



Complexation properties of the triaza, tetraaza and diaza-tetraoxa macrocyclic ligands containing acetyl-glycinate functional groups. Kinetic behaviour of lanthanide(III)-DOTA-tetraamide derivative complexes

PhD Thesis Abstract

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I. Introduction and research objectives

During the last 25-30 years the interest in the coordination chemistry of rare earth significantly increased because of the successful use of their polyaza-polycarboxylate and -polyphosphonate complexes in medical diagnosis and therapy.

There was a great progress in the use of some radioactive isotopes in the diagnosis and therapy of cancer. The complexes of ^{90}Y and ^{177}Lu formed with some DOTA derivatives, attached to monoclonal antibodies or to their fragments, are used for the treatment of cancer. The EDTMP complex of ^{153}Sm and DOTP complex of ^{166}Ho are successfully used as palliative agents in the case of bone metastases. Another fast developing field is the use of the complexes of Eu^{3+} and Tb^{3+} as fluorescent probes in biology and immuno-analysis, where the determination of biologically important molecules can be carried out with high sensitivity ($10^{-14} - 10^{-15} \text{ mol/dm}^3$) and selectivity.

Some of the macrocyclic polyaza-polycarboxylate ligands, developed for the complexation of lanthanides, have been proposed to use for the acceleration of the excretion of the toxic heavy metals (Pb^{2+} , Cd^{2+}) and radioactive fission products (^{90}Sr , ^{144}Ce). The ligands EDTA and DTPA, proposed earlier for the treatment of metal intoxication, show practically no selectivity for the toxic and radioactive metal ions.

Magnetic Resonance Imaging (MRI) is one of the most effective medical diagnostic tool in which paramagnetic Gd(III)complexes are used for increasing the image contrast. The effect of contrast agents is based on the increase of the relaxation rate of tissue protons (mainly water protons) in the presence of Gd(III)complexes. Recently a new type of contrast agents is under development, which contains exchangeable protons. The signal of the exchangeable protons is

selectively irradiated with a RF pulse and the chemical exchange with the surrounding water protons results in a decrease in the signal intensity of the water, which leads to a negative contrast (CEST agents). A larger effect resulted in with some lanthanide complexes of DOTA-tetraamide derivatives, which contain a slowly exchangeable water molecule in the inner sphere. The signals of this water and the amide NH protons of the ligand, which are strongly shifted by the paramagnetic Ln³⁺ ion (*e.g.* Eu³⁺), result in large CEST effect and the paramagnetic complexes are known as PARACEST agents.

In the research and development of PARACEST agents the complexes of lanthanides formed with the ligand 1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetrakis(acetyl-glycinate) (DOTA-Gly) and its derivatives are very promising. While the relaxation properties and the rates of the water exchange of these complexes have been studied in detail, there are quite a few data in the literature about the equilibrium and kinetic behaviour of the complexes, which are also important in their possible practical application.

The aim of this work is to obtain more detailed information on the equilibrium and kinetic properties of the complexes formed with the macrocyclic ligands DOTA-Gly, NOTA-Gly and ODDA-Gly, containing acetyl-glycinate functional groups, attached to the nitrogen atoms of the macrocycles. Some structural data will also be obtained on the coordination mode of the ligands by NMR spectroscopy and for a solid state Cu(II)complexes by X-ray diffraction method. The kinetics of the formation of the Ln(III)complexes of the negatively charged ligand DOTA-Gly and neutral DOTAM, will also be compared.

This work forms the past of the research work of the Department of Inorganic and Analytical Chemistry of the University of Debrecen, where the complexes of lanthanides with open chain and macrocyclic polyaza-polycarboxylate and polyphosphonate ligands have been studied for several decades. The results of my work are interesting first of all in the filed of

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coordination chemistry of lanthanides and the functionalised macrocyclic ligands but some aspect of the work can also be important for the development of the new type of MRI contrast agents.

II. Experimental methods

The **pH-potentiometric titrations** were performed in thermostated vessels at 25 ± 0.2 °C, with a Radiometer PHM 93 Reference pH-meter, Radiometer ABU 80 autoburette, PHG 211 glass and K401 calomel electrodes. The samples were in 0.1 M; 1.0 M KCl or KNO₃ to maintain constant ionic strength. The titrated samples were stirred and inert gas (N₂, Ar) was bubbled through them. The titrations were made in the pH range 1.7-11.7. For the calibration of the pH meter, KH-phtalate (pH=4.005) and borax (pH=9.180) buffers were used. For the calculation of [H⁺] from the measured pH values, the method proposed by Irving et al. was used. The protonation and stability constants were calculated with the program PSEQUAD.

The deprotonation of the amide-NH donor group in the presence of the Cu²⁺, the coordination arrangement of the Ce(DOTAM)³⁺ and Ce(DOTA-Gly)⁻ complexes, the formation and dissociation kinetics of the Ln(DOTAM)³⁺ and Ln(DOTA-Gly)⁻ complexes were studied by **spectrophotometry** at 25 °C in a thermostated cell holder with a Cary 1E spectrophotometer in 1.0 M KCl. The formation rates of the Ce(DOTAM)³⁺, Eu(DOTAM)³⁺, Ce(DOTA-Gly)⁻ and Eu(DOTA-Gly)⁻ complexes have been studied at 320 and 250 nm, respectively. For following the formation of the Gd³⁺-, Er³⁺- and Yb(DOTAM)³⁺ the release of the H⁺ from the ligand was monitored at 616 nm in weakly buffered solutions with bromocresolgreen indicator. The rates of dissociation of the complex

Eu(DOTA-Gly)⁻ in 0.1-1.0 M HClO₄ ([HClO₄]+[NaClO₄]=1.0 M) were studied at 250 nm.

The **relaxivity** values were calculated from the longitudinal relaxation times of H₂O protons (T_1) measured with an MS-4 NMR spectrometer (Institute Jožef Stefan, Ljubljana) at 9 MHz. The temperature of the sample holder was controlled with thermostated air stream. The longitudinal relaxation time were measured by the ‘inversion recovery’ method ($180^\circ - \tau - 90^\circ$) by using 6-8 different τ values.

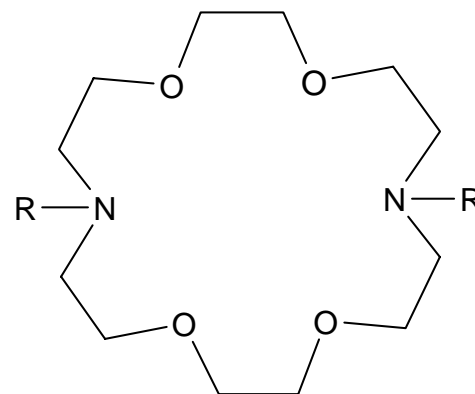
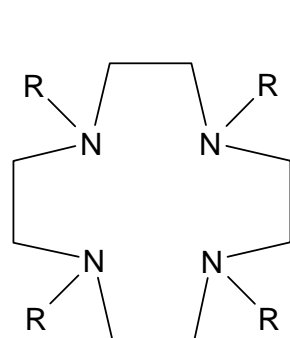
The study of the coordinated ligands was made by **¹H-NMR spectroscopy**. The spectra were recorded with a Bruker Avance 360 spectrometer. The temperature of samples was controlled with the Bruker VT-1000 unit. The spectra were processed by the Winnmr[®] software package.

The **solid state structure of the Cu(HNOTA-Gly) complex** has been established by single crystal X-ray diffraction study. Crystals of Cu(HNOTA-Gly)×4H₂O was mounted onto a glass fibre using epoxy resin. Data were collected at 293±1 K on an Euraf Nonius MACH 3 diffractometer using monochromated Mo K_α radiation ($\lambda=0.71073 \text{ \AA}$), ω -2 θ motion. Absorption correction was made using psi scans. The structure was solved using direct method with the SIR-92 software and refined on F² by full matrix least square method with the use of the program SHELX-97.

III. Ligands used for the studies

The synthesis of the two novel ligands NOTA-Gly and ODDA-Gly were made similarly to DOTA-Gly according to the published methods at our Department by Dr. István Lázár and Tímea Iványi. The DOTAM ligand was provided by Dr. Éva Tóth (Institut de Chimie Minérale et Analytique, BCH, Faculté des Sciences, Université de Lausanne, Switzerland).

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R= -CH₂-COOH

R= -CH₂-CO-NH-CH₂-COOH

R= -CH₂-CO-NH₂

:H₄DOTA

:H₄DOTA-Gly

:DOTAM

R= -CH₂-COOH

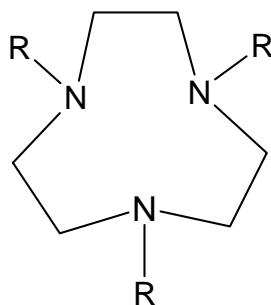
R= -CH-(CH₂-COOH)₂

R= -CH₂-CO-NH-CH₂COOH

:H₂ODDA

:H₄ODDM

:H₂ODDA-Gly



R= -CH₂-COOH

R= -CH₂-CO-NH-CH₂COOH

:H₃NOTA

:H₃NOTA-Gly

H₃NOTA: 1,4,7-triazacyclononane-1,4,7-triacetic acid, **H₃NOTA-Gly:** 1,4,7-triazacyclononane-1,4,7-tris(acetyl-glycine), **H₄DOTA:** 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, **H₄DOTA-Gly:** 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(acetyl-glycine), **DOTAM:** 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetamide, **H₂ODDA:** 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diacetic acid, **H₄ODDM:** 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-bis(malonate) **H₂ODDA-Gly:** 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-bis(acetyl-glycine), ,

IV. Results

In this work we studied the complexation properties of the 9-membered triaza, 12-membered tetraaza and 18-membered diaza-tetraoxa macrocyclic ligands, containing pendant acetyl-glycinate functional groups. The metal ions used for the complexation studies were the lanthanide(III) ions and some endogenous (Mg^{2+} , Ca^{2+} , Cu^{2+} , Zn^{2+}) and toxic (Pb^{2+} , Cd^{2+}) divalent metal ions.

The stability constants of the complexes of NOTA-Gly, DOTA-Gly and ODDA-Gly formed with the divalent metals and lanthanide(III) ions are significantly lower than those of the analogous NOTA, DOTA and ODDA complexes. The large differences in the stability constants are probably the result of the differences in the quality of the coordinated donor atoms. In the complexes of NOTA-Gly, DOTA-Gly and ODDA-Gly all the coordinated donor atoms – the ring nitrogens and amide oxygens – are neutral, while in the complexes of NOTA, DOTA and ODDA, beside the ring nitrogens, three, four and two carboxylate O^- oxygens are coordinated, respectively. The carboxylate groups of the NOTA-Gly, DOTA-Gly and ODDA-Gly are far from the coordination sites and so they can be protonated or can be coordinated to another metal ion with the formation of dinuclear complexes. In the lower stability constants of the amide derivative complexes the lower basicities of the ring nitrogens also play an important role which is the result of the electronwithdrawing effect of the amide groups.

The stability constants of the lanthanide complexes of NOTA-Gly and DOTA-Gly increase from La^{3+} to the middle of the series and slightly decrease with the further decrease of the ionic size. This trend indicates that the most favourable fit of the Ln^{3+} ions into the coordination cage, formed by the ring N and amide O donor atoms, occurs for the medium size Ln^{3+} ions. The $\log K_{\text{ML}}$ values of the $\text{Ln}(\text{ODDA-Gly})^+$ complexes are higher for the ions of larger size and slightly increase from the La^{3+} to Nd^{3+} , then definitely decrease with

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lowering ionic size. The trend of the $\log K_{ML}$ values indicates that the ring oxygens also participate in the complexation and the best "size match" of the ring and Ln^{3+} ion is at the Nd^{3+} . The ligand ODDA-Gly has a high size selectivity for Pb^{2+} over Zn^{2+} , since the stability constant of $Zn(ODDA-Gly)$ is very low. However, in contrast to the ligand ODDAM, which show a selectivity for the Sr^{2+} over Ca^{2+} , the stability constants of the Ca^{2+} and Sr^{2+} complexes of ODDA-Gly are similar.

The solid state structure of the protonated complex $Cu(HNOTA-Gly) \times 4H_2O$ is distorted octahedral, as it was found by single crystal X-ray diffraction studies. The ligand is coordinated with three ring N and three amide O to the Cu(II) and one of the carboxylate groups is protonated.

The N-acetylglycinate functional groups may behave like diglycinate groups in the Cu(II) complexes formed with the flexible NOTA-Gly and ODDA-Gly. In these complexes the amide NH hydrogens dissociate at higher pH values and instead of the amide O, the amide N^- is coordinated to the Cu^{2+} , when the ring nitrogens play the role of the "anchor" atom. The coordination of the amide N^- has been detected by the changes in the visible spectra of the Cu(II) complexes. The behaviour of the complex $Cu(ODDA-Gly)$ is very unique, since parallel with the dissociation of the amide hydrogens a dinuclear complex Cu_2LH_3 is spontaneously formed with the release of a half equivalent of the ligand, in which presumably an OH^- group is in bridging position between the two Cu^{2+} . In the complex Cu_2LH_3 dipolar antiferromagnetic coupling exists between the two Cu^{2+} -ions which leads to a lower magnetic moment ($\mu_{eff}/Cu = 1,16$ B.M.) and the 1H -NMR spectrum of the complex can also be recorded.

The relaxivity of $Gd(DOTA-Gly)^-$ is very low at room temperature since it is determined by the outer sphere relaxivity because of the slow exchange of the

coordinated water molecule. The increase of temperature between 10 °C and 25 °C results in a decrease of the outer sphere relaxivity but above 25 °C the rise of temperature leads to the increase of the relaxivity because of the growing exchange rate of the inner sphere water molecule. The pH dependence of the relaxivities indicates the contribution of the H^+ and OH^- catalyzed proton exchange at lower ($pH < 5$) and higher pH values ($pH > 9$), respectively.

The 1H -NMR spectra of the complex $Zn(NOTA-Gly)^-$ indicate that the protonation of the complex occurs at the carboxylate groups, which has practically no influence on the coordination of the ring nitrogens and amide oxygens.

The pH dependence of the 1H -NMR spectra of the DOTA-Gly shows that in the monoprotonated ligand the proton exchange is slow in the NMR time-scale. The 1H -NMR spectra of the $La(DOTA-Gly)^-$ indicate the coordination of the four amine nitrogens and amide oxygens and also the formation of H-bond between the amide hydrogen and carboxylate oxygen atom. The protonation of the complex occurs at the carboxylate groups.

The complex $Eu(DOTA-Gly)^-$ slowly dissociates in 0.1 – 1.0 M HCl solution. The dissociation of the predominating species $Eu(H_4DOTA-Gly)^{3+}$ occurs via spontaneous and proton assisted pathways. The proton assisted dissociation is slower than that of $Eu(DOTA)^-$ but the spontaneous dissociation in acidic solution occurs faster. However, the dissociation of $Eu(DOTA-Gly)^-$ under physiological condition is extremely slow and the complex is a safe potential contrast agent in CEST investigations.

The kinetics of formation of the complex of $Ln(DOTA-Gly)^-$ is very similar to those of $Ln(DOTA)^-$. The reactions take place via the fast formation of mono- and diprotonated intermediates, in which the carboxylate groups are coordinated to the Ln^{3+} ion and it is outside of the coordination cage. The rate determining step is the loss of the last proton and the rearrangement of the

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intermediate. The loss of the proton occurs mainly with the assistance of OH⁻ ions.

The kinetics of formation of the lanthanide complexes with the ligand DOTAM, which does not contain charged functional groups, differs considerably from that of Ln(DOTA-Gly)⁻ or Ln(DOTA)⁻. In these reactions the formation of intermediates can not be detected. The kinetic data indicate that the formation of the complexes Ln(DOTAM)³⁺ occurs with the direct reaction between the deprotonated ligand and Ln³⁺ ions in a second order process (k_L). The relatively slow proton exchange reactions of the protonated species H₂DOTAM²⁺ and HDOTAM⁺ have no effect on the rates of complex formation. The formation of the complexes Ln(DOTAM)³⁺ occurs with the successive replacement of the coordinated water molecules on the Ln(H₂O)_n³⁺ ion. The low k_L values observed can be interpreted by assuming that the rate determining Ln³⁺-amide bond formation is probably quite early in the reaction sequence, probably being located in the second or third step of the complex formation.

V. Potential use of the results

The results of this work may have some interest for coordination chemistry, for basic research, but some aspects of the work can be valuable for the possible “*in vivo*” medical or biological use of some lanthanide(III)complexes for the development of contrast agents for Magnetic Resonance Imaging (MRI).

Results of the equilibrium and kinetics studies of the Ln(DOTA-Gly)⁻ complexes suggest that the thermodynamic stability and kinetic inertness of the Ln(DOTA-Gly)⁻ is high enough for medical or biological *in vivo* applications. The Eu(DOTA-Gly)⁻ can be used as a safe PARACEST MRI contrast agent,

which is predicted from the very low rate of the proton-assisted dissociation of the complex. The spontaneous formation of the complex $[\text{Cu}_2(\text{ODDA-Gly})(\mu\text{-OH})]^+$ is very interesting since similar phenomenon was not observed earlier and this result shows a way for the formation of dinuclear Cu(II) complexes, which are often used as enzyme models.

IV. Publications

VI.1. Publication connected to the thesis

Articles:

3. Zs. Baranyai, E. Brücher, R. Király, T. Iványi and I. Lázár; *Complexation properties of the 1,4,7-triazacyclononane-1,4,7-tris(acetylglycinate) and 7,16-diaza-1,4,7,13-tetraoxacyclooctadecane-7,16-bis(acetylglycinate) ligands*
to be published
2. Zs. Baranyai, I. Bányai, E. Brücher, R. Király, E. Terreno; *Kinetics of the formation reactions of the lanthanide(III)DOTAM complexes*
Eur. J. Inorg. Chem., 2007, to be published
1. Zs. Baranyai, E. Brücher, T. Iványi, R. Király, I. Lázár and L. Zékány; *Complexation properties of N,N',N'',N''' -[1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayltetrakis(1-oxoethane-2,1-diyl)]tetrakis[glycine] ($H_4\text{dotagl}$), equilibrium, kinetic and relaxation behaviour of the lanthanide(III) complexes*
Helv. Chim. Acta, **2005**, 88, 604-617

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Conferences:

6. Zs. Baranyai, E. Brücher, T. Iványi, R. Király, I. Lázár,
Equilibrium and kinetic properties of the lanthanide(III) complexes formed with the tetraamide derivatives of DOTA
COST D-18 Annual Workshop (“Lanthanide Chemistry for Diagnostic and Therapy”), September 23-26, 2004, A Coruña, Spain (poster)
5. Baranyai Zs., Brücher E., Király R., Iványi T., Lázár I.,
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4. Baranyai Zs., Brücher E., Király R., Iványi T., Lázár I.,
Az 1,4,7,10-tetraazacilkooktadekán-1,4,7,10-tetrakis(acetilglicin) ligandum komplexképző sajátosságai
XXXVIIIth. Colloquim on Coordination Chemistry, May 21-23, 2003, Gyula, Hungary. (lecture in Hungarian)
3. E. Brücher, Zs. Baranyai, A. Bényei, T. Iványi, R. Király, I. Lázár, A. Simon,
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Eurobic-6, July 29-August 3, 2002, Lund, Sweden. (poster)
2. Baranyai Zs., Brücher E., Király R., Iványi T., Lázár I. és Simon A.,
Acetilglicinát funkciós csoportokat tartalmazó tetraaza- és diaza-tetraoxa makrociklusos ligandumok komplexképző sajátosságai
XXXVIIth. Colloquim on Coordination Chemistry, May 29-31, 2002, Mátraháza, Hungary. (lecture in Hungarian)

1. Baranyai Zs., Bényei A., Bücher E., Iványi T., Király R. és Lázár I.,
Az 1,4,7-triaza-ciklononán-1,4,7-trisz(acetil-glicin) előállítása és komplexképző sajátosságai
XXXVIth. Colloquim on Coordination Chemistry, May 23-25, 2001, Pécs, Hungary. (lecture in Hungarian)

VI.2 Publication not connected to the thesis

Articles:

3. Zs. Baranyai, E. Gianolio, K. Ramalingam, R. Swenson, R. Ranganathan, E. Brücher and S. Aime; *The effects of intramolecular H-bond formation on the stability constant and water exchange rate of the Gd(III)-diethylenetriamine-N'-(3-amino-1,1-propylenephosphonic)-N, N,N'',N''-tetraacetate complex*
Contr. Med. Mol. Imag., 2007, to be published
2. É. Csajbók, Zs. Baranyai, I. Bányai, E. Brücher, R. Király, A. M. Fahrnow, J. Platzek, B. Radüchel and M. Schafer; *Equilibrium, ¹H and ¹³C NMR Spectroscopy, and X-ray Diffraction Studies on the Complexes Bi(DOTA)⁻ and Bi(DO3A-Bu)*
Inorg. Chem., **2003**, 42, 2342-2349
1. I. Lázár, A. Egri, R. Király, Zs. Baranyai, T. Iványi and E. Brücher; *Synthesis, conformation and equilibrium study of new piperazine and azamacrocyclic ligands with N-(tetrahydrofuran-2on-3-yl) and N-[(carboxy)(2-hydroxyethyl)methyl] pendant arms,*
Eur. J. Org. Chem., **2002**, 351-360

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Conferences:

4. Zs. Baranyai, E. Gianolio, K. Ramalingam, E. Brücher and S. Aime
The effect of intramolecular H-bonding on the exchange rate of inner-sphere water in a Ln(III)-(phosphate containing)- DTPA derivative
COST D-18 Final workshop (“Lanthanide Chemistry for Diagnostic and Therapy”), May 31-April 1, 2006, Orleans, France. (poster)
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(lecture)
2. Király R, Baranyai Zs., Bányai I. és Brücher E.
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XXXVth. Colloquim on Coordination Chemistry, May 24-26, 2000, Kecskemét, Hungary. (lecture in Hungarian)
1. I. Lázár, A. Egri, R. Király, Zs. Baranyai, E. Brücher and A. D. Sherry
Synthesis, NMR and coordination chemistry study of 2-(2'-hidroxiethyl)-acetat pendent arm containing macrocycles,
XXIVth. International Symposium on Macrocyclic Chemistry, July 18-23, 1999, Barcelona, Spain. (poster)