Interaction between Pb(II) and Cd(II) heavy metal ions and hydroxamic acids

PhD theses

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1 INTRODUCTION AND THE AIM OF THE WORK

Hydroxamic acids are widely known, good chelators for iron(III). Natural hydroxamate based siderophores play role in the iron(III) uptake and transfer in the microorganisms via complexation. In order to form a complex with high stability and selectivity, they contain mostly three functional groups having special backbone which allows ideal coordination of the chelate functions to the compact iron(III). One of these natural compounds, DFB is used as a therapeutic agent to sequester the iron(III) excess or aluminum(III) from the human body. The complex forming behaviour of numerous hydroxamates with transition metal ions were studied extensively, however their interactions with some toxic heavy metal ions are studied just in a few articles. Both Cd(II) and Pb(II) are frequent environmental pollutants via industrial use, however, since the toxicity of their compounds have became known from the ’80-s, their utilization were substituted, even so the contamination is decreasing slowly. Therefore, there is an increasing interest how to remove these toxic elements from waste-water, soil and from the human body. Most of the methods used nowadays are based on complex formation. One type of these chelators used is the above mentioned hydroxamic acid, DFB. The selectivity of the hydroxamates is influenced by several interesting features (e.g. the length, rigidity or orientation of the linker, relative orientation of the linked chelating groups, substituents, etc.). Since there is a lack of information, we decided to study the interaction between the toxic Cd(II)- and Pb(II)-ions and hydroxamic acids, in order to get insight whether they are able to bind these ions in stable complexes, and which factors have influence on the selectivity and stability (e.g. substituents of hydroxamic acid group, the structure of the connecting chain between the two groups in the case of di- and trihydroxamic acids).

Besides siderophores, hydroxamic acids and their derivatives have a wide variety of biological activities, e.g. aminohydroxamic acids have enzyme inhibitory effect. They are able to inhibit several metalloenzymes (collagenase, MMP-s, etc.)

via complexation and thereby blocking the activity of the transition metal ion in the active centre. These ligands contain other donoratoms besides the hydroxamate, which play crucial role in the molecular recognition processes or they can also act as donoratoms forming other types of chelates, beside the one with hydroxamate. With aminohydroxamic acids, and their imidazole analogues (imidazolehydroxamic acids) our aim was to explore the effects of these substituents (amino-N and imidazole-N) on the stability and structure of complexes formed. (Based on literature data for Cd(II)-α-alaninehydroxamic acid, we exclude the examination of the Cd(II)-amino- and imidazolehydroxamic acid systems.

2 EXPERIMENTAL METHODS

pH-potentiometry: The most common experimental method for determination of the protonation and the complex formation processes in aqueous solution. The aim of the measurements is to determine the composition and the stability constants of the complexes formed (T = 278 K, I = 0.2 M KNO_3). The stability constants from the experimental data were calculated by SUPERQUAD and PSEQUAD programs.

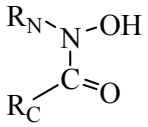
^{1}H-NMR spectroscopy: this method was used to check the purity of the newly synthesized ligands, to get deeper insight into the protonation steps of the ligands, and furthermore it was used to get structural information for Pb(II) complexes.

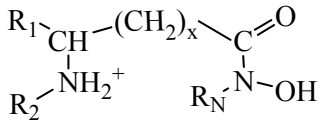
Mass-spectroscopy (ESI-MS): This technique, using soft ionization ion-source (ESI), allows collecting information about the molecular mass of the metal complexes confirming the assumed structures in solutions. The ions formed by ionization are separated by mass/charge (m/Z).

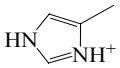
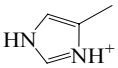
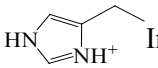
Some of the ligands studied were purchased from SIGMA or CIBA-GEIGY. Others were synthesized at the University of Debrecen, or within the frame of collaborations.

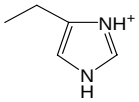
The structures of the ligands studied are listed below:

Monohydroxamic acids:

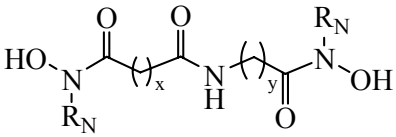




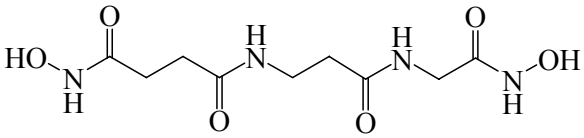
R_N	R_C	abbrev.
Monohydroxamic acids		
-H	$-CH_3$	Aha
-H		Bha
$-CH_3$	$-CH_3$	N-Me-Aha
	$-CH_3$	N-Ph-Aha
Imidazolehydroxamic acids		
-H		Im-4-Cha
$-CH_3$		*N*-Me-Im-4-Cha
-H		Im-4-Aha

R_1	R_2	x	R_N	abbrev.
Aminohydroxamic acids				
$-CH_3$	-H	0	-H	α-Alaha
$-CH_3$	-H	0	$-CH_3$	*N*-Me-α-Alaha
-H	-H	1	-H	β-Alaha
-H	-H	1	$-CH_3$	*N*-Me-β-Alaha
-H	$-CH_3$	0	-H	Sarha
-COOH	-H	1	-H	Asp-β-ha
-COOH	-H	2	-H	Glu-γ-ha
	-H	0	-H	Hisha

Dihydroxamic acids:



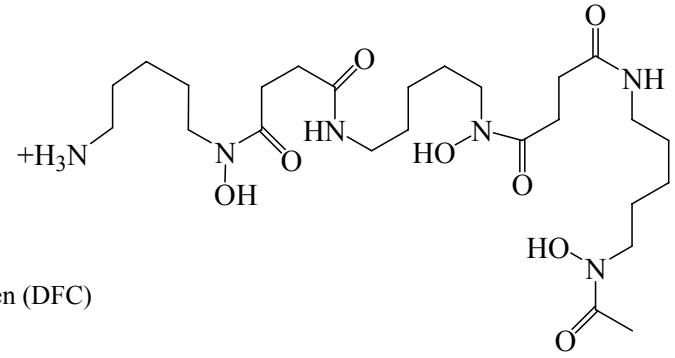
x	y	R_N	abbrev.
2	5	$-CH_3$	2,5-DIHA
3	4	$-CH_3$	3,4-DIHA
2	4	$-CH_3$	2,4-DIHA
3	3	$-CH_3$	3,3-DIHA
2	3	$-CH_3$	2,3-DIHA
2	2	$-CH_3$	2,2-DIHA
2	5	-H	2,5-*H,H*-DIHA
2	2	-H	2,2-*H,H*,-DIHA



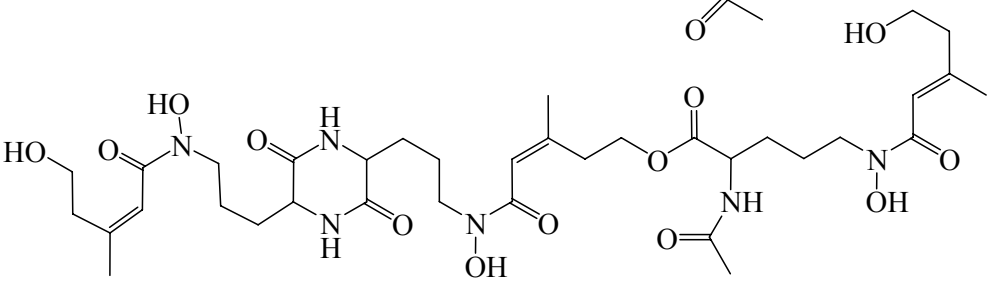
2,2,1-*H,H*-DIHA

Trihydroxamic acids:

Desferrioxamine B (DFB)



Desferriocoprogen (DFC)



3 NEW SCIENTIFIC ACHIEVEMENTS

Equilibrium data for complexation of 26 ligands with Pb(II) and 15 with Cd(II) have been determined. Hydrolysis of Pb(II) under our conditions was studied. New methods were developed for synthesis of three ligands. Additional three ligands have been re-synthesized by using known methods. Attempts were made for the use of ^{113}Cd-NMR or ^{207}Pb-NMR spectroscopy in order to get insight into the stoichiometry and structure of the complexes formed.

3.1 Acid-base properties of the ligands

Protonation constants of the ligands were already known at an ionic strength of 0.2 M KCl (expect the newly synthesized ones) from earlier works. Comparing the corresponding protonation constants of the ligands measured at an ionic strength of 0.2 M KCl and 0.2 M KNO_3, one can conclude that the change of the medium does not have measurable effect. Based on this, the former conclusions established at 0.2 M KCl became valid at 0.2 M KNO_3. Protonation constants of the newly synthesized ligands were also determined. They are in good agreement with the former trends. Namely: (1) Comparing the basicity of the imidazole-N in Im-4-Aha and Im-4-Cha

(where the imidazolering is connected to the hydroxamate with one bond less than the former one), one can conclude, that it has a definitely higher basicity value than Im-4-Aha has, but lower than the free imidazole group. Therefore the protonation processes of the imidazole and hydroxamate groups do not overlap. (2) The protonation constants of *N*-Me-β-Alaha can be compared with the corresponding values of the β-Alaha, *N*-Me-α-Alaha and α-Alaha. The results show, that the lower basicity of the hydroxamate in β-Alaha was decreased by the change of the R_N substituents (-H to $–CH_3$), therefore the protonation processes of the amino and hydroxamate groups take place in a separated pH region. (3) In the case of 2,2,1-*H,H*-DIHA, the difference between the stepwise protonation constants is somewhat higher than that of *N*-Me-DIHA derivatives, which indicates stronger interaction between hydroxamic acid groups in the former case. The reason might be the formation of an (or two) H-bond(s) between the hydroxamate-NH(s) and peptide group(s) besides the through-space interaction.

3.2 Cd(II)- and Pb(II)-monohydroxamate systems

Equilibrium studies for Cd(II)- and Pb(II)-monohydroxamates show comparable stability of the mono- and bis-hydroxamato complexes of the two studied metal ions. Tris-chelated ones could be fitted in none of the monohydroxamate systems studied. Replacing the proton with methyl or phenyl group in R_N or R_C position does not affect the stability of the complexes. Out of the results one can obtain, that Pb(II)-ion forms more stable complexes than Cd(II) ($\Delta\log\beta_{MA}$ ~2). The stability of the Pb(II)-species are as high as the Cu(II) ones ($\log\beta_{PbA}$ ~ 6).

3.3 Cd(II)- and Pb(II)-DFB model-dihydroxamate systems

Among the synthetic DFB model dihydroxamic acids, 2,5-DIHA has the same arrangement of the connecting chain between the two hydroxamic acid groups as in the natural DFB. The other ligands studied contain this chain modified compared to the 2,5-DIHA, which may mean either different position of the peptide group, the

presence of a second peptide moiety, or decreased length of the backbone. In two ligand pairs the change of the hydroxamate-N substituent (R_N = -H → $-CH_3$) was investigated. Among the studied dihydroxamic acids 2,5-DIHA forms the most stable complexes with Cd(II), but 2,2-*H,H*-DIHA does with Pb(II). It was proven by ESI-MS that the complexes with Pb(II) are only monomeric type species. For Cd(II), however, the ESI-MS results show that neither 2,5-DIHA nor any of the studied dihydroxamic acids have ideal length of the linker or relative orientation of the linked chelating groups for the formation of a mononuclear bis-chelated complex ([MA]). Therefore dinuclear, double-bridged [M_2A_2] with decreased stability is formed. The main conclusion is that the stoichiometry and stability of the bis-chelated DIHA-complexes depend much more on the geometry of the complex formed than either on the ionic radius of the metal ion or on the length of the linker situating in the studied dihydroxamic acids.

3.4 Cd(II)- and Pb(II)-trihydroxamate systems

According to the results on the two natural trihydroxamate based siderophores (DFB and DFC) we can conclude, that none of them are good ligands for Cd(II). It is characteristic for the interaction that, at 1:1 metal ion to ligand ratio almost 30% of Cd(II) is *not* bound to the ligand at physiological pH. These siderophores bind to the Pb(II) via two hydroxamate only, similarly to other systems investigated previously. The third hydroxamate of the ligand coordinates to a second metal ion forming trinuclear type complexes, thus both DFB and DFC are able to bind metal excess (3/2). Out of the two trihydroxamate, DFC forms more stable complexes with both metal ions studied. These experimental findings could fit well in the former trend: DFC favors metal ions with bigger ionic radius a little more compared to the DFB. Extra stability of the Pb(II)-DFC complexes can be detected, similarly as it was found with Mg(II). This can be explained with the interaction between the valence-shell electrons of the metal ion and the π-electrons of the double bonds of the ligand.

3.5 Pb(II)-aminohydroxamate systems

The aminohydroxamic acids were investigated only with Pb(II) (such as imidazolehydroxamic acid systems). In these cases besides hydroxamate type (O,O) binding mode, coordination via amino and hydroxamate nitrogen donor atoms is also possible due to the presence of the amino group. According to the results, different behaviour of the aminohydroxamic acids can be obtained depending on the position of the amino group compared to the hydroxamic one, and the substituent (R_N) of the latter moiety. In the Pb(II)-primary α-aminohydroxamic acids (α-Alaha, Sarha, Hisha) above pH 6 a complex with high stability can be detected ($[PbAH_{-1}]_x$ where x = 1-4). By ^{1}H-NMR and ESI-MS studies we know that this is a polymeric type complex, where the ligand is coordinated via ($O_{hydroxamate}$,$O_{hydroxamate}$) and (N_{amino},$N_{hydroximate}$) type chelates to the metal ion.

For ligands having the amino group in β- or γ-position, or in the cases of both N-methyl analogues only hydroxamate type mono- and bis-hydroxamato complexes are formed. If the ligand contains a third donor group, as it is for Asp-β-ha, Glu-γ-ha and Hisha, a weak interaction is proved (the ligand binds tridentate mode).

3.6 Pb(II)-imidazolehydroxamate systems

Substituting the amino-N with a less basic imidazole-N causes other behaviour, which was investigated in the Pb(II)-imidazolehydroxamic acid systems. The complex formation processes between the Pb(II) and imidazolehydroxamic acids starts at lower pH compared to the former hydroxamate systems investigated, in addition, the role of the imidazole-N was proved.

The formation of a complex, $[MAH_{-1}]_x$, with high stability could not be observed for Im-4-Cha, which is analogous to the α-Alaha, due to solubility problems.

According to the results, in *N*-Me-Im-4-Cha which is an analogue of the aminohydroxamic acid *N*-Me-α-Alaha, the fundamental effect of the imidazole-N have became proven, forming ($N_{imidazole}$,$O_{carbonil}$) coordination mode in the complexes below pH ~5.5 and ($N_{imidazole}$,$O_{hydroxamate}$) above that pH.

Im-4-Aha (analogous to β-Alaha) forms mainly hydroxamate type complexes but at higher pH the ligand coordinates in a tridentate ($N_{imidazole}$,$O_{hydroxamate}$,$O_{hydroxamate}$) manner.

4 POSSIBLE APPLICATION OF THE RESULTS

The aim of our studies was first of all exploring the interaction between the toxic Cd(II) or Pb(II) ions and hydroxamic acids. The importance of the hydroxamic acids are based on their good complex forming ability. This work, as a basic research focused mostly on the determination of stability, stoichiometry and structure of the hydroxamate complexes formed. These results can be used for biological studies, where the mobilization of these metal ions is in focus.

5 PUBLICATIONS

Articles connected to the thesis:

3. E. Farkas, D. Bátka, P. Buglyó and M. A. Santos: **Hydroxamic acids as potential lead(II) sequestering agents: Factors affecting lead(II) binding properties of hydroxamic acids,** *Metal Ions in Biology and Medicine, vol 9. eds: M. C. Alpoim, P. V. Morais, M. A. Santos, A. J. Cristovao, J. A. Centero, P. Collery. 2006.* (21th – 24th may, 2006, Lisboa, Portugal)

2. D. Bátka and E. Farkas: **Pb(II)-binding capability of aminohydroxamic acids: Primary hydroxamic acid derivatives of α-Amino acids as possible sequestering agents for Pb(II),** *J. Inorg. Biochem.,* **100** (2006) 27

1. E. Farkas, D. Bátka, Z. Pataki, P. Buglyó and M. A. Santos: **Interaction of desferrioxamine B (DFB) model dihydroxamic acids with some essential (Cu^{2+}, Ni^{2+}, Zn^{2+}) and toxic (Cd^{2+}, Pb^{2+}) metal(II) ions: effects of the structure and length of connecting chains on the metal ion selectivity,** *Dalton Trans.,* (2004) 1248

Article not detailed in the thesis

1. E. Farkas, D. Bátka, E. Csapó, P. Buglyó, W. Haase and D. Sanna: **Synthesis and Characterization of Cu^{2+}, Ni^{2+} and Zn^{2+} Binding Capability of Some Amino- and Imidazole Hydroxamic Acids: Effects of Substitution of Side Chain Amino-N for Imidazole-N or Hydroxamic-N-H for -N-CH_3 on Metal Complexation,** *Polyhedron*, **26** (2007) 543

Lectures and posters:

8. D. Bátka, E. Farkas (poster): **Effect of imidazole and amino nitrogen atoms of hydroxamic acid derivatives on lead(II) binding properties**, *X. International Symposium on Bioinorganic Chemistry*, 20th – 25th September, 2005, Szklarska Poreba, Poland

7. D. Bátka, E. Farkas (english lecture): **Lead(II) binding capabilities of hydroxamic acids,** *XXI. International Conference on Coordination and Bioinorganic Chemistry*, 5th – 10th June, 2005, Smolenice, Slovakia

6. D. Bátka, E. Farkas (lecture): **Az ólom(II) - aminohidroxámsavak közötti kölcsönhatás tanulmányozása (Study of the interaction between aminohydroxamic acids and Pb(II) ion),** *XL. Komplexkémiai Kollokvium (Colloquium on Coordination Chemistry),* 18th – 20th May, 2005, Dobogókő, Hungary

5. E. Farkas, D. Bátka, P. Buglyó and M. A. Santos (poster): **Factors affecting the metal binding ability of new model dihydroxamic acids: effect of the peptide moieties situating in the connecting chain,** *7th Europian Biological Inorganic Chemistry Conference,* 29th August – 2nd September, 2004, Garmisch-Patenkirchen, Germany

4. D. Bátka, E. Farkas (poster): **Interaction between new desferrioxamine B (DFB) model dihydroxamic acid and M(II) ions: effect of the complex geometry on the stoichiometry of the bis-chelated complex**, *IX International Symposium on Inorganic Biochemistry*, 4th-7th September, 2003, Szklarska Poreba, Poland

3. D. Bátka, E. Farkas (poster): **Interaction between new desferrioxamine B (DFB) model dihydroxamic acid and M(II) ions: effect of the complex geometry on the stoichiometry of the bis-chelated complex***, 28th International Conference on Solution Chemistry,* 23th – 28th August, 2003, Debrecen, Hungary

2. D. Bátka, E. Farkas (lecture): **Kadmium(II) és Ólom(II) toxikus nehézfémionok kölcsönhatása hidroxámsavakkal** (**Interaction between toxic heavy metal Cd(II) and Pb(II) ions and hydroxamic acids**), *XXVI. Kémiai Előadói Napok, (Young Chemist's Conference),* 27th – 29th October, 2003, Szeged, Hungary

1. D. Bátka, E. Farkas (lecture): **Kadmium(II) és Ólom(II) toxikus nehézfémionok kölcsönhatása hidroxámsavakkal** (**Interaction between toxic heavy metal Cd(II) and Pb(II) ions and hydroxamic acids**), *XXXVIII. Komplexkémiai Kollokvium (Colloquium on Coordination Chemistry)*, 19th - 22th May, 2003, Gyula, Hungary

Lectures and posters not detailed in the thesis:

3. D. Bátka, H. Csóka, E. Farkas (lecture): **Az imidazolcsoport szerepe a vas(III) – hidroxamát kölcsönhatásban (The role of the imidazol-moiety on the interaction between the iron(III) and hydroxamic acids),** *XLII. Komplexkémiai Kollokvium (Colloquium on Coordination Chemistry)*, 31th May – 2nd June, 2006, Mátrafüred, Hungary

2. E. Farkas, D. Bátka, É. A. Enyedy (poster): **Some factors affeting metal ion selecivity of natural siderophores, desferrioxamine B and desferriocoprogen**, *X. International Symposium on Bioinorganic Chemistry*, 20th – 25th September, 2005, Szklarska Poreba, Poland

1. É. A. Enyedy, D. Bátka, E. Farkas (lecture): **Az összekötő lánc hatása hidroxámsav alapú sziderofórok fémmegkötő sajátságára: a deszferriokoprogén és a deszferrioxamin B eredményeinek összevetése, (The effect of the connecting chain of the siderophores on the metal-binding ability: Comparison of the results for Desferrioxamine B and Desferricoprogen),** *XXXIX. Komplexkémiai Kollokvium (Colloquium on Coordination Chemistry)*„ 26th -28th May, 2004, Agárd-Gárdony, Hungary