Investigations into the relaxivity and complexation behaviour of GdDOTA-4AmP, a pH responsive MRI contrast agent

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The ability to produce pH maps of tissue *in vivo* could be quite important in diagnosing metabolic disturbances such as tumor acidosis or ion-pump imbalances in functioning kidneys. MRI is ideal for this purpose since its signal originates largely with water. To generate such pH maps with MRI, it is first necessary to have a contrast agent that modulates the water signal (either *via* altered relaxation or total intensity) as a function of pH. We reported previously one such pH responsive contrast agent, GdDOTA-4AmP^{5-}, that exhibits a change in relaxivity over a pH range that is nearly ideal for generating pH maps *in vivo*.[1,2,3] Since those first reports, more details about structure of the complex, its relaxivity behaviour, and optimal preparation methods have been further investigated.

Materials and Methods

Relaxivity measurements were made at 20 MHz and 25 °C and the pH adjusted by addition of solid LiOH. Potentiometric titrations were performed on acidic solutions by incremental additions of 0.1679 M KOH.

Results and Discussion

The pH of response of GdDOTA-4AmP^{5-} was originally reported to reach a maximum of 10 mM^{-1}s^{-1} at pH 6. However, subsequent studies on the relaxivity and structure of the complex found that the profile varied somewhat from one sample preparation to another. Upon preparation of the Eu^{3+} complex of DOTA-4AmP, the product was found to be critically dependent upon the reaction pH. If the complex is prepared at an unusually low reaction pH (below ~5), a different structural isomer is formed than when complexation is performed at higher pH values (above ~8). As this "low pH" species is quite stable (Fig. 1), it can contribute to the relaxivity profile of the agent even if imaging is performed at physiological pH. However, the "high pH" species is the structural isomer that yields "correct" pH profile shown in Fig 2. The pH range over which the relaxivity change is observed is almost identical to that of the original profile, but the maximum relaxivity is limited to 5.3 mM^{-1}s^{-1}. This has been pointed out in recent imaging papers.[2,3] The reason for this difference appears to have been in the way the original complex was prepared.

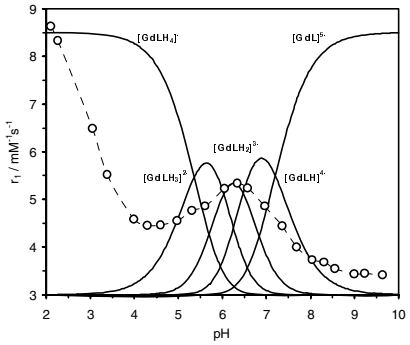

Fig 2. pH profile and speciation diagram for GdDOTA-4AmP^{5-}

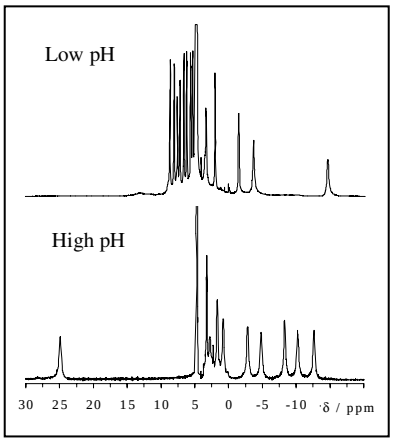

Fig 1. NMR spectra of EuDOTA-4AmP^{5-} prepared at two different pH values.

Potentiometric titrations of the complex yielded a speciation diagram for GdDOTA-4AmP^{5-} that shows the relaxivity contibutions of each protonated form of the agent (Fig 1). This anaylsis demonstrated that the diprotonated species GdLH$_2^{3-}$ that contributes to the peak maximum observed near pH 6. This supports our long-held hypothesis that it is the protonation state of the phosphonate side-arms that modulates prototropic exchange of protons from the single long-lived Gd^{3+}-bound water molecule to bulk solvent. Based upon these current data, the most likely mechanism involves rapid deprotonation/reprotonation of the Gd^{3+}-bound water molecule by the two unprotonated and two protonated phosphonate side-arms. This catalytic prototopic exchange enhances the relaxivity of bulk water with an optimal increase observed at pH 6. Because the relaxivity enhancment in this complex is associated with a change in water exchange rate, it was reasoned that it should improve if the rotation of the complex were slowed. The complex was modified and conjugated to a G5-PAMAM dendrimer. The relaxivity was found to increase substanitally, from 5.3 to a maximum of 25 mM^{-1}s^{-1}, improving the effectiveness of the pH responsive agent.

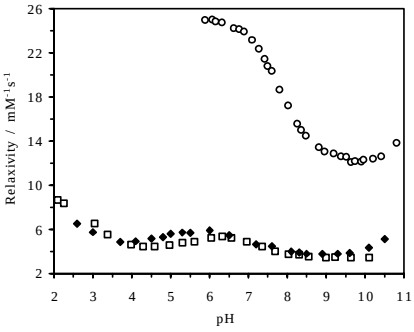

Fig 3. pH profiles of GdDOTA4-AmP^{5-} free in solution and covalently attached to G5 dendrimer.

References

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3) Raghunand *et al. Magn. Reson. Med.*, **2005**, *51*, In Press