Equilibrium, structural and kinetic properties of lanthanide(III), copper(II) and zinc(II) complexes formed with DTPA-amide derivative ligands

PhD Thesis Abstract

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I. Introduction and research objectives

Nowadays, *in vivo* applications of lanthanide(III) complexes formed with chelating ligands is one of the most important fields of the coordination chemistry research of rare earth elements. Consequently, the number of biological and medical applications of lanthanide(III)-poliaza-policarboxylate and –polyphosphonate complexes has been increasing significantly. One of the most powerful techniques in medical diagnostics is magnetic resonance imaging (MRI) with the use of Gd^{3+}-poliaza-polycarboxylate complexes as contrast agents to enhance the intrinsic contrast of the image. Moreover, there is still a continuing search for lanthanide(III) complexes which could provide a means of measuring *in vivo* enzyme activity, partial pressure of oxygen, temperature and pressure with the use of magnetic resonance imaging and there is also a great interest in the development of the organ specific contrast agents.

For lanthanide(III) complexes, fluorescence immunoassay is another important medical diagnostic application, since this method is able to determine antigens, synthetic and biologically important compounds using the fluorescent properties of the metal complexes.

Medical therapy is also a relevant use beside diagnostic applications for the lanthanide(III) complexes. For instance, ^{90}Y and ^{177}Lu complexes of polyaza-polycarboxylates linked to monoclonal antibodies or proteins can be used as tumour targeting agents, because of their β emission.

Recently, promising experiments have been performed to link together the magnetic resonance imaging (MRI), as a diagnostic method and the gadolinium neutron capture therapy of cancer (Gd-NCT). In this new method, the Gd^{3+}-compleces are targeted to the tumours and irradiated by slow neutrons. The nuclear reaction between the $^{157}Gd^{3+}$ and the slow neutrons is able to kill the tumour cells and the efficacy of the therapy is followed by MRI technique.

In the Department of Inorganic and Analytical Chemistry of the University of Debrecen (Hungary) the research area of the lanthanide(III) complexes with open-chain

polyamino-polycarboxylate ligands has been active for a long time in order to study the equilibrium, structural and kinetic properties of the complexes. For a few years amide derivatives have also been studied, some of them are already used in the medical diagnosis. The research in the group is mainly related to coordination chemical problems of the usage of the Gd^{3+}-complexes as MRI contrast agents. These investigations are also interesting from the scientific point of view, because new information can be obtained for the coordination chemical properties of the metal complexes. My work is connected to this field and my research objective was to study in details the properties of the metal complexes formed with DTPA-amide derivative ligands.

Our primary aim was to determine the thermodynamic and kinetic stability of the GdDTPA-bis- and –tris(amide) derivative complexes, because these two parameters have high importance on the *in vivo* toxicity. We were curious about the influence of several factors not only on thermodynamic and kinetic stability but also on the relaxivity (presents the efficacy of the MRI contrast agents) such as the substitution degree of the amide groups, the presence of stable nitroxide free radicals on the amide groups and the replacement of the third carboxylate arm by an amide group in the ligand. We wanted to answer coordination chemistry questions like, how the water-exchange rate of the Gd^{3+}-complexes changes with the variation of the substitution degree of the amide groups or the charge of the complexes and what kind of isomers exist in solution of the simplest GdDTPA-bis(amide) complex.

II. Experimental methods

The **solution structure measurements** were performed using one-dimensional ^{1}H, ^{13}C-NMR and two-dimensional NMR spectroscopy. The spectra were recorded by Bruker Avance 360 spectrometer and processed by the Winnmr software package.

The **equilibrium measurements** were carried out by pH-potentiometric titrations in double-walled vessels thermostated at 25±0.2°C, with a Radiometer PHM 93 Reference pH-meter, ABU80 autoburette and PHG 211 glass and K401 calomel electrodes. The titrated samples were stirred and inert gas was bubbled through the solutions. The

concentration of the H^+-ion was obtained from the measured pH values according to the method proposed by Irving et al. The protonation and stability constants were calculated with the program PSEQUAD.

For the **relaxivity measurements** the longitudinal relaxation times (T_1) of the water protons of the GdDTPA-bis- and –tris(amide) complexes were measured with an MS-4 NMR spectrometer (Institute Josef Stefan, Ljubljana) at 9 MHz by the inversion recovery method.

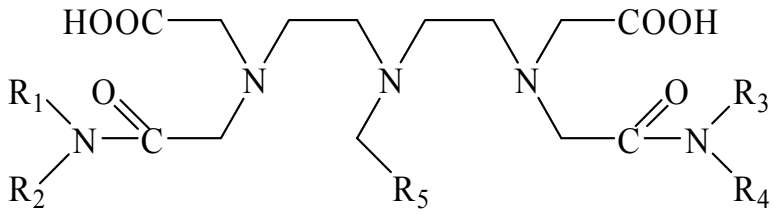
In order to determine the **water-exchange rate** of the Gd^{3+}-complexes, variable temperature ^{17}O NMR measurements were performed using a Bruker AM-400 (9.4 T, 54.2 MHz) spectrometer. Longitudinal relaxation rates, $1/T_1$, were obtained by the inversion recovery method and transverse relaxation rates, $1/T_2$, were measured by the Carr-Purcell-Meiboom-Gill spin echo technique. Variable pressure ^{17}O NMR measurements up to a pressure of 200 MPa were performed using a Bruker ARX-400 spectrometer equipped with a home-built high pressure probe head. The temperature was controlled by circulating fluid from a temperature bath. The X-band EPR spectra were recorded on a Bruker ESP 300 spectrometer (9.425 GHz, 0.34 T). The peak-to-peak linewidth was measured from the recorded spectra using MATLAB program. The analysis of ^{17}O NMR and EPR data was performed with Scientist® for Windows™ by Micromath®, version 2.0.

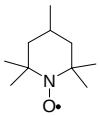
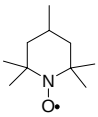
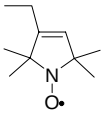
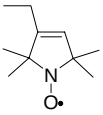
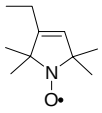
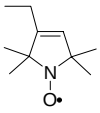
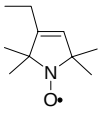
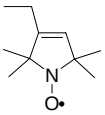
The **metal-exchange reactions** of the GdDTPA-bis- and –tris(amide) complexes with Eu^{3+} or Cu^{2+} were studied by spectrophotometry at 250 or 300 nm with a Cary 1E spectrophotometer. The exchange reactions with Lu^{3+} or Zn^{2+} were followed by measuring the relaxation rates since there is no UV-Vis absorption either of the reactants or the products, but the relaxivities of the complexes GdL and the Gd^{3+}·aq ion formed in the exchange reactions differ considerably.

III. Ligands

Two novel DTPA-amide derivative ligands (DTPA-bbBA and DTPA-tra) were synthesized. The DTPA-bA and DTPA-bBA ligands have been prepared according to the literature. The three spin labelled ligands containing nitroxide free radicals were provided

by Prof. Kálmán Hideg from the Institute of Organic and Medicinal Chemistry of University of Pécs (Hungary).



R_1	R_2	R_3	R_4	R_5	Ligands
H	H	H	H	COOH	**H_3DTPA-bA**
H	n-Bu	H	n-Bu	COOH	**H_3DTPA-bBA**
n-Bu	n-Bu	n-Bu	n-Bu	COOH	**H_3DTPA-bbBA**
nBu	n-Bu	n-Bu	n-Bu	CONH-Me	**H_2DTPA-tra**
H		H		COOH	**H_3DTPA-bNOPA**
H		H		COOH	**H_3DTPA-bNOPMA**
				COOH	**H_3DTPA-bbNOPMA**

[**H_3DTPA-bA**: DTPA-N,N''-bis(amide), **H_3DTPA-bBA**: DTPA-N,N''-bis(n-butylamide), **H_3DTPA-bbBA**: DTPA-N,N''-bis[bis(n-butylamide)], **H_2DTPA-tra**: DTPA-N,N''-bis[bis(n-butylamide)]-N'-methylamide, **H_3DTPA-bNOPA**: DTPA-N,N''-bis(1-oxyl-2,2,6,6-tetramethyl-4-pyrolidine)amide, **H_3DTPA-bNOPMA**: DTPA-N,N''-bis(1-oxyl-2,2,5,5-tetramethyl-3-pyroline-3-methylene)amide, **H_3DTPA-bbNOPMA**: DTPA-N,N''-bis(bis(1-oxyl-2,2,5,5-tetramethyl-3-pyroline-3-methylene)amide]

IV. Results

In this work equilibrium, structural and kinetic properties were studied for lanthanide(III), copper(II) and zinc(II) complexes formed with DTPA-amide derivative ligands. The new scientific results are the following,

The solution structure of the Y(DTPA-bA) was studied at 323 K. Four diastereomer pairs (anti, cis, trans and syn) were detected almost in the same concentration.

The stability constants of Gd^{3+}-complexes increase significantly with increasing number of substituents on the amide groups (DTPA-bA, DTPA-bBA, DTPA-bbBA, DTPA-tra). The results are the same for the stable nitroxide free radical containing ligands (DTPA-bNOPA DTPA-bNOPMA, DTPA-bbNOPMA), because the overall basicity ($\sum logK_i^H$) is higher for the disubstituted amide group containing ligands than for the mono- or the non-substituted amide group containing ones.

The trend in the stability constants of CuL and ZnL is similar to that found for the Gd^{3+}-complexes, namely the $logK_{ML}$ values are also increasing, with increasing number of substituents on the amide groups. In the case of Cu^{2+}- and Zn^{2+}-ions we determined the stability constants of dinuclear complexes also from the equilibrium studies.

The replacement of the third carboxylate with an amide group (DTPA-tra) decreases the stability of the Gd^{3+}-, Cu^{2+}- and Zn^{2+}-complexes, in contrast to that of the metal complexes of DTPA-bbBA. This diminution in the stability constants originates from the lower negative charge and the decreased amine basicity of the DTPA-tra ligand.

Selectivity constants were calculated under physiological conditions to characterize the Gd^{3+} binding properties of the ligands over the endogenous metal ions. The examined DTPA-bBA and DTPA-bbBA complexes present higher selectivity (or much higher for DTPA-tra) for Gd^{3+} over Cu^{2+} and Zn^{2+} as the $Gd(DTPA)^{2-}$. Therefore, according to the equilibrium data, the Gd^{3+}-complexes of these ligands could be used *in vivo* successfully, like Gd(DTPA-bis(methylamide)), Gd(DTPA-BMA) which is a safe MRI contrast agent.

According to the relaxivity measurements of the complexes Gd(DTPA-bNOPA), Gd(DTPA-bNOPMA) and Gd(DTPA-bbNOPMA), we can conclude that the attachment of the nitroxide free radicals to the complexes Gd-DTPA-bis(amide) resulted in only a weak relaxivity increase. The interaction between the unpaired electron of the free radicals and Gd^{3+} must be negligible because the free radicals are not coordinated in the inner sphere of Gd^{3+} and some magnetic interaction is possible only if the free radical is in the inner sphere.

The temperature dependence of the relaxivities can be used to estimate the water-exchange rate of the water molecule in the inner coordination sphere. The k_{ex} is slightly higher for [Gd(DTPA-bA), Gd(DTPA-bBA), Gd(DTPA-bbBA) and Gd(DTPA-tra)$^+$ than the water-exchange rates of the spin-labelled Gd^{3+}-complexes.

In the examined Gd^{3+}-complexes of DTPA-bis- and (tris)amides the protons of the coordinated water molecule and the amide protons can both take place in the proton-exchange. From the pH dependence of the relaxivity values we could decide that in the proton-exchange the protons of the coordinated water molecule play a predominant role.

In the case of Gd(DTPA-bA), Gd(DTPA-bBA), Gd(DTPA-bbBA), with the increasing number of hydrophobic substituents (n-butyl group) in the ligand the relaxivity values are slightly increasing in human serum albumin (HSA) which show a little interaction between the Gd^{3+}-complexes and the plasma proteins.

The water-exchange rates on the [Gd(DTPA-bbBA)(H_2O)] and [Gd(DTPA-tra)(H_2O)]$^+$ complexes are smaller than that observed for [Gd(DTPA)(H_2O)]$^{2-}$. It can be explained in terms of a decreased negative charge and steric crowding around the water binding site in the amides in comparison to the carboxylate complexes. This diminution in the water-exchange rates persists in the case of the three amide group containing Gd^{3+}-complex, compared to the GdDTPA-bis(amides). The activation volumes of the water-exchange reactions indicate a dissociative interchange mechanism for the trisamide [Gd(DTPA-tra)(H_2O)]$^+$ and a limiting dissociative mechanism for the bisamide [Gd(DTPA-bbBA)(H_2O)].

According to our metal-exchange (Ln^{3+}, Cu^{2+} or Zn^{2+}) kinetic measurements, the replacement of two carboxylates of DTPA with the non-ionic amide groups leads to the decrease of the reactivity of the coordinated ligands, but there is a weak additional effect of the replacement of the third carboxilate by an amide group. The gradual substitution of the amide hydrogens with butyl or nitroxide groups results in an additional increase in the kinetic inertness of the Gd^{3+} complexes. The presence of the butyl or nitroxide substituents presumably results in a steric hindrance (under also physiological conditions) for the formation of the kinetically active protonated or dinuclear intermediates.

V. Potential use of the results

Our results correspond to the coordination chemical properties of the examined metal complexes and have a basic research character, but this work is interesting and useful for the development of MRI contrast agents and for the estimation of the *in vivo* dissociation of the studied complexes.

It can be concluded as a result of our kinetic measurements that the metal-exchange reactions of the disubstituted amide groups containing Gd^{3+}-DTPA-bis- and -tris(amide) complexes are much slower than that of the Gd^{3+}-complexes with the similar donor atoms which are already used as MRI contrast agents. Moreover, the high selectivity values for Gd^{3+} of these ligands (coming from the stability constants) and their other favourable coordination chemical properties show that especially the Gd(DTPA-bbBA), Gd(DTPA-tra)$^{+}$ and Gd(DTPA-bbNOPMA) complexes can be considered for use as MRI contrast agents or as a building block in a molecule for any other medical or biological *in vivo* applications. (According to our published results an American company has started to produce DTPA-bbBA-type ligands for complexing ^{90}Y which is used in the medical therapy.)

VI. Publications

VI.1. Publications connected to the thesis

Articles:

3. Z. Jászberényi, I. Bányai, E. Brücher, K. Hideg, T. Kálai and R. Király, *Equilibrium and NMR spectroscopic studies on the gadolinium(III), copper(II) and zinc(II) complexes formed with the DTPA-N,N''-bis(amide), -bis(butylamide) and -bis(bis-butylamide) ligands. Kinetic stabilities of the gadolinium(III) complexes*
   To be published

2. Z. Jászberényi, É. Tóth, T. Kálai, R. Király, L. Burai, E. Brücher, A. E. Merbach and K. Hideg; *Synthesis and complexation properties of DTPA-N,N''-bis[bis(butyl)]-N'-methyl-tris(amide). Kinetic stability and water exchange of its Gd^{3+} complex*
Dalton Transactions, 2005, 694 – 701

1. Z. Jászberényi, E. Brücher, J. Jekő[1], K. Hideg, T. Kálai, R. Király; *Synthesis, equilibrium and kinetic properties of the Gd^{3+} complexes of three DTPA-bis(amide) derivatives containing stable nitroxide free radical substituents*
European Journal of Inorganic Chemistry, 2003, 3601-3608.

Conferences:

6. E. Brücher, R. Király, Z. Jászberényi and L. Sarka: *Substituent effects on the thermodynamic and kinetic stabilities of the complexes of Gd^{3+}, Cu^{2+} and Zn^{2+} formedwith DTPA-mono(amide), -bis(amide) and -tris(amide) derivative ligands* (lecture). 5th International Conference on f-elements, August 24-29, 2003, Geneva, Switzerland.

5. Jászberényi Z., Brücher E., Király R., Hideg K., Kálai T.: *DTPA-bisz(amid) és –trisz(amid) származék ligandumok gadolínium(III), réz(II) és cink(II) komplexeinek egyensúlyi és kinetikai sajátsága* (lecture in Hungarian). XXXVIIIth. Colloquim on Coordination Chemistry, May 21-23, 2003, Gyula, Hungary.

4. E. Brücher, Z. Jászberényi, R. Király and L. Sarka: *Equilibrium and kinetic parameters for the prediction of in vivo stability of Gadolinium(III) complexes formed with DTPA derivative ligands.* (lecture) 10th. International Conference on Bioinorganic Chemistry, August 26-31, 2001, Florence, Italy.

3. Jászberényi Z., Brücher E., Hideg K., Kálai T., Király R. és Sár P. C.: *Néhány DTPA-bisz(amid) származék Gd^{3+}-komplexének egyensúlyi és kinetikai sajátságai* (lecture in Hungarian). XXXVIth. Colloquim on Coordination Chemistry, May 23-25, 2001, Pécs, Hungary

2. Z. Jászberényi, E. Brücher, R. Király, K. Hideg, T. Kálai, C. P. Sár: *Equilibrium and kinetic properties of the Gd^{3+}-complexes formed with some DTPA-bis(amide) derivative ligands* (poster). Cost D8 Final Workshop, March 29-31, 2001, Dublin, Ireland.
1. Z. Jászberényi, E. Brücher, R. Király, K. Hideg, T. Kálai, C. P. Sár: *Equilibrium and kinetic properties of the Gd^{3+}-complexes formed with some spin labeled DTPA-bis(amide) derivatives* (lecture). Cost D8/D18 European Workshop, September 14-17, 2000, Prague, Czech Republic.

VI.2. Publications not connected to the thesis

Conferences:

2. Z. Jászberényi, A. Sour, R. Ruloff, É. Tóth, A. E. Merbach: *Optimization of the water exchange rate on DOTA- and DTPA-type Gd(III) complexes* (poster). Fall meeting of the Swiss Chemical Society, October 7, 2004, Zurich, Switzerland.

1. Z. Jászberényi, A. Sour, R. Ruloff, É. Tóth, A. E. Merbach: *Effect of steric crowding on the physico-chemical properties of Gd(III) chelates* (poster). COST D18 Annual Workshop ("Lanthanide Chemistry for Diagnosis and Therapy"), September 23-25 2004, A Coruña, Spain.