



BIOLÓGIAILAG AKTÍV HIDROXÁMSAVAK ÉS MODELLJEIK
KÖLCSÖNHATÁSA
KÉTÉRTÉKŰ FÉMIONOKKAL

INTERACTION BETWEEN BIOLOGICALLY ACTIVE OR MODEL
HYDROXAMIC ACIDS AND BIVALENT METAL IONS

propositions of PhD thesis

Éva Anna Enyedy

University of Debrecen
Department of Inorganic and Analytical Chemistry

Debrecen, 2002.

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1. INTRODUCTION AND THE AIM OF THE WORK

It is well known that iron is an essential element for almost every living organism. Microbial siderophores are synthesised in order to solubilize and transport iron(III) into the cells in the required concentrations. Most of the hydroxamate type siderophores contain three metal binding groups, which are able to complete the octahedral coordination sphere of iron(III) forming very stable 1:1 complexes. It has been proved in the case of numerous species that the reduction of the metal centre of the iron(III)-siderophore complex in the cell plays an important role in the mechanism of iron release. Complexes of iron(II) formed by this reduction are more labile and less stable than those of iron(III). Consequently, iron(II) is more available for the cells. It means that characterisation of complexes formed between iron(II) and siderophores or different siderophore model hydroxamic acids may contribute to a better understanding of the above mentioned biological processes. However, only few studies on *complexation between iron(II) and hydroxamic acids* have been published previously. Therefore, the aim of this work is to reveal these interactions. The results have been compared with both those of iron(III) and the corresponding data of complexes formed with other $3d^{5-10}$ bivalent metal ions. These data were either available from the literature or in a number of cases, they were determined in the present work.

Besides serving as siderophores, hydroxamic acids have a wide spectrum of biological activities, *e.g.* antitumor, antibiotic activity and numerous hydroxamic acids and aminohydroxamic acids (derivatives of amino acids) are effective inhibitors of metalloenzymes such as urease, collagenase, 5-lypogenase. In the active centre of these enzymes bivalent transition metal ions *e.g.* nickel(II), zinc(II), copper(II), cobalt(II), manganese(II) can usually be found and the basis of the inhibition is the direct interaction between these metal ions and hydroxamic acids. Consequently, systematic equilibrium studies on complexation between hydroxamic acids and bivalent metal ions may give an insight into understanding of the inhibition. Moreover, investigations of the effects of substituents on the stability of hydroxamate complexes can help in the selection of a suitable hydroxamic acid inhibitor. This is the reason why we have studied *complexation of different mono and aminohydroxamic acids with the above mentioned bivalent metal ions*. Mixed ligand complexes are more adequate models for the more complicated biological systems. Since data for the formation of mixed ligand complexes of monohydroxamic acids cannot be found in

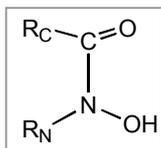
the literature, detailed investigations of *systems containing nickel(II), copper(II), or zinc(II), monohydroxamic acid and a secondary (B) ligand* have been performed.

2. EXPERIMENTAL

Different ligands such as monohydroxamic acids, aminohydroxamic acids, dihydroxamic acids and trihydroxamic acids have been studied. Out of the ligands dimerum acid, DFB, and coprogen are natural compounds. (Formulae for the hydroxamic acids can be seen on p.3)

Proton dissociation constants of the ligands, the stoichiometry and the stability constants of the complexes have been determined by pH-potentiometric or spectrophotometric techniques. For characterisation of the binding modes in the complexes UV-vis spectrophotometric, EPR, calorimetric methods have been applied. Since iron(II) can be easily oxidised to iron(III) by atmospheric oxygen in aqueous solution, a suitable equipment for equilibrium measurements under anaerobic conditions has been constructed. To reveal the nature of the redox reaction between iron(II) and trihydroxamic acids, pH-potentiometry, pH-stat measurements, spectrophotometry, gas chromatography, and capillary electrophoresis have been used.

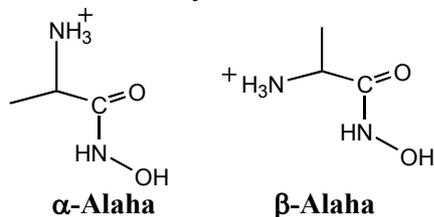
Monohydroxamic acids and analogue compounds:



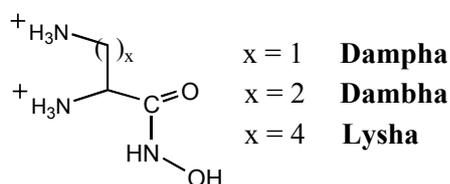
R_C :	R_N :	name:
-CH ₃	-H	Aha
-CH ₂ -CH ₃	-H	Pha
-(CH ₂) ₄ -CH ₃	-H	Hha
	-H	Bha
-CH ₃		MAha
-CH ₃		PhAha
		PYRha

Aminohydroxamic acids:

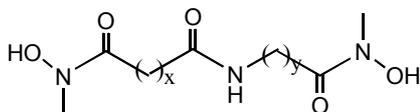
a. monoaminohydroxamic acids:



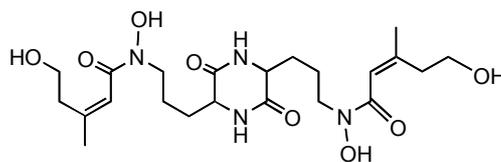
b. diamino hydroxamic acids:



Dihydroxamic acids:

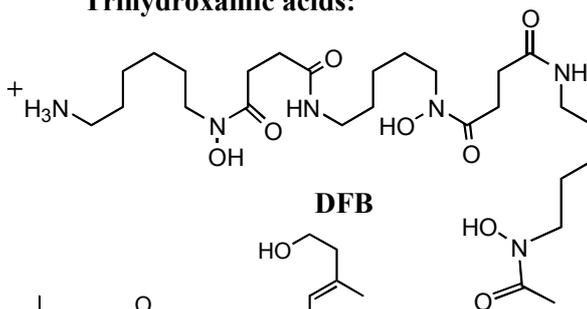


$x = 2, y = 5$ **2,5-DIHA**
 $x = 3, y = 4$ **3,4-DIHA**
 $x = 3, y = 3$ **3,3-DIHA**

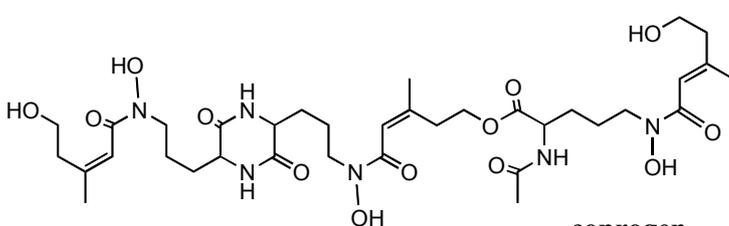


dimerum acid

Trihydroxamic acids:



DFB



coprogen

Formulae for the hydroxamic acids studied

3. NEW SCIENTIFIC ACHIEVEMENTS

1. Acid-base properties of the ligands

Proton dissociation constants of coprogen, dimerum acid and the synthetic 2,5-DIHA, 3,4-DIHA, and 3,3-DIHA have been not known before. The pK values determined for these ligands are in good agreement with the corresponding values for analogous compounds. Furthermore, in the case of a given di, or trihydroxamic acid the differences between the stepwise proton dissociation constants are somewhat larger than the statistical values showing a weak interaction between the hydroxamic acid groups. Considering the length of the connecting chain between the functional groups in these hydroxamic acids the interaction is probably realised through space but not through the chain.

2. Metal ion – hydroxamate interactions

2.1. Monohydroxamic acids

Equilibrium studies for complexation between nickel(II), copper(II) and zinc(II) and a series of monohydroxamic acids containing different R_C- and R_N-substituents have shown that the stability of the complexes follows the basicity order of the ligands. In other words, the electron withdrawing or donating effect of the substituents is not measurable in the case of bivalent metal ions which form lower stability complexes compared to iron(III). However, the heterocyclic PYRha forms more stable complexes in the fairly acidic pH range than it can be expected based on its proton dissociation constants, which can be explained by a more favoured charge distribution in the chelates due to the ring π - electrons.

It has been pointed out that in the copper(II)–Pha system the deprotonation of the coordinated hydroxamate in the bis complex above pH = 11 results in the formation hydroximato chelate, similarly to other primary hydroxamic acids, where the formation of hydroximato chelates was proved with EPR.

2.2. Ternary complexes of monohydroxamic acids

The formation of mixed ligand complexes between bivalent metal ions (nickel(II), copper(II) or zinc(II)), monohydroxamic acids and two donor atoms containing B ligands is favoured if the coordinating ligands have different types of donor atoms. Namely, the coordination of hydroxamate oxygens is favoured if B ligand has nitrogen donor atoms (*e.g.* ethylenediamine (en), or 2,2'-bipyridine (bipy)) and unfavoured if B ligand has oxygen donor

atoms (*e.g.* Tiron). It has been proved with spectrophotometry and EPR that in copper(II)–en/ or bipy–primary hydroxamic acid systems mixed ligand hydroximato complexes are formed at high pH.

Diethylenetriamine (dien) and 2,2':6,2''-terpyridine (terpy) B ligands occupy three of the equatorial positions of copper(II) and in the [CuAB] complex, which is formed in the copper(II)–dien/or terpy–monohydroxamic acid ternary systems in pH range 7.0-10.5, copper(II) is five-coordinated. It means that one of the donor atoms is coordinated at axial position, which is proved by the red shift of the electron absorption spectra parallel to the formation of this complex. At higher pH the replacement of hydroxamate by hydroxide is documented by both pH-potentiometry and spectrophotometry. The EPR results indicate that [Cu(AhaH.₁)₂]²⁻ bis hydroximato complex is formed only in the Cu(II)–terpy–Aha system above pH = 11, that is, the hydroxamic acid comes back to the coordination sphere replacing terpy. This phenomenon cannot be observed in systems containing the relatively flexible dien or secondary hydroxamic acids (which do not have dissociable –NH protons).

2.3. Aminohydroxamic acids

Amino groups can be found at α -, and β -positions in the aminohydroxamic acids studied. In addition to the hydroxamate type (O,O) binding mode, the coordination of amino and hydroxamate nitrogen donor atoms is also possible due to the presence of the amino group. According to the results these ligands coordinate as simple hydroxamic acids through the hydroxamate oxygens to iron(II), cobalt(II) and manganese(II).

If there is a second amino group at various positions in an α -aminohydroxamic acid (Dampha, Dambha and Lysha) only hydroxamate type complexes are formed with the above mentioned metal ions, similarly to the simple α -derivatives. However, the side chain amino group is an effective binding group for nickel(II) or copper(II) if it is at chelatable position, *e.g.* Dampha and Dambha. Nickel(II) forms only octahedral complexes with Dampha and the [Ni(Dambha)₂] complex is also octahedral, which is unusual in the case of aminohydroxamic acids, and this geometry suggests that Dampha and Dambha coordinate tridentatly. These two diamino hydroxamic acids can coordinate tridentatly to copper(II) when one of the axial positions is also occupied, or bidentatly when the side chain and the α position amino nitrogen donor atoms bind at the equatorial plane replacing the hydroxamate from the

coordination sphere. It has been pointed out that Dampha is a more effective chelator for nickel(II) and copper(II) at a wider pH range than for iron(III), although hydroxamic acids and also aminohydroxamic acids form more stable complexes with iron(III) than with the above mentioned bivalent metal ions.

2.4. Complexes formed between iron(II) and mono- or dihydroxamic acids compared to those of iron(III)

Iron(II)–hydroxamic acid systems have been studied under anaerobic conditions. Stability constants have been determined for the complexes formed in iron(II)–Aha, – α -Alaha, – β -Alaha, –Dambha, –Lysha, –dimerum acid, –2,5-DIHA, –3,4-DIHA, –3,3-DIHA systems and have been compared to those of the other $3d^{5-10}$ bivalent metal ions. The stability order corresponds to the Irving-Williams series. The stability constants of iron(II) complexes are always much lower than those of iron(III), and the difference between the logarithm of the constants of each hydroxamate chelate of iron(II) and iron(III) is *ca.* 6.

Complexation of dimerum acid, 2,5-DIHA, 3,4-DIHA, 3,3-DIHA and coprogen has been investigated also with iron(III) in the present work.

A study of the complexes of iron(II)/or iron(II) formed with the natural dimerum acid has been shown that double bounds at β position to the hydroxamate residues in the ligand result in higher stability of mono complexes ([FeAH]) due to the electron donating effect. In bis and tris complexes, however, this effect is compensated by the steric effect of the bulky piperazine-2,5-dione ring.

Among the synthetic dihydroxamic acids (2,5-DIHA, 3,4-DIHA, 3,3-DIHA) the arrangement of the connecting chain between the two hydroxamic acid groups, *e.g.* the position of the peptide bond, is the most favoured in 2,5-DIHA, since the stability of the [FeA] complexes in which both hydroxamates of the ligand are coordinated is higher in the case of 2,5-DIHA than with 3,4-DIHA, 3,3-DIHA. 2,5-DIHA has the same special connecting chain like the natural DFB has.

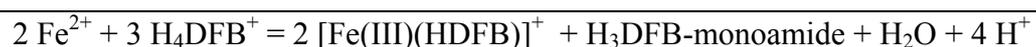
2.5. Complexes of coprogen formed with some transition metal ions

Complexation processes between coprogen and iron(III), nickel(II), copper(II), zinc(II) have been also studied. Results have been compared to those of DFB. Although, the structures of DFB and coprogen are different, the stoichiometry and the stability of the complexes of coprogen are similar to those of DFB. The stability increasing effect of the β -keto bonds is

probably compensated by the steric effect of the piperazine-2,5-dione ring as in the case of dimerum acid.

2.6. Interaction between iron(II) and trihydroxamic acids

It has been observed at the first time that DFB and coprogen oxidise iron(II) to iron(III) under strictly anaerobic conditions while the trihydroxamic acid is reduced. The possibility of the oxidation by water could be precluded by gas chromatography measurements. In the case of DFB the reaction equation has been determined by spectrophotometric and pH-stat measurements at pH = *ca.* 6. In addition to the stoichiometry of the redox reaction, the dependence of the reaction rate on pH has also been studied. According to these kinetic measurements, the reaction proceeds only in a given pH range (pH ~5,5-8,5) and the reaction rate is the highest at physiological pH. It has been suggested that $[\text{Fe(II)H}_3\text{DFB}]^{2+}$ mono complex acts as the reactive species in the reaction.



4. SOME ASPECTS OF POSSIBLE APPLICATIONS OF THE RESULTS

The main goal of this work has been to reveal the interaction between some bivalent metal ions or iron(III) and different hydroxamic acids in aqueous solution. Results may give useful contribution to understanding certain biological processes or can be applied for planning new synthetic hydroxamic acids, since any use of these compounds, which have strong complex forming ability, is connected to their metal complexes. Therefore investigations of the effects of substituents or the side chain nitrogen donor atoms of aminohydroxamic acids on the stability of hydroxamate complexes can help in the selection of a suitable hydroxamic acid inhibitor of metalloenzymes.

On the basis of the results we have concluded that the redox reaction between iron(II) and trihydroxamic acids may play an important role in microbial iron uptake under anaerobic conditions.

5. PUBLICATIONS:

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5. E.Farkas, P.Buglyó, É.A.Enyedy, V.Gerlei, A.M.Santos
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6. POSTERS AND LECTURES:

13. É. A. Enyedy, E. Farkas, I. Pócsi (poster)

Complexation of a natural siderophore, coprogen produced by *Penicillium chrysogenum* fungus

10th International Conference on Bioinorganic Chemistry, 2001.08.31-26., Florence, Italy

12. E. Farkas, P. Buglyó, É. A. Enyedy, V. Gerlei and A.M.Santos (poster)

Syntheses and metal ion binding strength of desferrioxamine B model dihydroxamic acids

10th International Conference on Bioinorganic Chemistry, 2001.08.31-26., Florence, Italy

11. É.A.Enyedy, E.Farkas (lecture)

A *Penicillium chrysogenum* gomba által szintetizált természetes sziderofór, a koprogén fémmegkötő sajátosságának vizsgálata

XXXVI. Komplexkémiai Kollokvium, 2001.05.23-25, Pécs, Hungary

10. É.A.Enyedy, E.Farkas (poster)

Effects of side chain imidazole and amino nitrogen donor atoms on complexation of aminohydroxamic acids with 3d bivalent metal ions

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2. É.A.Enyedy, E. Farkas (lecture)

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1. É. A. Enyedy, E.Farkas (lecture)

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