek egyensúlyi és szerkezeti vizsgálata

XXXIV. Komplexkémiai Kollokvium, 1999. május 19-21. Tata, Hungary

6. Beáta Bóka and Imre Sóvágó (poster)

Palladium(II) Complexes of Peptides Containing Thioether Side Chains

Vth Symposium on Inorganic Biochemistry, 23-27 September 1999, Wroclaw, Poland

7. B. Bóka and I. Sóvágó (poster)

Metal ion dependence of thioether coordination

10th International Conference on Bioinorganic Chemistry, 26-31 August 2001, Florence, Italy

8. Bóka Beáta, Nagy Zoltán, Sóvágó Imre, Alexandra Myari, Nick Hadjiliadis (lecture)

A Cu,Zn-SOD aktív centrumát modellező peptidek Cu(II)- és Zn(II)komplexei

XXXVIII. Komplexkémiai Kollokvium, 2003. május 21-23. Gyula, Hungary