THESIS OF THE DOCTORAL (PHD.) DISSERTATION

FINE-TUNING OF THE ENVIROMENT OF THE METAL ION BY USING TRIPODAL LIGANDS

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Szeged, 2019

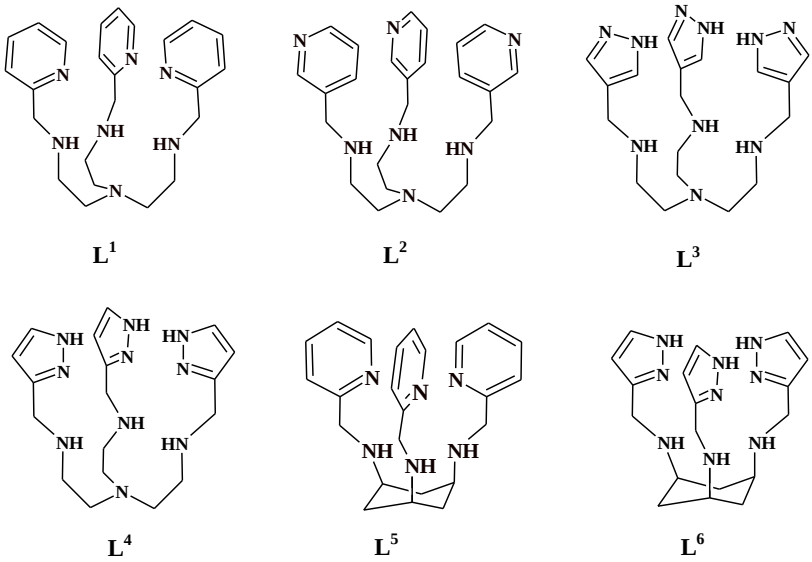
I. INTRODUCTION

Enzymes are special proteins, whose primal role is the catalysis of biochemical processes in the living body. About third of them has one of more metal ions in the structure, so called metalloenzymes. The function of the metal ion can be structural and/or catalytical. One of the important challanges in modern bioinorganic chemistry is the understanding of the operation of these enzymes, what can be greatly assisted by the structural/functional modelling of the active centers by small molar weight complexes. These investigation can also result in catalysts, what can be used in practice. The position and geometry of the metal ion(s) in the enzymes is highly fixed by the tertiary structure of the protein what can be really hard to mimic with linear polidentate ligands. This problem can be helped by the use of preorganized, so-called tripodal ligands. Tripodal ligands are organic molecles, what consists of a central part and three „legs", what has donor groups in them. Suitable funcionalization of simple tripodal pratfomrs can lead to increased metal ion affinity or the potential to bind two or more metal ions.

II. OBJECTIVES

The topic of my dissertation is the investigation of the substituted derivatives of two simple tripodal platforms (tris(aminoethyl)amine (tren) and 1,3,5-triaminocyclohexane (tach)). The goal was to explore the effect of the variation of the platform itself and the subtituents to the coordinational chemical properties of the ligands and the possibilities of multinuclear complexes. Thus four tren-based (N,N',N''-tris(2-pyridylmethyl-aminoethyl)amine (trenpyr, L1); N,N',N''-tris(3-pyridylmethyl-aminoethyl)amine (tren3pyr, L2) N,N',N''-tris(4-pyrazolylmethyl-aminoethyl)amine (tren4pyz, L3); N,N',N''-tris(5-pyrazolylmethyl-aminoethyl)amine (trenpyz, L4), 1. figure), and two tach-based (N,N',N''-tris(2-pyridylmethyl)-1,3,5-cis,cis-triaminocyclohexane (tachpyr, L5) és N,N',N''-tris(5-pyrazolylmethyl)-1,3,5-cis,cis-triamino-cyclohexane (tachpyz, L6), 1. figure) polidentate tripodál ligand was prepared and the coordinational chemical and enzyme-mimetical properties were studied

During these investigation the structure and stability of the Mn(II), Fe(II), Co(II), Cu(II) és Zn(II)-complexes of the ligands were determined. The protonation constants of the ligands and the formation constants of the complexes were determined with pH-potentiometry and in the case of coloured complexes, UV-Vis spectrophotometry. The structure of the formed comlexes were verified with mass spectrometry. Complex structure was determined by using UV-Vis, EPR and NMR spectroscopies and – if suitable crystac could be grew – X-ray diffractometry. The enzyme-mimicing abilites were determined using 3,5-ditertbuthyl-catechol oxidation model reaction



1. figure: Structure of the ligands presented in the dissertation

III. INVESTIGATION METHODS

Synthesis of the ligands

The general process of the synthesis of the ligands were the folowing: Schiff-base condensation between the tripodal platform and the respective carboxaldehyde, followed by the reduction of the intermedier. Further purification included extraction, hydrochloride salt formation, and if needed, preparative HPLC.

pH-potentiometry

Our pH-potentiometry studies were carried out in water, at 25 °C with 0,10 M (NaCl) ionionic strength under argon atmosphere. The determination of the protonation constants of the ligands (pK_a) and the formation constants of the complexes ($\lg\beta$) was carried out using PSEQUAD software.

UV-Vis spectrophotometry

Our potentiometry studies were Potenciometriás méréseinket a a fémion d-d átmeneteinek, esetleg a fém-ligandum töltésátviteli sávok pH-függő vizsgálatával egészítettük ki. A potenciometriás és spektrofotometriás mérések együttes kiértékeléséből a komplexek stabilitási állandóit számítottuk ki a PSEQUAD programmal, a sávok alakja és helyzete ugyanakkor értékes információkat szolgáltatott a képződő komplexek szerkezetére nézve is.

EPR-spectroscopy

Important information can be gathered from the structure of the copper(II)-complexes using EPR-spectroscopy, so the spectra of these type of complexes of the ligands in the dissertation were recorded at both room temperature and 77K. The evaluation of the spactra was performed by Dr. May Nóra, researcher of the MTA TTK.

^{1}H NMR spektroscopy

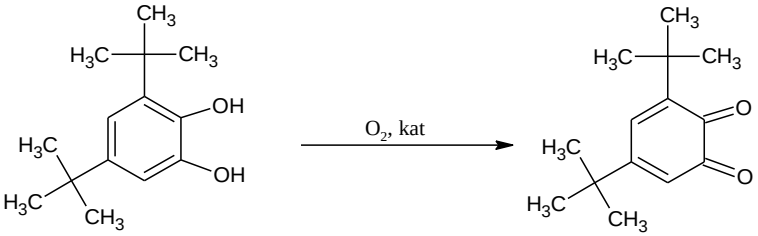
To confirm the speciation of the ligands and their zinc(II)-complexes, and to gain insight into the laters' structure, pH-dependent ^{1}H-NMR spectra were recorded. Samples contained 10% (V/V) D_2O-t and WATERGATE pulse sequence was used.

X-ray diffractometry

One of the most informative structural determination method is the single crystal X-ray difftactomerty, so if suitable crystal could be grew from the ligands or their complexes, diffractograms were recorded. The measurements and the evaluation were performed by Dr. Attila Bényei, Dr. Éva Kováts és Dr. Nóra May végezték.

Measurment of catechol oxidase activity

The catechol oxidase enzyme, what is widely investigated and modeled, catalysis the oxidation of orto-diphenols to quinones with molecular oxygen. The oxidised for of the enzyme contains to hydroxo-bridged copper(II)-centers. The model reaction applied was the oxidation of 3,5-ditertbuthyl-catechol (H_2dtbc) resulting di-tertbuthyl-o-kinon keletkezik (2. ábra). Subsequent oxidation of the substrate doesn't follow caused by the bulky electron donating groups.



2. ábraA 3,5-ditercbutil-pirokatechin dioxigén általi oxidációja

IV. NEW SCIENTIFIC RESULTS

1. A clear relation was determined between the ligands' protonations constants and the substituents' quality and position

The studied ligands goes through 3-6 deprotonation steps in the studied pH-range. 3 of these processes were assigned to the secondary nitrogens of the platforms, the others to the nitrogens of the aromatic rings. The tertiary nitrogen of the tren subunit never goes through protonation in our pH-range. It can generally be said that the pKs of both the aromatic and amino nitrogens of the substituted derivatives were lower then in the base molecules, and the position of the nitrogens in the ring had essential effect on this decrease. This can be attributed to numerous factors, the electron withdrawing effect of both the platform and the aromatic ring (this effect is different depending on the position of the pyridine nitrogen relative to the platform) and the position of the heteroatom may enable intramolecular H-bonds

2. The ligands, where the aromatic nitrogen is not in chelate-forming position creates only mononuclear complexes in solution, however in solod phase 3D-polimer structures were also detected

The tren3pyr és tren4pyz ligands form 4N, ML composition complexes in neutral pH-range in water, where the coordination mode is tren-like trignonal bipyramidal. On higher pH after one extra deprotonation step mixed hydroxido complexes are formed with the composition ML(OH). Stability of the complexes follows the Irving-Williams order. These ligands may form polymer complexes in the presence of copper(II), and a single crystal could be obtained from a MOF (Metal organic Framework)-like complex of tren3pyr. There are 3 copper(II)-ion enviromens in this polymer structure (i) there is a metal ion bound to the tren-like binding place of every ligand (ii) one pyridine nitrogen of four different ligand is bound to a copper(II)-ion, forming a Cu6L4 subunit with big inner cavity. (iii) and three of these subunits are connected via tetrahedrally coordinated copper(II)-ions.

3. Correlation was established for the complexes of the ligands, where there is one nitrogen in chelate forming position for a leg between the relative stability and the structure of the complexes

The trenpyr and tachpyr ligands forms highly stable monocomplexes with copper(II), zinc(II) and manganese(II). Though the stability of the complexes follow the Irving-Williams order, the pKs of the MHL complexes resulted some interesting observations. The pKs of the MH(tachpyr) complexes resulted expected differences: M = Cu(II) < Zn(II) < Mn(II), however the order was reversed for the MH(trenpyr) complexes. The explanation can be found at the different denticity of the ligands and the different geometry of their complexes. For tachpyr the MH(tacpyr) are always

5N, and during the deprotonation the count of the donor atoms increase by one, so this process is always favoured. In contrast, the coordination number for copper(II) and zinc(II) stays the same for the trenpyr complexes (5 and 6 respectively), so the amino nitrogen replaces an already coordinating nitrogen in the coordination sphere, so the process is less favoured and the pK values for CuH(trenpyr) and ZnH(trenpyr) are much higher then for their respective tachpyr complexes. For the three complex, the lowest pK is of the MnH(trenpyr), contary to the lowers Lewis-acidity of the metal ion. This can be explained by the increase of the coordination number of the metal ion from six to seven.

4. It was found out, that the ligands, where there are two nitrogen for a leg, and one is in chelate forming position creates mono- and trinuclear comlplexes with high stability. The structure and the factors that effects thermodynamical stability were determined

Both tachpyz and trenpyz forms highly stable monocomplexes with copper(II). In the copper(II)-tachpyz 1/1 system above pH = 8 the formation of the trinuclear $Cu_3H_{-4}L_2$ was found, what suggests the extremely high thermodinamical stability of this complex. For both ligands at M/L = 3/2 ratio the formation of trinuclear complexes were found with the composition of $Cu_3H_{-x}L_2$ (x = 2-4). The structure of these complexes are similar. We were able to recrystallize the $Cu_3H_{-4}(tachpyz)_2\times 2ClO_4\times 2H_2O$ complex. From XRD studies it was determined that the complex is linear trinuclear, where the metal ions are bound with pirazolate bridges and the Cu(II)-Cu(II) distance is about 3.8 Å. The enviroment of the terminal copper ions are distorted tetragonal pyramidal due to the Jahn-Teller effect. The central copper is bound by four pyrazolate-rings in tetrahedral geometry. Although the composition of the complexes are similar for trenpyz, the relative stabilities of these species are quite different due to the denticity of their respective platforms. Due to the two free pirazolate rings in the Cu(trenpyz) complex the twice deprotonated trinuclear complex has extra stability. Party because there are already enough donor groups to strongly (6N) bind the central copper ion, on the other hand amino groups has to leave the coordination sphere of the terminal copper ions for the formation of new pirazolate bridges. Therefore the formation of pretty similar trinuclear complexes can be shifted by 2-3 pH-units.

5. Relation was determined between the catechol-oxidase activity of the trinuclear complexes and the quality of the tripodal platform

Our kinetic studies showed that the monocomplexes of trenpyz and tachpyz didn't enhance the speed of the oxidation of H_2dtbc, on the other hand the $Cu_3H_{-x}L_2$ complexes were very efficient catalizators. The differences of their formation, what was previosly mentioned were present in the kinetic studies also. The pH-profile of the catechol oxidase activity resulted in a distorted bell-curve in both cases, but the

pH-optimum was quite different (pH = 5,7 for tachpyz, what is the lowest result in the literature so far and pH = 7,3 for trenpyz), what corresponds with the difference in their speciation. We proved that some enzime-mimetic effects of the complexes, like the pH-optimum can be fine-tuned with changing the tripodal platform. However at optimal pH, both system has outstanding activity, and – ensued by the similar structure – almost the same activity.

V. LIST OF PUBLICATIONS

Publications what the dissertation is based upon ΣIF: 11,127

1. Attila Szorcsik, Ferenc Matyuska, Attila Bényei, Nóra V May, Róbert K Szilágyi, Tamás Gajda: A novel 1,3,5-triaminocyclohexane-based tripodal ligand forms a unique tetra(pyrazolate)-bridged tricopper(II) core: solution equilibrium, structure and catecholase activity

Dalton Transactions, 45, 2016, 14998-15012, IF: 4,029, Citations: 9

2. Ferenc Matyuska, Attila Szorcsik, Nóra V. May, Ágnes Dancs, Éva Kováts, Attila Bényei and Tamás Gajda: Tailoring the local environment around metal ions: a solution chemical and structural study of some multidentate tripodal ligands

Dalton Transactions, 46, 2017, 8626-8642, IF: 4,029, Citations: 7

3. Ferenc Matyuska, Nóra V May, Attila Bényei and Tamás Gajda: Control of structure, stability and catecholase-like activity by the denticity of tripodal platforms

New Journal of Chemistry, 41, 2017, 11647-11660, IF: 3,069

Lectures, posters about the topic of the dissertation:

1. F. Matyuska, Á. Dancs, A. Szorcsik, Nóra V May, T. Gajda: Metal ion binding of some multidentate tripodal ligands, ICCBIC24, 2013
2. Ferenc Matyuska, Tamás Gajda, Nóra V May: Metal ion complexes of a TREN-based tripodal ligand , ICBIC16, 2013, poszter
Attila Szorcsik, Ferenc Matyuska, Ágnes Dancs, Tamás Gajda: Metal ion binding of TACH-based multidentate tripodal ligands, EuroBIC12, 2014, poszter
3. Tamás Gajda, Ágnes Dancs, Ferenc Matyuska, Attila Szorcsik, Péter Boros, Vanessza Dokonpil, Adrián Traj: Metal ion complexes of some tren- and tach-based tripodal ligands, Internationan Symposium on Metal Complexes, 2015
4. Tamás Gajda, Ágnes Dancs, Attila Szorcsik, Ferenc Matyuska, Nóra V May, Attila Bényei: Tuning the metal binding properties by derivatization of tripodal platforms, EuroBIC13, 2016
5. Ferenc Matyuska, Nóra V May, Attila Szorcsik, Róbert K Szilágyi, Tamás Gajda: Transition metal complexes of pyridine-substituted TREN-based tripodal ligands, EuroBIC13, 2016, poszter

6. Attila Szorcsik, Ferenc Matyuska, Nóra V May, Tamás Gajda, Transition metal complexes of TACH-based polydentate tripodal ligands, EuroBIC13, 2016, poszter

7. Ferenc Matyuska, Attila Szorcsik, Nóra V May, Ágnes Dancs, Éva Kováts, Attila Bényei, Tamás Gajda: Transition metal complexes of mono- and trisubstituted tren-derivatives, ICCBIC26, 2017

8. Ferenc Matyuska, Attila Szorcsik, Nóra V May, Éva Kováts, Attila Bényei, Tamás Gajda: Tailoring the local environment around metal ions by multidentate tripodal ligands, ISMEC 2017

9. Matyuska Ferenc, May Nóra Veronika, Gajda Tamás: Egy tren-alapú tripodális ligandum átmenetifémkomplexeinek vizsgálata, KKK, 2013

10. Szorcsik Attila, Matyuska Ferenc, Nagy Nóra Veronika, Gajda Tamás: Triamino-ciklohexán alapú tripodális ligandumok előállítása és komplexeinek vizsgálata, KKK, 2013

11. Matyuska Ferenc, Dokonpil Vanessza, Gajda Tamás: TREN-alapú tripodális ligandumok komplexképző sajátságainak összehasonlítása, KKK, 2014

12. Szorcsik Attila, Matyuska Ferenc, Dancs Ágnes, Gajda Tamás: TACH alapú tripodális polidentát ligandumok réz(II) és cin(II) komplexei, KKK, 2014

13. Matyuska Ferenc, Traj Adrián, May Nóra Veronika, Gajda Tamás (SzTE): Pirazol-szubsztituált TREN-származékok átmenetifém komplexei, KKK, 2015

14. Matyuska Ferenc, May Nóra V., Szorcsik Attila, Szilágyi Róbert K., Gajda Tamás: Piridin-szubsztituensek számának és a nitrogén helyzetének hatása tripodális ligandumok komplexképzésére, KKK, 2016

15. Matyuska Ferenc, May Nóra Veronika, Selmeczi Katalin, Gajda Tamás (SzTE): Új eredmények a trenpyz-réz(II) kölcsönhatásban, KKK, 2017

Publication related to the dissertation, but not included in it:

1. Ágnes Dancs ; Nóra V May ; Katalin Selmeczi ; Zsuzsanna Darula ; Attila Szorcsik ; Ferenc Matyuska ; Tibor Páli ; Tamás Gajda: Tuning the coordination properties of multi-histidine peptides by using a tripodal scaffold: solution chemical study and catechol oxidase mimicking
New Journal of Chemistry, 41, 2017, 808-823.

2. Gajda Tamás ; Szorcsik Attila ; Dancs Ágnes ; Matyuska Ferenc
Polidentát tripodális ligandumok biomimetikus fémkomplexei
Magyar Kémiai Folyóirat – Kémiai Közlemények, 123, 2017, 94-100