Multimeric Gd-based Contrast Agents for high field MR-imaging

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Introduction.
MR imaging is increasingly moving to higher fields as the majority of MRI scanners used in clinics work at 3 T. Imaging at high fields brings greater signal-to-noise ratio (SNR) and its concomitant benefits of higher spatial resolution and/or reduced acquisition times. Currently employed Gd-based contrast agents are small, fast tumbling molecules with low relaxivity that decreases slowly with the field strenght. Improved systems with high efficiency were prepared for MR-angiography exploiting their binding to slow tumbling molecules (i.e. Human Serum Albumin), but this strategy gives high relaxivity between 0.5 and 1.0 T and then sharply drops with increasing field. Therefore, it is important to synthetise new T_1 agents that have excellent relaxation properties over a broad range of imaging field strengths in order to take advantage of current 1.5 – 3 T scanners.[1] Multimeric Gd^{III} agents with medium molecular weight (~3-4 KDa) have been devised as one possible solution as they show improved r_1 values also at high fields.[2] Starting from a recently published bifunctional chelating agent based on the structure of AAZTA,[3] a well known ligand that result in Gd-complexes with excellent properties in terms of stability and relaxivity, we synthesised a series of di- tri- tetra- hexa- and octameric Gd^{III} complexes covering a broad range of molecular weights (1200-6000 Da). This allowed us to systematically investigate their relaxometric behaviour and find the best parameters to design optimal high field contrast agents.

Methods.
An AAZTA-like chelator with a free hydroxymethyl group and the carboxylates protected as *t*-butyl esters (AAZTA-OH)[3] was used as base ligand for the contruction of multimeric systems. The ability of hydroxyl groups to react readily with isocyanates was exploited, thus forming dimer and trimer by reacting AAZTA-OH with 1,4-diisocyanato and 1,3,5-triisocyanato-benzene. For larger multimeric systems we designed a dimeric module containing two AAZTA-OH ligands bearing an isothiocyanate group, that readily reacts with amines. With this funtionalised dimeric ligand, tetra- and hexameric ligands were synthesised by reaction with ethylendiamine and tris-(2-aminoethyl)amine, respectively. Finally, the octamer was prepared by reaction of generation-I PAMAM dendrimer with the monomeric AAZTA-OH containing an isothiocyanate functional group. The multimers were characterized by ^1H and ^{13}C NMR, by ESI and MALDI-TOF MS and by HPLC. A complete ^1H NMR relaxometric study was carried out on all multimeric Gd-complexes to obtain information on the relaxivity at different field strenghts (range: 0.01- 9.4 T) and different temperatures, on the stability with pH and on the critical parameters of MR-contrast agents such as reorientational correlation time (τ_R) and exchange lifetime (τ_M). T_1-weighted phantom MR-images at 1 and 3 T were also acquired on ProHance® and each multimeric complex to demonstrate the enhanced efficiency of the multimeric contrast agents.

Results.
The synthesis of the Gd^{III} di-, tri-, tetra-, hexa- and octameric complexes is reported. The relaxivity (r_{1p}) at 20 MHz and 298 K ranges from 12 to 25 $mM^{-1}s^{-1}$ per Gd unit and is almost constant over a broad range of field strenghts (0.5 -3 T). r_{1p} values follow a linear behaviour with the molecular weight of the Gd-multimer and for example they increase of about 250% moving from the monomer to the hexamer. Full relaxometric characterization was carried out by analysing the ^1H relaxivity data as a function of magnetic field strength and temperature. Remarkable signal enhancement at different magnetic fields was observed in T_1-weighted phantom MR-images of Gd-multimers in respect to commercial agent ProHance®.

Conclusions.
In conclusion, Gd-multimeric complexes show improved efficiency for both clinical MRI and Molecular Imaging applications. Mass relaxivities or densities of relaxivity are defined as the enhancement of the relaxation rate by a unit mass (gL^{-1}) of the contrast agent. High density of relaxivity is required for applications such as cell imaging in which a sufficient relaxation effect has to be produced by a limited mass of the agent. Future work will deal with the study of these Gd-multimeric systems as simple extracellular contrast agents as wel as cell labelling agents.

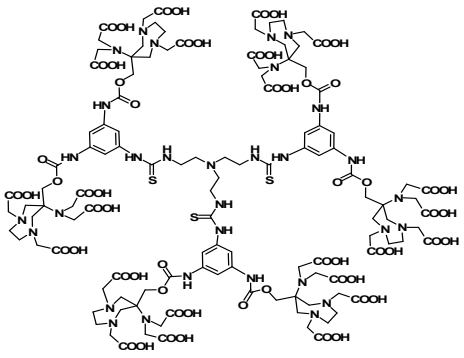

Figure 1. Structure of the hexameric ligand based on AAZTA chelator

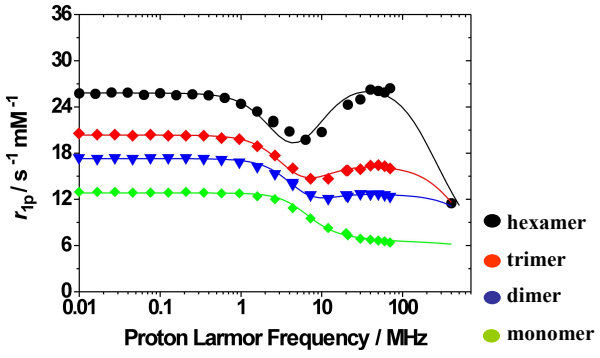

Figure 2. ^1H NMRD profiles of Gd-multimers recorded at 20 MHz and 298 K.

References
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