Elucidation of structure and dynamics of compounds related to medical imaging techniques

Ph. D. Thesis

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1. Introduction and objectives

Imaging of internal human organs with exact and non-invasive methods is very important for medical diagnosis, treatment and post-treatment follow-up. Before the recent advances in medical technology, our knowledge of the internal structure of the human body had mostly relied on surgical methods, hence the word anatomy (from Greek: cutting up). Nowadays, tomography, another word with Greek origin, is associated with a virtual "cutting up" process (*tomos*: a cut, *graph:* written).

Two of the widely applied methods are x-ray computer-assisted tomography (CT) and Magnetic Resonance Imaging (MRI). In CT, the signal intensity in the final picture reflects electron density. This technique applies ionising radiation, x-rays. The basic feature of the method is that the x-ray tube permits the rays to sweep in many directions through the cross-section of the body under examination. The detection is made by sensitive crystal detector and the signals are stored and analysed by a computer. Like its predecessor, the CT, the magnetic resonance imaging (MRI) is also a computer-based imaging modality. In MRI, ionising radiation is not used; the nuclear magnetic resonance (NMR) signal intensity of the ^{1}H nuclei is measured. The ^{1}H nuclei have the advantages of high NMR sensitivity, high natural abundance and their omnipresence (more than 70% in weight of the human body is water).

To gain better contrast in the images, contrast-enhancing agents (contrast agents, CAs) are often used in both techniques.

All x-ray contrast agents contain a heavy element (I, Ba, Ce, Gd, Tb, Dy, Yb, Au, Pb, Bi). Contrast enhancement mainly results from the photoelectron effect due to their large atomic numbers. The photoelectron effect induces sharp increase in the mass attenuation coefficient at the K-shell binding energy, therefore results in higher contrast.

Although MRI is regarded as a non-invasive technique, there are occasions when CAs are administered into the patient to enhance the contrast of the image. Paramagnetic ions, when administered, can reduce T_1 (and T_2) values of the water protons, even at low concentration. The most commonly used CAs are Gd^{3+} chelates.

Generally, the coordination sphere of the overall nine-coordinated Gd^{3+} consists of eight donor atoms of the chelate ligand and (at least) one inner-sphere water molecule. The protons of this coordinated water molecule in the immediate proximity of the paramagnetic metal centre relax fast due to the dipole-dipole interactions between the nuclear spins and the fluctuating local magnetic field raised by the unpaired electron spins. These inner-sphere water protons can exchange fast with the bulk water protons, thus the fastening of relaxation can be transferred to the bulk.

Both in CT and MRI, the used CAs are facing with strict requirements. They, when administered to a patient, should enter and pass through anatomic regions of interest to provide contrast enhancement. Afterwards, these agents must be essentially completely excreted without being metabolised. The undesirable biodistribution and excretion profile of heavy metal ions can be altered successfully by using metal chelate complexes. In general, the metal chelate complexes have lower *in vivo* absorption and therefore lower toxicity than the metal aqua ions themselves. All of these requirements (high stability and kinetic inertness, efficacy in contrast enhancement) are in connection with the solution structure (isomeric composition) and dynamics (intra- and intermolecular exchange reactions) of these compounds.

During my Ph.D. work at the University of Debrecen, Hungary and at the Technical University of Delft, The Netherlands, the structure and dynamics of several, inert and labile complexes formed with polyamino-polycarboxylate (EOB-DTPA) and macrocyclic (DOTA) ligands have been investigated, mainly by multinuclear NMR spectroscopy. Our aim was to unravel new structural and *dynamic properties according to the contrast agent applications, mainly focusing on the presence of various isomers in solution and the dynamic exchange processes among them. In the case of a new type of MRI contrast agent, Gd(III)-loaded zeolite nanoparticles, parameters influencing the relaxivity (efficiency of the MRI contrast agent) have been planned to study systematically, in order to clarify the mechanism of contrast enhancing effect of nanoparticles.*

2. Applied methods and equipments

The main tool for these studies was the NMR spectroscopy, but other methods, as x-ray diffraction, NMR relaxometry were also used to gain more information.

^{1}H and ^{13}C NMR measurements were performed on Bruker AM 360 NMR and Bruker DMX 500 spectrometers. The ^{1}H-^{1}H COSY spectra were made with the standard Bruker program, using 45° mixing pulse. The ^{1}H-^{13}C correlation spectra were recorded on Bruker DMX 500 spectrometer in inverse mode, using gradient pulses in the z direction with the usual Bruker HSQC or HMQC pulse sequences. Data processing was made by Bruker WinNMR software package. The line widths were determined by deconvolution, fitting Lorentz curves on the peaks.

The NMRD measurements were made on a Stelar equipment, the T_1 and T_2 measurements on 20 and 60 MHz were made on Bruker Minispec relaxometers, the 300 MHz measurements were made on a Varian Inova-300 spectrometer, using inversion recovery sequence for T_1 and CPMG spin-echo sequence for T_2 measurements. The 500 MHz measurements were made on a Bruker DMX 500 spectrometer, using inversion recovery sequence for T_1 and CPMG spin-echo sequence for T_2 measurements.

The XRD patterns were obtained with a D-5000 Siemens diffractometer, with Ni-filtered $Cu_{K\alpha}$ radiation (λ= 1.5406 Å). MAS NMR spectra were recorded on a Varian VXR-400S spectrometer with resonance frequencies of 79.460 MHz (^{29}Si) and 104.229 MHz (^{27}Al).

3. Results

We have studied: *i)* the structure and dynamics of an inert and a labile complex of the macrocyclic DOTA ligand ($(\mathrm{BiDOTA})^-$ and $\mathrm{K(H_xDOTA)^{(3-x)-}}$, *ii)* the effect of C5-substitution on the structure and dynamics of the DTPA-skeleton ($\mathrm{Lu(S\text{-}EOB\text{-}DTPA)^{2-}}$) *iii)* the effect of the pore size, the Si/Al ratio and the calcination on the relaxivity of Gd(III)-loaded zeolite nanoparticles.

3.1 We have found that the $\mathrm{Bi(DOTA)^-}$ prefers the twisted square antiprismatic (TSA) arrangement, similarly to DOTA complexes formed with the larger lanthanide ions. By VT ^{1}H NMR spectroscopy and the 2D EXSY spectra taken at 274 K, the dynamic behaviour of the complex has also been revealed. The observed fluxionalities correspond *i)* to the motion of the ring protons and *ii)* to the change of the acetate arms' helicity. With the notation introduced for DOTA complexes, these fluxionalities are *i)* the $(\delta\delta\delta\delta)\leftrightarrow(\lambda\lambda\lambda\lambda)$ rearrangement for the ring and *ii)* the $\Delta\leftrightarrow\Lambda$ rearrangement for the acetate arms. The similar activation parameters obtained for the ring inversion and for the change in the acetate arms' helicity support the suggestion that the two types of motions, *i.e.* the motion of the ring and the acetate arms, proceed at the same rate in a concerted way. Another explanation can be a two-step mechanism where the slow, rate-determining ring inversion is followed by a fast change in the acetate arms' helicity. Since the $\Lambda(\lambda\lambda\lambda\lambda)/\Delta(\delta\delta\delta\delta)$ enantiomer pair cannot be detected, both accounts can be applied to explain our results.

3.2 Besides these types of fluxionalities, a new type of ring motion appears in the case of $\mathrm{K(DOTA)^{3-}}$ at pH~13. This ring-proton exchange process requires metal-ligand bond breaking and leads to we called the ring-slewing motion. The analysis of the activation parameters showed that at lower temperatures the ring inversion is the main ring motion, while the simultaneously existing ring slewing occurs much slower. The change in the acetate arms' helicity accompanies the ring inversion with similar rate. However, as the temperature increases the K^+ ion can dissociate more easily from the DOTA, giving rise to the new type of ring motion, the ring slewing. Interestingly, at high temperature the change in the acetate arms' helicity follows the ring slewing; while the ring inversion has slower rate, compared to the other two motions. We may state that the change in helicity of acetate arms is not an independent form in the fluxionality of $K(DOTA)^{3-}$ complex.

3.3 By line shape analysis of the HDO- $K(H_xDOTA)^{(3-x)-}$ system at lower pH, we have found that besides the intramolecular dynamic processes, exchange occurs also between HDO and $K(H_xDOTA)^{(3-x)-}$. We supposed that an ion pair between H_2DOTA^{2-}, and K^+ could be responsible for this phenomenon. This $K(H_2DOTA)^-$ can be protonated, forming $K(H_3DOTA)$ assembly, which contains exchangeable protons with HDO. The potassium probably has an "out-of-cage" position, but it can also slow down the intramolecular motion of the macrocycle. At pH between 6 and 9.5, where H_2DOTA^{2-} and $HDOTA^{3-}$ (both partially containing K^+) species are present, the detected proton exchange with HOD is in the *slow exchange regime*, while the fluxionality is *in the fast exchange regime.*

3.4 Studying what are the structural and dynamic consequences of introduction of an ethoxybenzyl-group into the 5-position in the Lu(*S*-EOB-DTPA)$^{2-}$ complex, we found two sets of signals appearing both in ^{13}C and ^1H-NMR at 330 K, which were assigned to isomers differing in the configuration of the central nitrogen. The isomer ratio was found to be 35:65. At temperatures lower than 300 K, the lines of the two isomers showed separate broadening. This observation is a consequence of the change in the acetate arms' helicity, "wagging", which is observable on the NMR time scale. By analysing the coupling patterns of the ethylene-diamine protons we determined that in both cases the preferred (and only detectable) arrangement of the acetate arms corresponds to the Λ helicity.

3.5 In the case of Gd^{3+}-loaded zeolite nanoparticles, studying the the effect of changing various parameters as Si/Al ratio within the zeolite framework, pore size and Gd^{3+} distribution on the r_1 relaxivity, we found the following:

i) Dealumination of the Y framework resulted in higher relaxivities of the GdNaY system, which can be explained by the larger water content inside the zeolite pores, overcompensating the slower diffusion through the more hydrophobic zeolite pore windows, as it was shown by NMRD fittings.

ii) The GdNaA sample has significantly lower relaxivity compared to GdNaY with similar Gd^{3+}-loading, due to the smaller pores (lower water content inside the zeolite pores) and smaller pore windows (slower diffusion), as it was shown by NMR relaxometry.

iii) The calcination induced Gd^{3+}-"travelling" inside the Y framework from the larger pores towards the smaller ones, which have very slow water diffusion rate towards the larger pores and the bulk in the NMR time scale. It resulted in reduced r_1 values.

iv) At higher fields outer-sphere effects play significant role in the transverse relaxivities induced by these Gd^{3+}-loaded zeolite nanoparticles.

4. Possible application of the results

The results presented in 3.1-3.5 are of basic research. However, detailed information about the structure and dynamics of these compounds is required for designing more effective contrast agents. The results presented in 3.5 can be used to synthesize Gd^{3+}-loaded zeolite nanoparticles as potential targeted MRI contrast agents for the gastrointestinal MRI or MRI angiography. Another advantage of these particles that they show significant r_1 enhancement at lower fields, where the traditional MRI works. However, at the higher fields of the recently designed MRI equipments they can be used as r_2-enhancing contrast agents, due to their large superparamagnetic contribution.

5. Acknowledgement

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6. List of Publications

5.1 Papers related to the dissertation

Dynamic Properties of DOTA-ligand: ^{1}H NMR and ^{17}O NMR Studies
I. Bányai, É. Csajbók, E. Brücher
J. Inorg. Biochem. **2001,** *86,* 137 (Conf. abstract from the 10th International Conference on Bioinorganic Chemistry, Florence, Italy, 26th-31st of August, 2001)

Csajbók, É.; Baranyai, Z.; Bányai, I.; Brücher, E.; Király, R.; Müller-Fahrnow, A.; Platzek, J.; Radüchel, B.; Schäfer,
Equilibrium, ^{1}H-, ^{13}C-NMR-Spectroscopy and X-Ray Diffraction Studies on the Complexes Bi(DOTA)$^-$ and Bi(DO3A-Bu)
Inorg. Chem. **2003** *42* 2342-2349

É. Csajbók, I. Bányai and E. Brücher
Dynamic NMR Properties of DOTA Ligand: Variable pH and Temperature ^{1}H NMR Study on $[K(H_xDOTA)]^{(3-x)-}$ Species
Dalton Trans. **2004** 2152-2156

R. N. Muller, L. Vander Elst, A. Roch, J.A. Peters, É. Csajbók, P. Gillis, Y. Gossuin
Relaxation by Metal-Containing Nanosystems (review)
Adv. Inorg. Chem. (accepted for publication)

2.2 Lectures and posters presented at conferences and meetings

Lectures:

É. Csajbók, I. Bányai, E. Brücher:
The intramolecular motion of DOTA ligand as studied by ^{1}H NMR
XXXVI. Coordination Chemistry Conference, Pécs, 2001 (lecture in Hungarian)

É. Csajbók, I. Bányai, E. Brücher:
NMR studies on the dynamics of inert and labile DOTA complexes
EMT Conference, Félixfürdő, Rumania, 2001 (lecture in Hungarian)

É. Csajbók, I. Bányai, E. Brücher:
NMR structure of Ln-EOB-DTPA stereoisomers
XXXVII. Coordination Chemistry Conference, Mátraháza, 2002 (lecture in Hungarian)

É. Csajbók, E. Brücher, I. Bányai:
Dynamic NMR studies on labile complexes of DOTA (1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic-acid)
9th Nordic Symposium on Coordination Chemistry, Umeå, Sweden (lecture in English)

Poster presentations:

É. Csajbók, E. Brücher, I. Bányai:
Intra-and intermolecular exchange processes: the DOTA molecule as an example
„Tavaszi szél"-Meeting of Hungarian Ph.D. Students, Gödöllő, 2001 (Poster in Hungarian)

É. Csajbók, I. Bányai, J. A. Peters
Gd(III)-loaded zeolite nanoparticles as MRI contrast agents
Interdisciplinary Workshop of Marie Curie Fellows, Institute For Energy, Petten, The Netherlands, 2003 (Poster in English)

É. Csajbók, I. Bányai, J. A. Peters
Gd(III)-loaded zeolite nanoparticles as MRI contrast agents
NMR Meeting, Groningen, The Netherlands, 2003 (Poster in English)

É. Csajbók, I. Bányai

Rediscovery of ^{1}H NMR for studying complex systems: Lanthanides, macrocycles, polyamino-polycarboxylates
COST D18 ”Lanthanide Chemistry for Diagnosis and Therapy” Annual Workshop A Coruna, Spain, 2003 (Poster in English)

G. A. Pereira, C. F. G. C. Geraldes, D. Ananias, J. Rocha, É. Csajbók, J. A. Peters, L. Vander Elst, R. N. Muller
Magnetic, NMR and relaxivity studies of microporous lanthanide(III) silicates
COST D18 ”Lanthanide Chemistry for Diagnosis and Therapy” Annual Workshop A Coruna, Spain, 2003 (Poster in English)