Short Communication

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Towards MRI contrast agents responsive to Ca(II) and Mg(II) ions: metal-induced oligomerization of dota–bisphosphonate conjugates

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In magnetic resonance imaging (MRI), paramagnetic complexes are utilized as contrast agents. Much attention has been paid to the development of new contrast agents responsive to pH, temperature or concentration of various components of body liquids. We report a new type of MRI probe sensing the concentrations of calcium and magnesium in biological media. The ligand do3ap^{BP} combines a dota-like chelator with a bisphosphonate group. In the complex, the Gd(III) ion is entrapped in the macrocyclic cavity whereas the bisphosphonate group is not coordinated and therefore is available for coordination with endogenous metal ions. In the presence of metal ions, Gd–do3ap^{BP} appears to show formation of coordination oligomers leading to an unprecedented increase in r_1 up to 200–500%. The extremely high relaxivity response makes this type of compound interesting for further studies as MRI ion-responsive probes for biomedical research. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: MRI; gadolinium complexes; bisphosphonate; responsive agents

1. INTRODUCTION

Magnetic resonance imaging (MRI) is an important diagnostic method in medicine. The principle of MRI relies on measurement of the water abundance in body tissues and on the relaxation rates of water protons. In order to improve the contrast of MRI images, paramagnetic complexes are utilized as contrast agents (1,2). The contrast agents are mostly Gd(III) complexes and their efficiency is expressed as millimolar relaxivity r_1, the reciprocal value of the decrease of the longitudinal relaxation time of water protons in a 1 mM solution of a particular contrast agent. Contrary to other imaging techniques, MRI is able to provide not only anatomical but also physiological information. For this purpose, much attention has been paid to the development of new contrast agents responsive to pH, temperature or concentration of various components of body liquids (3–6). In living organisms, metal ions play an important role in regulation of many biochemical processes. Among divalent metal ions, calcium and magnesium are present in relatively high concentrations in all body liquids. Thus, development of MRI contrast agents responsive to the concentration of these ions has attracted much attention in order to follow their concentration changes during various physiological processes. Most of the studied probes are based on changes in the coordination sphere of the Gd(III) ion, i.e. changes in hydration state (7–17). The sensing efficiencies of the reported probes are usually rather low. A relaxivity increase of more than 50% is exceptional, usually even lower increases are observed in biological media.

Here, we report on a new type of MRI probe sensing the concentrations of calcium and magnesium in biological media. Recently, we have synthesized the new ligand, do3ap^{BP} (Scheme 1), combining a dota-like chelator with a bisphosphonate group (18). In the complex, the Gd(III) ion is entrapped in the macrocyclic cavity whereas the bisphosphonate group is not coordinated and, therefore, is available for coordination with endogenous metal ions (18). In the presence of metal ions, the Gd–do3ap^{BP} complex appears to show increases in r_1 up to 200–500%.

2. RESULTS AND DISCUSSION

In order to evaluate the potential of the Gd–do3ap^{BP} complex as an ion-sensing MRI agent, we studied its interactions with Zn(II), Ca(II) and Mg(II) ions. In the presence of each of these ions, a significant increase in relaxivity was observed. Upon addition of 3 equiv. or more of the metal ions, the ^{1}H NMRD profiles (Fig. 1 and

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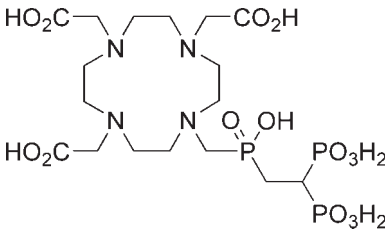

Scheme 1. Ligand H_8do3ap^{BP}.

Supporting Fig. S1) of Gd–do3ap^{BP} showed 200–500% increase along the whole frequency range. No changes were observed in fluorescence spectra of Eu–do3ap^{BP} under the same conditions during a period of a week. This indicates that the divalent metal ions did not change the lanthanide(III) coordination sphere and, thus, a possible release of free lanthanide(III) ion can be ruled out. The local maximum that appeared in the ^{1}H NMRD profiles at Larmor frequencies 10–100 MHz upon addition of the divalent metal ions is typical for complexes with a long rotational correlation time. This indicates an increase in molecular size upon interaction with the studied metal ions, which may be explained by the formation of coordination oligomers that are typical for phosphonate and bisphosphonate complexes in the solid state (19). A self-association process has been also reported for complexes of phosphonate-modified macrocycles in solution (20,21). The high relaxivity at low magnetic fields cannot be attributed exclusively to the increase in the rotational correlation time. The coordination spheres of divalent metal ions are not fully saturated by bisphosphonate oxygen atoms and the remaining positions are occupied by water molecules. As these ions are located in the proximity of the paramagnetic center, exchange of the coordinated water molecules improves the transfer of magnetic information. This phenomenon, which resembles an extremely rich 'second' hydration sphere, may contribute significantly to the overall value of relaxivity.

A relaxometric titration of Gd–do3ap^{BP} complex with Zn(II) ions gave surprising results (Supporting Fig. S2A). The first equivalent of metal ion gave rise to only a small relaxivity increase. The main response was obtained upon addition of 2–3 equiv., whereas the relaxivity remained constant at higher Zn(II) concentrations. The sharp break of the curve at 3 equiv. of Zn(II) indicates a well-defined structure. As bisphosphonate complexes show a

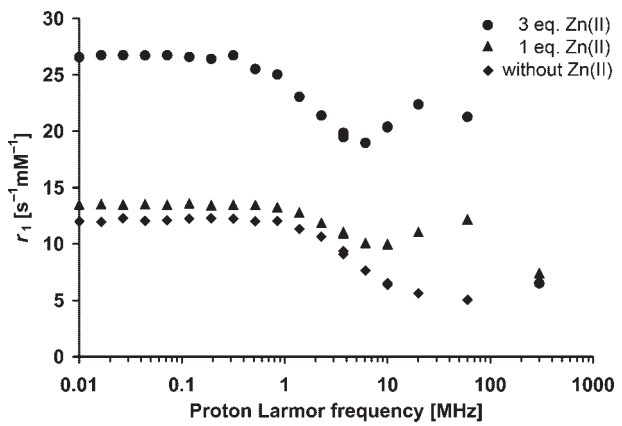

Figure 1. ^{1}H NMRD profiles (37°C, pH = 7.5) of Gd–do3ap^{BP} in the presence of 3 or 1 equiv. of Zn(II) ions. The profile of Gd–do3ap^{BP} is displayed for comparison.

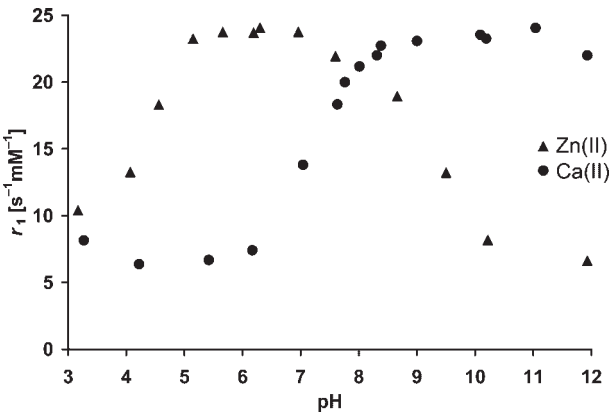

Figure 2. Relaxivity pH profiles (37°C, 20 MHz) of Gd–do3ap^{BP} in presence of 3 equiv. of Zn(II) or Ca(II) ions.

large variety of structural motifs (19), any structural prediction of the studied oligomers would be highly speculative. A similar trend was observed in the titration curves for Ca(II) ions (Supporting Fig. S2B), although it should be noted that the transition to the plateau value was more gradual in this case. This may be explained by a somewhat lower stability of the Ca(II) complex.

The pH dependences of the relaxivity at 20 MHz of solutions containing 3 equiv. of divalent metal ions reflect differences in the coordination properties of the studied ions (Fig. 2 and Supporting Fig. S3). The Zn(II) ion forms the most stable complexes and, thus, the oligomers are formed already at pH = 4–6. At pH > 8, these oligomers are decomposed due to the formation of zinc-hydroxo species. The Ca(II) and Mg(II) complexes are formed at higher pH as result of lower stability constants and are not decomposed at high pH. The lower relaxivities observed for the system with Mg(II) ions may be explained by the smaller ionic radius resulting in a steric crowding around the Mg(II) ion, which disfavors oligomer formation.

3. CONCLUSION

In conclusion, we can state, that the interaction of the Gd–do3ap^{BP}complex with divalent metal ions results in the formation of oligomeric phosphonate complexes and, consequently, leads to an unprecedented 200–500% increase in relaxivity along the whole ^{1}H NMRD profile. Relaxometric titrations indicate formation of oligomers with a well defined 3:1 ion:complex stoichiometry. The pH dependences of the relaxivities reflect different coordination behaviors of the studied divalent ions – Zn(II), Ca(II) and Mg(II). Similar behavior could be expected also for other dota-like bisphosphonate-containing chelators. In principle, the Gd–do3ap^{BP}complex would be an interesting Zn-sensitive MRI contrast agent. Unfortunately, the required concentration of MRI contrast agents in the tissues (1) is much higher than the biological zinc concentration (22,23). On the other hand, the application of MRI for a sensing of Ca(II) and Mg(II) ions using this complex is more realistic, as the biological concentrations of these ions are comparable with the concentration of MRI agents in tissue, although it should be mentioned that the selectivity is low, since the stabilities of the Ca(II) and Mg(II) complexes of Gd–do3ap^{BP} are comparable (24).

4. EXPERIMENTAL

The complex Gd–do3ap^{BP} was prepared as reported previously (18). ^{1}H NMRD profiles were measured at magnetic field range 4.7×10^{-4} to 0.35 T (0.02–15 MHz) using a Stelar SpinMaster FFC-2000 relaxometer. Measurements at 0.47 and 1.42 T (20 and 60 MHz) were performed with a Bruker Minispec mq20 and Bruker Minispec mq60, respectively. Aqueous solutions of the Gd–do3ap^{BP} complex (2 mM) were measured at 37°C in the presence of $Zn(NO_3)_2$, $Ca(NO_3)_2$ or $Mg(NO_3)_2$ at 37°C. For pH adjustment KOH and HCl solutions were used.

5. SUPPORTING INFORMATION

Supporting information can be found in the online version of this article.

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