Interaction of transition metal ions with small biomolecules. New results on the metal ion selectivity of the ligands.

Ph.D. thesis abstract

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Debrecen, 2006

1. INTRODUCTION AND AIM OF THE WORK

The biochemical role of vanadium in the living systems is not well understood yet. In spite of that vanadium is accumulated by few low forms of life, it is toxic over a given concentration for the higher animals and the human. The biological interest in vanadium has been increased for the last twenty years, when several compounds of vanadium were tested *in vivo* and proved to have insulin-mimetic activity. At the beginning inorganic vanadium(V) salts and oxovanadium(IV) complexes were studied, which were able to normalize the glucose level in the blood serum and alleviate the other symptoms of *diabetes mellitus*, too. However, the toxicity of the metal ion limits the potential use of vanadium as a therapeutical agent. For this reason the research works were continued toward the less toxic form of vanadium with lower oxidation state. In order to control and enhance their transport via biological membranes in the body and to reduce the required effective dosage below the toxic level, numerous complexes with different structures were studied. A number of bis complexes of VO(IV) with small molecular mass ligands containing oxygen, nitrogen or sulphur donor atoms have been reported as having glucose lowering effects. The solution equilibrium studies are necessary to understand the fate of the complexes in the body. In our work joining to this research field we have planned to study the complex formation of VO(IV) with 5-methyl-carboxy-picolinic acid. The ligand is a derivative of the picolinic acid with the ester group in position 5, which has no role in the complex formation but it may have the effect for the stability of the complexes and for the transport properties via membranes. In order to assess the stability of the complexes in the presence of competitive low molecular mass ligands present in the blood serum (oxalate, lactate, citrate and phosphate) we have also planned to study mixed ligand complexes.

By the recent results the V(III) complexes containing the ligands from VO(IV) complexes having insulin-like properties were also found to normalize the serum glucose level. Although the free V(III) ion is very sensitive to get oxidized, the low oxidation state of the metal ion can be conserved in the solid compounds. The difference in the coordination chemistry of V(III) and VO(IV) or V(V) results in different behaviour of the complexes. The solution equilibrium studies on V(III) systems are quite difficult to carry out because of the strong tendency of V(III) to get oxidized under aerobic conditions and its intensive hydrolysis in aqueous solution. The amount of equilibrium data with V(III) systems is very limited in the literature, although solution speciation results are necessary to understand the properties of the complexes in the living system.

Firstly, our aim was to work out the technical details of the preparation and storage of V(III) stock solution, and to carry out the measurements under anaerobic conditions. We also planned to study the hydrolysis of the metal ion under our experimental conditions, and the solution equilibria of V(III) complexes having insulin-like properties. Furthermore, the complex formation between V(III) and some amino acids and their derivatives was also planned to study in detail. Our results showed that all the ligands in insulin mimetic compounds can form stable complexes with V(III), the amino acids and accordingly peptides are not able to hinder the hydrolysis of the metal ion even at high ligand excess. Therefore we thought to design a new, modified peptide derivative with a functional group linked to the peptide chain, which can be an anchor group for V(III) binding and hindering the hydrolysis effectively. In this way the transport of the metal ion in the body would be possible, while the peptide chain in the complex might have an important role in the molecular recognition.

The new model ligand designed by us was the peptide hydroxamic acid, which can be considered to be derivatives of hydroxylamine and carboxylic acid of the corresponding peptide. The coordination chemistry of simple hydroxamic acids with different metal ions is well-known. The ligand has strong interaction with hard metal ions mainly, such as Fe(III) and Al(III). The similarity of their character in the coordination chemistry with V(III) may lead to the parallel properties. However, it is also well-known that simple hydroxamic acids oxidize the low oxidation state vanadium. In the case of modified hydroxamic acids by the peptide chain the properties of the original molecule can be changed basically.

The complex formation between peptides and metal ions has also been studied, but the role of the side chains has not been described unambiguously in all cases. It is especially true for the guanidinium group of arginine, whereas a numbers of important roles of Arg-containing peptides in biological processes and molecular recognitions were published. Hence one of our aims was to study the properties of different peptide residues on the complexation with Cu(II), as the most common used metal ion in the coordination chemistry. Because it is thought that the positively charged side chain of Arg has an effect on the stabilization of the peptide with weak non-covalent interaction, monodentate ligands with a polar, negatively charged side chain as B ligand in ternary systems have also been planned to study. In order to understand that guanidinium group can take part as an anchor group for amide binding, the complexation of the fragment (17–29) of rat amylin with Cu(II) has also been proposed.

Peptide hydroxamic acids are known as effective inhibitors of metalloenzymes. These ligands are very versatile in coordination chemistry because of the two different part of the molecules: the hydroxamate group coordinates to hard metal ions strongly; on the contrary the peptide

chain with nitrogens is a good ligand for soft metal ions. In the literature only a few results have been published on metal ion – peptide hydroxamic systems, because these ligands are not commercially available. First of all, we planned the synthesis of several di- and tripeptide hydroxamic acids with different non-coordinating side chains, and then to study the complexation of the ligands with different metal ions, as Fe(III), Al(III), Zn(II), Cu(II) and Ni(II), and to characterise the binding modes in the complexes with different spectroscopic methods.

2. EXPERIMENTAL METHODS

pH-potentiometry: the most common experimental method for determination of the complex formation processes in aqueous solution. The condition of its application is that the metal ion coordination has an effect on the protonation equilibrium of the ligand, the complex formation has pH effect. The aim of the measurements is to determine the composition and the stability constants of the complexes formed. The stability constants from the experimental data were calculated by PSEQUAD.

UV–vis spectrophotometry: was carried out with Cu(II)-, Ni(II)-, Fe(III)- and V(III) complexes. The maximum value of the electron absorption spectrum (λ_{max}) depends on the type of the donor atoms coordinating to the metal ion, and the geometry of complexes. Analysing of the given spectra the structure, the geometry of the complex, the number and the quality of the coordinating ligands can be determined.

CD spectroscopy: the optically active molecules deflect the plane of the polarized light diversely. The optical activity of the metal complexes may be generated by the asymmetry of the ligand or the asymmetry of the complex formed with the coordination. The absorption of the both components of the polarized light is called circular dichroism. The measurements were carried out with Cu(II) complexes of peptide hydroxamic acids and peptide residues containing Arg.

EPR spectroscopy: is an important experimental method to study paramagnetic molecules and ions, e.g. Cu(II) and VO(IV) complexes. The method is based on the interaction of the electron and the magnetic field. The hyperfine structure of EPR spectra is sensitive to even small structural changes in the complexes. The geometry of the complex, the number and the quality of the coordinating ligands can be concluded from the EPR parameters.

^{1}H-NMR spectroscopy: was used to refer the peptide hydroxamic acids synthetized and to check their purity. The method was also used to identify the overlapping protonation steps and to determine the protonation microconstants using the pD dependence of the chemical shifts of the NMR active nuclei. Furthermore we used it to characterise the structures of Zn(II) complexes.

ESI-MS spectroscopy: the ions formed by ionization are separated by mass/charge (*m/Z*). The method gives information about the molecular mass of the metal complexes confirming the assumed structures from other spectroscopic methods. In addition, fragmentation patterns may provide more structural information.

3. NEW SCIENTIFIC ACHIEVEMENTS

The equilibrium data for complexation of 31 ligands in parent complexes and 9 ligands in mixed ligand systems with VO(IV), V(III), Fe(III), Al(III), Zn(II), Cu(II) and Ni(II) have been determined. The techniques of the preparation and storage of V(III) have been worked out, and the anaerobic conditions for the required measurements have also been carried out. The hydrolysis of V(III) has been studied in details. The synthesis of 12 new peptide hydroxamic acids has been done and their interaction with several transition metal ions has been characterized.

1. The VO(IV) – 5-methyl-carboxy-picolinic acid system:

- The composition and the stability constants of the complexes have been determined by pH-potentiometry and their structures defined by EPR spectroscopy. The ligand, similar to picolinic acid, has formed mono and bis complexes with different protonation degree with VO(IV) coordinating via the pyridine-nitrogen and carboxylate and forming five-membered chelate(s).
- All the four serum bio-ligands (oxalate, lactate, citrate and phosphate) were competitive binders for VO(IV). In the ternary systems stable mixed ligand complexes dominating in a wide pH range have been found. The structures of the complexes have been defined by EPR spectroscopy. The model calculation of the distribution of VO(IV) among picolinates and the four serum ligands close to physiological pH has shown that the picolinates ligands were partly or completely displaced from the coordination sphere of the metal ion by citrate and phosphate in ternary complexes and by phosphate and lactate in binary complexes.

2. <u>Equilibrium studies of V(III)</u>:

- The stability constants of the hydroxo complexes of V(III) have been determined under our experimental conditions (t = 25,0 °C, I = 0,2 mol/dm^3 (KCl), $C_{V(III)} = 5 \cdot 10^{-4} - 8 \cdot 10^{-3}$ mol/dm^3).
- The solution equilibrium studies on V(III) complexes having insulin-like properties have been carried out. All the 7 ligands (picolinic acid, 6-methylpicolinic acid, pyridine-2,6-dicarboxylic acid, 4-hydroxypyridine-2,6-dicarboxylic acid, maltol, 1,2-dimethyl-3-hydroxy-4-(1H)-pyridinone and tiron) have yielded stable complexes with V(III), the hydrolysis of the metal ion was hindered strongly in the acidic pH range.
- The V(III) binding capabilities of some amino acids (glycine, aspartic acid, penicillamine, histidine) have been characterized. The results have shown a very weak interaction, which was parallel with the hydrolysis of the metal ion. We could not obtain reliable models and stability constants with these ligands because of the very complicated equilibrium systems.

3. <u>Complexation of peptides containing arginine residues with Cu(II)</u>:

- Interaction between different oligopeptides containing arginine and Cu(II) (AlaArg, ArgAla, ArgSer, ArgArg, ArgArgArg and ArgArgArgArg) have been studied. The results have shown that the deprotonation and coordination of the guanidinium group to the metal ion did not take place. The metal ion speciation of all the systems was similar to the corresponding glycine derivatives.
- The weak ligand – ligand interaction in ternary systems, using monodentate ligands as B ligand (1-methyluracil, uridine, phenylalanine-ethylester, tyrozine-methylester) with a polar, negatively charged side chain has been studied. The formation of mixed ligand complexes was favoured and a slight stability increase was observed, caused by the weak interaction between the positively charged guanidinium group and negatively charged side chain.
- The complexation on the fragment (17–29) of rat amylin, protected at N and C termini and having only the guanidinium group in the side chain for coordinating to the metal ion has also been investigated. The amide-nitrogens were found to take part in metal binding, the coordination sites of Cu(II) were occupied by the peptide backbone. The important role of guanidinium group as an anchor donor has been confirmed in the lack of other side chain donor function.

4. <u>Complex formation of peptide hydroxamic acids with transition metal ions</u>:

- The solution equilibrium studies on peptide hydroxamic acids, primary and secondary, di- and tripeptide derivatives containing protected and free N terminus have been carried out.
- Proton dissociation constants of all the ligands have been determined. With non-protected ligands the two deprotonation steps overlap considerably each other. Therefore the microscopic protonation processes were also explored from combined pH – ^{1}H-NMR titrations of the primary dipeptides. The data have shown that the ammonium group is more acidic than the hydroxamic acid group.
- The stability constants of the complexes with Fe(III), Al(III), Zn(II), Cu(II) and Ni(II) have also been determined. For characterisation of the binding modes in the complexes UV–vis, EPR, CD and ^{1}H-NMR spectroscopic techniques have been applied.

» In the case of *N-protected derivatives* the coordination of the ligands via hydroxamate chelate(s) alone has been found. In all systems precipitation occurred as a consequence of the large Z group yielding sparingly soluble complexes.

» The *non-protected derivatives* can coordinate to the metal ions via hydroxamate oxygen and/or peptide nitrogens.

› The results with hard Fe(III) and Al(III) have shown that the ligands formed complexes with the exclusive involvement of [O,O] hydroxamate chelates, the peptide chain did not take part in the coordination. Comparing to the simple hydroxamic acids, in these complexes, however, the peptide chain might be of importance in the transport of the metal ion, or in the molecular recognition by non-covalent weak interactions with macromolecules in the body.

› The coordination of the ligands to Zn(II) either via [O,O] or [NH_2,CO] chelating set has been found and linkage isomers can be formed. Although the deprotonation and coordination of amide-nitrogen(s) has not been occurred.

› In the case of Cu(II) and Ni(II) the amino group as a real anchor donor has been found and the re-arrangement of the coordination mode to nitrogens has been observed. With the borderline Cu(II) the complex formation started via hydroxamate group and it remained in a wide pH range in many instances yielding oligomeric species with mixed coordination mode. With the soft Ni(II) the hydroxamate coordination was uncommon. Both metal ions were able to induce the deprotonation and coordination of amide-nitrogen(s) and also the hydroxamate-nitrogen in primary derivatives, yielding complexes with high stability. The metal ion selectivity of the ligands with the peptide chain has been confirmed.

4. POTENTIAL USE OF RESULTS

Nowadays *diabetes mellitus* is a widespread disease in the world. Insulin, which is necessary for patients with type I diabetes can only be used in the form of daily injection. The scientists have been looking for possibilities for a long time in order to displace insulin with pharmaceutical preparations having capability for oral administration. These compounds with similar effect, as against insulin, are able to make a more comfortable therapeutics for the patients. For the adaptation of a compound to use in the therapy the basic researches are absolutely necessary, which may help understand the properties and the fate of the compounds in the body, and provide an opportunity to design new synthetic ligands. The equilibrium studies on metal complexes and the determination of their structures in solution are such a basic research.

In our work we have joined to the research field on vanadium complexes having insulin-mimetic activity. We have got new results on the equilibrium studied with difficulty investigating V(III). Additionally, new ligands having metal ion selectivity have been designed, synthesised and their complex formation with different metal ions has been studied. Our results and the drawing conclusions may help greatly to understand the fate and the effect of the complexes in biological systems.

5. PUBLICATIONS (ARTICLES)

1. J. Gätjens, B. Meier, T. Kiss, E.M. Nagy, P. Buglyó, H. Sakurai, K. Kawabe and D. Rehder
A New Family of Insulin-Mimetic Vanadium Complexes Derived from 5-Carboalkoxy-picolinates
Chem. Eur. J., **9/20** (2003) 4924–4935.

2. P. Buglyó, E.M. Nagy and I. Sóvágó
V(III) Binding Strengths of Small Biomolecules
Pure and Appl. Chem., **77** (2005) 1583–1594.

3. P. Buglyó, D.C. Crans, E.M. Nagy, R.L. Lindo, L. Yang, J.J. Smee, L.-H. Chi, M.E. Godzala III and G.R. Willsky
Aqueous Chemistry of the Vanadium^{III} (V^{III}) and the V^{III} – Dipicolinate Systems and a Comparison of the Effect of Three Oxidation States of Vanadium Compounds on Diabetic Hyperglycemia in Rats
Inorg. Chem., **44** (2005) 5416–5427.

4. P. Buglyó, E.M. Nagy, E. Farkas, I. Sóvágó, D. Sanna and G. Micera
Metal Complexes of Primary Hydroxamic Acid Derivatives of Common Dipeptides in Aqueous Solution
J. Inorg. Biohem., (submitted)

5. P. Buglyó, E.M. Nagy, Á. Seprényi, E. Farkas, D. Sanna and G. Micera
Metal Ion Binding Capabilities of Secondary Hydroxamic Acid Derivatives of Simple Dipeptides in Aqueous Solution: *the Effect of the Methyl Substituent at the Hydroxamate Nitrogen*
(manuscript in preparation)

6. E.M. Nagy, D. Sanna and I. Sóvágó
Co(II) Complexes of Oligopeptides Containing Arginyl Residues
(manuscript in preparation)

7. P. Buglyó, E.M. Nagy, Á. Seprényi, E. Farkas, D. Sanna and G. Micera
Metal Ion Binding Capabilities of Simple Tripeptide Hydroxamic Acids
(manuscript in preparation)

6. PUBLICATIONS (POSTERS AND LECTURES)

12. E.M. Nagy, P. Buglyó, E. Farkas, I. Sóvágó and D. Sanna (poster)
Fe^{3+}, Al^{3+}, Ni^{2+}, Cu^{2+} and Zn^{2+} complexation by dipeptide hydroxamic acids
10^{th} International Symposium on Inorganic Biochemistry,
20 – 25 September 2005, Szklarska Poreba, Poland

11. E.M. Nagy, P. Buglyó, E. Farkas, I. Sóvágó and D. Sanna (poster)
Metal binding strengths of peptide hydroxamic acids
29^{th} International Conference on Solution Chemistry,
21 – 25 August, 2005, Portorož, Slovenia

10. P. Buglyó, E.M. Nagy, E. Farkas and I. Sóvágó (lecture)
Metal ion binding capability of peptide hydroxamic acids
20^{th} International Conference on Coordination and Bioinorganic Chemistry,
5 – 10 June, 2005, Smolenice, Slovakia

9. P. Buglyó, E.M. Nagy, Á. Seprényi, E. Farkas, I. Sóvágó and D. Sanna (lecture)
Metal ion binding capability of common dipeptide hydroxamic acids
40^{th} Colloquium on Coordination,
18 – 20 May, 2005, Dobogókő, Hungary

8. E.M. Nagy, P. Buglyó and I. Sóvágó (lecture)
Complexation of vanadium(III) and [O,O] or [N,O] donor ligands
10^{th} International Chemistry Conference,
12 – 14 November, 2004, Kolozsvár, Roumania

7. P. Buglyó and E.M. Nagy (lecture)
Interaction of vanadium(III) with small molecules of potential biological importance
4^{th} International Symposium on Chemistry and Biological Chemistry of Vanadium
3 - 5 September, 2004, Szeged, Hungary

6. E.M. Nagy, P. Buglyó and I. Sóvágó (lecture)
Complexation of vanadium(III) and small bioligands
26^{t} Young Chemists' Conference,
27 – 29 October, 2003, Szeged, Hungary

5. P. Buglyó, E.M. Nagy and I. Sóvágó (lecture)

Interaction between vanadium(III) and small biomolecules

9th International Symposium on Inorganic Biochemistry,

4 - 7 September, 2003, Szklarska Poreba, Poland

4. E.M. Nagy, P. Buglyó and I. Sóvágó (poster)

Vanadium(III) complexes of (O,O) or (N,O) donor bioligands

9th International Symposium on Inorganic Biochemistry,

4 - 7 September, 2003, Szklarska Poreba, Poland

3. E. M. Nagy, P. Buglyó and I. Sóvágó (poster)

Speciation studies on vanadium(III) – (O,O) or (N,O) donor ligand systems

28th International Conference on Solution Chemistry,

23 – 28 August, 2003, Debrecen, Hungary

2. P. Buglyó and E.M. Nagy (poster)

Interaction between vanadium(III) and pyridinecarboxilates

EUROBIC-6,

29 July – 3 August, Lund, Sweden

1. P. Buglyó and E.M. Nagy (lecture)

Interaction between vanadium(III) and pyridinecarboxilates

37th Colloquium on Coordination Chemistry, 29 – 31 May, 2002, Mátraháza, Hungary