Thesis points of PhD dissertation

Copper(II)-, zinc(II)- and nickel(II)-complexes of imidazole containing ligands and inositol derivatives: equilibrium, structure, hydrolytic activity

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1. Introduction: the aim of the work

Small molecular model compounds are extensively applied to imitate the active sites of biomolecules. Coordination chemical and kinetic studies of low molecular weight metal complexes contribute to understand better the structural and functional properties of metalloproteins and metalloenzymes. These investigations are not only simpler and cheaper than those of the native macromolecules but provide also an opportunity for developing so called artificial enzymes by means of highly active model systems. Molecular structure of the active centres of metalloproteins are fundamentally determined by coordination of side-chain donor groups. In this respect the imidazole ring is one of the most important residues because of its versatility offering an extremely stable metal-binding site in physiological pH range. Imidazole moiety can be found in almost all copper(II)- and zinc(II)enzymes, its presence is often related to hydrolytic reactions even in absence of metal ions. One of the most abundant classes of hydrolytic enzymes are phosphatases, which hydrolyse phosphate ester bonds and are involved in a number of biological processes. Besides crystallographic and kinetic studies of these native enzymes, low molecular weight functional models have attracted great interest due to their applications in biotechnology and chemotherapy.

In the present Ph.D. work copper(II)-, zinc(II)- and nickel(II)-complexes of several imidazole containing ligands and some methylated derivatives of 1,3,5-trideoxy-1,3,5-triamino-*cis*-inositol ensuring rather rigid, preorganised structures have been studied. Imidazole-based ligands having very different basicity and metal binding ability are good models for studying the coordination behaviour of the imidazole ring in the presence of varied donor groups and substituents. Most of the systems have been investigated by solution equilibrium and structural methods. In case of some imidazole derivatives ternary complex formation was studied with catecholamines which play an essential role in various neurological and biochemical processes. Kinetic measurements have been performed at different pH, concentrations and metal-to-ligand ratios in those systems which proved to be highly active on the cleavage of phosphate esters. Kinetic data combined with the equilibrium and structural results allowed to identify the active species and to draw mechanistic conclusions. The aim of the present work were the followings:
– description of the solution equilibrium speciation of metal complexes
– structure determination of species formed by means of several solution structural methods
– kinetic analysis of model systems towards hydrolysis of phosphate esters

2. Experimental methods applied for the investigations

Solution equilibria, speciation and composition of species formed have been studied by pH-metric and combined pH-metric/spectrophotometric methods. Experimental data have been evaluated by a computer program PSEQUAD.

Several solution structural methods (UV/VIS-, EPR-, CD-, NMR-spectroscopy) have been applied to characterise the solution structure of metal complexes. EPR-spectroscopy have been used to get information on copper(II)-complexes (e.g. number of nitrogen atoms, structure distortion etc.) and to identify dinuclear species. ^{1}H NMR measurements have been performed to characterise the zinc(II)-complexes. This method proved to be very useful in case of slow ligand exchange (as compared to the NMR time-scale).

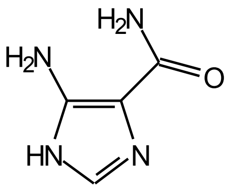
The hydrolysis of activated and nonactivated phosphate esters have been followed by spectrophotometry and HPLC techniques respectively. Spectrophotometric experiments were based on the appearance of p-nitrophenolate ion at 400 nm which is the hydrolytic product of the activated esters. In case of the nonactiveted esters reversed phased HPLC have been applied to separate the original substrate and the products and their quantity was monitored at 260 nm.

3. New scientific results

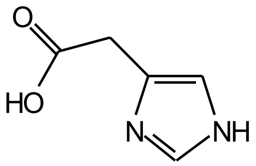
1. All of the three bidentate imidazole-containing ligands (aic, iaa, biim) form ML and ML_2 parent complexes with copper(II)-, zinc(II)- and nickel(II)ions. In spite of the low basicity of the imidazole donor groups, the ligands are able to form particularly stable complexes due to the formation of five- or six-membered chelate ring and the strong π-acceptor property of the imidazole ring. In the copper(II)–iaa system further deprotonations were observed at higher pH, which resulted in a formation of oligomer complexes.
2. The biib ligand, which contains bis-imidazolyl unit, offers extremely stable bi-, tri- and tetradentate coordination to copper(II) in its mono- and dinuclear complexes. In equimolar solution two major species (CuLH és Cu_2L_2) are formed and in case of metal excess a dinuclear Cu_2LH_{-1} complex is dominant. The low temperature EPR spectrum of the CuLH complex were assigned to the two coexisting binding isomers having identical compositon but different structures. The two dinuclear complexes show magnetic interaction between metal centres. The EPR spectra of the Cu_2L_2 is a characteristic triplet with high zero-field splitting, which indicates symmetric structure with indirectly coupled metal centres. The biib is a tripodal ligand and according to the results the coordination of the four donor groups to one metal ion can be excluded because of steric hindrance. However the structure of the ligand is favourable to the formation of dinuclear species.
3. The tripodal, tetradentate bimha containing also three imidazole rings forms mono- and bis-complexes with different protonation states, but in the zinc(II)–bimha system only mono-complexes have been detected. Most of these species possess (distorted) octahedral geometry, but pentacoordinated structure is preferred in the ML complexes. The CuL presumably has square pyramidal geometry, while trigonal bypiramidal structure is proposed for ZnL. The geometrical difference between the ML complexes can help to explain the diverse behaviour in terms of bis-complex formation. The square pyramidal CuL complex, with the bridgehead nitrogen in one of the basal positions, seems to transform easily into an octahedral species through the substitution of an equatorial imidazole nitrogen by the second ligand. The trigonal bypiramidal ZnL species, with the bridgehead nitrogen in the apical position, is less susceptible to the coordination of a second ligand.
4. The two studied structurally similar tripodal ligands (biib and bimha) have basically different coordination chemical behaviour. The bimha has longer arms than the biib and thus the four donor groups are able to coordinate to one metal ion, too.
5. The linear tetradentate bimdm and mbimdm form extremely stable ML complexes with copper(II)- and zinc(II)ions in wide pH range having $\{2N_{im}, 2N_{am}\}$ coordination. In the presence of both metal ions further deprotonation can be observed above pH 9, which results water soluble MLH_{-1} species only in case of mbimdm. The ZnL(OH) complex of mbimdm proved to be active towards the hydrolysis of activated bis(4-nitrophenyl)phosphate. The metal complexes of the bimdm and mbimdm form $MLAH_2$, MLAH and MLA mixed complexes with catecholamines. In these mixed complexes catecholamines are coordinated in a catechol like bonding mode with the exception of the $MLAH_2$ complex of L-dopa, where the aminoacid type coordination is dominant. In the copper(II)-containing mixed complexes at least three nitrogens of tetradentate ligands

bound to metal ion, while in case of zinc(II) presumably all of the four nitrogens are coordinated.

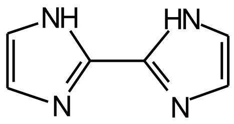
6. The hexadentate dhen containing histamine like donor sets form considerably stable dinuclear M_2L_2 complexes with copper(II)- and zinc(II)ions having bis(histamine-like) coordination in the psychological pH. The MLH_{-2} complex, also formed in both systems above pH 10, has different structures with the two metal ions. A presumably hydroxo mixed-ligand complex is formed in case of zinc(II), while the base-consuming processes are assigned to metal-promoted deprotonation of amide nitrogens in the copper(II) containing system. Between these two dominant species tetranuclear, cyclic oligomer complexes are formed in both case, as suggested by the EPR, CD and NMR results, in which the four metal ions are bounded by bidentate imidazolate anions. The hydrolytic activity of the zinc(II)–dhen system towards the hydrolysis of 2',3'-cUMP is constant in wide pH range and increases only at higher pH.
7. The zinc(II)–histamine complexes efficiently hydrolyse the non-activated 2',3'-cUMP as opposed to our expectations. Kinetic data, obtained at different pH, concentrations and metal-to-ligand ratios combined with the concentration distribution of five species provided by equilibrium data have been used to determine the individual contributions of all species to the hydrolysis rate. Not only the ZnL(OH) complex, but also the Zn(OH) formed in small amount is highly active towards the hydrolysis of the 2',3'-cUMP.
8. The copper(II)–tdci system was proved to be extremely active towards the hydrolysis of the examined two activated DNA model substrate – 2,4-dinitrophenyl-ethyl-phosphate and bis(4-nitrophenyl)phosphate (bnpp). The second-order rate constant is ($0.95\ M^{-1}s^{-1}$) is ca. 47600-fold higher than that of the hydroxide ion catalysed hydrolysis and the rate acceleration is $3{,}5\times10^7$-fold compared to the autohydrolysis in the presence of 4 mM dinuclear complex at pH 8.6. On the basis of equilibrium and kinetic data the dinuclear Cu_2LH_{-3} complex is responsible for the hydrolytic activity. The hydrolysis was proved to be catalytic and selective for the phosphodiester bnpp. The proposed bifunctional mechanism of the hydrolysis includes double Lewis acid activation and intramolecular nucleophilic catalysis. The Cu_2LH_{-3} species is more active for bnpp hydrolysis than any other divalent metal complex as well as most of the lanthanide(III)-complexes.
9. The copper(II)–tmci system is extremely active for the hydrolysis of 4-nitrophenyl-phosphate (npp) and selective for the phosphomonoester because it was proved to be inactive towards the hydrolysis of the diester bnpp. Only few metal containing systems are able to accelerate the hydrolysis of npp having two negative charges in the psychological pH range owing to the significant electrostatic repulsion hindering the nucleophilic hydroxide. Similarly to the copper(II)–tdci system the hydrolytic activity is also assigned to a dinuclear complex. The initial rate – substrate concentration profile refers to inhibition, which is well-known in case of enzymes, but remarkably rare in terms of model complexes. The catalytic rate constant is about 200000-fold higher than the autohydrolysis of npp at pH 8 and this data is much more higher than those of the metal complexes of similar bivalent metal ions.
10. According to the results imidazole derivatives are good structural models, but only moderately active functional models. The hydrolytic activity of the two inositol compounds is extremely high and they are selective for the hydrolysis of phosphate mono- or diesters.



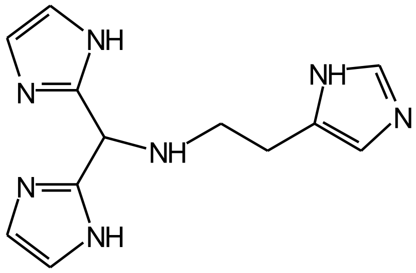
aic



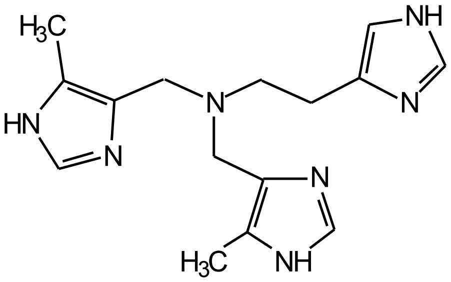
iaa



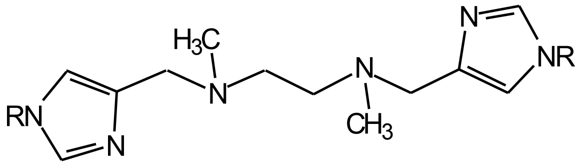
biim



biib

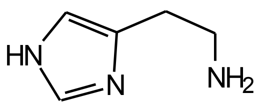


bimha

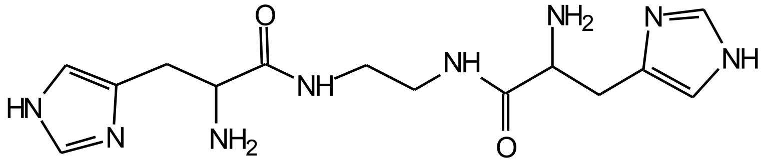


(1) bimdm R = H

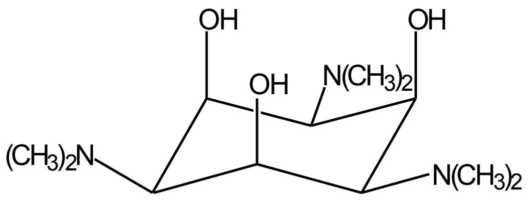
(2) mbimdm R = CH_3



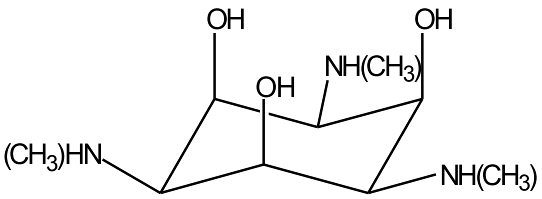
ha



dhen



tdci



tmci

Structure of the studied ligands

Publications related to the subject of the dissertation

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2. **I. Török**, T. Gajda, B. Gyurcsik, G. K. Tóth, A. Péter, "Metal complexes of imidazole-ligands containing histamine-like donor sets: equilibrium, solution structure and hydrolytic activity", *J. Chem. Soc. Dalton Trans.,* 1998, 1205 (Imp. fakt.: 1,955)

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Other publications

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5. J. Costa Pessoa, T. Gajda, S. M. Luz, T. Kiss, J. J. G. Moura, **I. Török,** ”The system Gly-L-Asp+VO^{2+} and related ligands; a potentiometric and spectroscopic study”, *ICBIC-8,* 27 July - 1 August 1997, Yokohama, Japan

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10. **I. Török**, T. Gajda, ”Metal complexes of imidazole containing ligands: equilibrium, solution structure and hydrolytic activity”, *XXXIII. International Conference on Coordination Chemistry,* 30 August - 4 September 1998, Florance, Italy

11. Jancsó A., **Török I.**, Gajda T., ”A donorcsoportok számának és elhelyezkedésének hatása poliimidazol ligandumok átmenetifém-komplexeinek szerkezetére és stabilitására”, *XXXIV. Komplexkémiai Kollokvium,* 1999. május 19-21. Tata

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15. T. Gajda, A. Jancsó, **I. Török**, K. Hegetschweiler, H. Sirges, ”Nuclease activity of some alkoxo-bridged copper(II) and zinc(II) complexes”, *5th Europian Biological Inorganic Chemistry Conference*, 17-20 July 2000, Toulouse, France

16. **I. Török**, T. Gajda, K. Hegetschweiler, ”Highly efficient and selective hydrolysis of phosphate esters promoted by dinuclear copper(II) complexes”, *5th Europian Biological Inorganic Chemistry Conference,* 17-20 July 2000, Toulouse, France