A bio-activated paramagnetic Gd(III) complex Gd-TTDA-CGP for MRI

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Abstract

A β-galactopyanose-containing gadolinium(III) complex, $[Gd(TTDA\text{-}CGP)]^-$ (TTDA-CGP = ((3,10-di(carboxymethyl)-6-β-galactopyranosyl)-3,6,10-triazadodecanedioic acid)), was synthesized and characterized. The stability toward Zn(II) transmetallation of $[Gd(TTDA\text{-}CGP)]^-$ in the presence and absence of β-Galactosidase (β-gal) is less than that of $[Gd(DTPA)]^{2-}$ but apparently higher kinetically stable than that of [Gd(DTPA-BMA)]. The number of inner sphere water was significantly decreased (about 33%) in the presence of β-gal. The water exchange rate k_{ex}^{298} value of $[Gd(TTDA\text{-}CGP)]^-$ is significantly higher than those of $[Gd(TTDA\text{-}CHE)]^-$ (TTDA-CHE = (3,10-di(carboxymethyl)-6-hydroxyethyl)-3,6,10-triaza-dodecanedioic acid) and $[Gd(DTPA)]^{2-}$ but slightly lower than that of $[Gd(TTDA)]^{2-}$. The rotational correctional time τ_R value of $[Gd(TTDA\text{-}CGP)]^-$ is significantly higher than those of $[Gd(TTDA)]^{2-}$ and $[Gd(TTDA\text{-}CHE)]^-$. Relaximetric studies shows that the T_1 values of $[Gd(TTDA\text{-}CGP)]^-$ is significantly decrease in the presence of β-gal. The enhancement (77.6 ± 1.2%) indicated by MR imaging is higher than before the galactopyranose residue is removed.

Introduction

β-gal is frequently used as a reporter gene in animals to determine the transfection efficiency of gene expression by a colorimetric staining assay. However, the application of β-gal is limited by requiring invasive tissue sampling for in vivo monitoring in living animals. To exploit the advantage of MRI for biological studies, the study of the innovative bio-activated Gd(III) complexes for MRI has intensified recently.[1,2] Hence, Gd(III) chelate with ligand TTDA (3,6,10-tri(carboxymethyl)-3,6,10-triazadodecanedioic acid) is chosen because the water-residence lifetime is significantly lower than that of DTPA (3,6,9-tri(carboxymethyl)-3,6,9-triazaundecanedioic acid) and also reaches the optimal value.[3,4] A novel ligand TTDA-CGP, the derivative of TTDA, for bio-activated MRI contrast agent containing galactopyranose residue, was designed and synthesized. The kinetic stability of its Gd(III) complex was studied to understand its toxicity. The galactopyranose residue of $[Gd(TTDA\text{-}CGP)]^-$ removed in the presence of β-gal to obtain $[Gd(TTDA\text{-}CHE)]^-$ ($[Gd(TTDA\text{-}CGP)]^-$ without the galactopyranose residue) was proved by HPLC analysis. The water-exchange rate and rotation correction time of $[Gd(TTDA\text{-}CGP)]^-$ were obtained from the reduced ^{17}O relaxation rate ($1/T_{1r}$ and $1/T_{2r}$) and chemical shifts (ω_r) of $H_2{}^{17}O$. Finally, We described the MR imaging of the $[Gd(TTDA\text{-}CGP)]^-$ complex in the absence and presence of β-gal to compare the signal enhancement.

Methods

The kinetic stability of $[Gd(TTDA\text{-}CGP)]^-$ chelate containing phosphate buffer and $ZnCl_2$ in the presence and absence of β-gal to measure the relaxation rate (R_1) at 20 MHz was studied by transmetallation with Zn(II).[5]. The number of inner-sphere water was determined by ^{17}O-NMR chemical shift of the water as a function of Dy(III) concentration. The reduced ^{17}O-NMR transverse and longitudinal relaxation rate and chemical shifts data were analyzed together to determine the water-exchange lifetime and the rotational correlation time. The experimental setup of HPLC analysis is as follows: a reverse phase HPLC analytical C18 column (5 μm, 250 × 4 mm), fluorescence detector at λ_{ex} = 310 nm and λ_{em} = 420 nm, isocratic elution with CH_3CN/H_2O = 9/1; flow rate = 0.3 mL/min. The $[Gd(TTDA\text{-}CGP)]^-$ solution was incubated with β–gal (7.2 nM) for 14 days at 37 ± 0.1°C in Tris buffer(0.1M, pH 7.3) and performed imaging using a MR scanner at 3.0T (GE Medical system, Gyroscan), head coil and spin echo pulse sequence. Other imaging parameters were as follows: slice thickness = 10 mm, matrix = 128 x 128, field of view = 24 cm, TE =14 msec, TR=200 msec and signal acquisition.

Results and Discussion

The sequence of the kinetic stability decreases in the following order: $[Gd(DTPA)]^{2-}$ > $[Gd(TTDA\text{-}CGP)]^-$ > $[Gd(TTDA\text{-}CGP)]^-$ + 2.4 nM β–gal >> [Gd(DTPA-BMA)]. Therefore, the kinetic stability toward Zn(II) transmetallation of $[Gd(TTDA\text{-}CGP)]^-$ in the presence and absence of β-gal is less than that of $[Gd(DTPA)]^{2-}$ but is strikingly higher than that of [Gd(DTPA-BMA)]. The cleavage of galactopyranose residue from $[Gd(TTDA\text{-}CGP)]^-$ in the presence of β-gal was examined by HPLC analysis. When the $[Gd(TTDA\text{-}CGP)]^-$ was incubated with β-gal (2.4 nM) at pH 7.3 in 0.1 M Tris buffer for 3 days, the peaks with the retention time of 6.2 min and 9.1 min appeared for $[Gd(TTDA\text{-}CGP)]^-$ and $[Gd(TTDA\text{-}CHE)]^-$, respectively. The HPLC data shows that the galactopyranose residue is removed by β-gal even at the extremely much lower concentration of β-gal (2.4 nM).

The number of inner-sphere water of $[Dy(TTDA\text{-}CGP)]^-$ with and without β-gal was contain 1.2 and 0.8 inner-sphere water molecules per Dy(III) ion, respectively. The inner-sphere water molecules do not change after incubation for 14 days. The number of Ln(III)-bound water molecules in this complex provides information about concerning the coordination mode of the ligand. The probable cause of this is that the position of the galactopyranose residue does hinder the water-binding site of Dy(III) ion. These results are similar to that of smart MRI agent Egad (4,7,10-tri(acetic acid)-1-(2-β-galactopyranosylethoxy)-1,4,7,10-tetraazacyclododecane) gadolinium).[2] Therefore, the number of inner-sphere water molecule of $[Gd(TTDA\text{-}CGP)]^-$ is noticeably changed (about 33%) in the presence of β-gal.

The effect of the presence of β-gal cleavage on the galactopyranose residue from the $[Gd(TTDA\text{-}CGP)]^-$ chelate on the T_1 was assessed by 400 MHz(9.4T) NMR spectroscopy. The results indicate that the presence of β-gal is significantly decreasing the T_1 value of the $[Gd(TTDA\text{-}CGP)]^-$. The higher concentration of β-gal results in a huge decrease in the T_1 value of about 25%. The control experiment using $[Gd(TTDA\text{-}CGP)]^-$ was incubated with heat-inactivated β-gal. It shows that the T_1 value increases slightly. The enzymatic $[Gd(TTDA\text{-}CGP)]^-$, therefore, renders to cleavage the galactopyranose residue by the active β-gal enzyme.

The water exchange rate k_{ex}^{298} value of $[Gd(TTDA\text{-}CGP)]^-$ (125×10^6 s^{-1}) is significantly higher than those of $[Gd(TTDA\text{-}CHE)]^-$ (32.2×10^6 s^{-1}) and $[Gd(DTPA)]^{2-}$ (4.1×10^6 s^{-1}) but lower than that of $[Gd(TTDA)]^{2-}$ (146×10^6 s^{-1}). The substitution of an acetate arm with a hydroxyl group on the linear poly(aminocarboxylate) ligand (TTDA-CHE) caused a meaningful effect in the exchange lifetime. The τ_R value of $[Gd(TTDA\text{-}CHE)]^-$ (114 ps) is similar to that of $[Gd(TTDA)]^{2-}$ (104 ps) and is significantly lower than that of $[Gd(TTDA\text{-}CGP)]^-$ (175 ps). The higher τ_R value for $[Gd(TTDA\text{-}CGP)]^-$ compared to $[Gd(TTDA)]^{2-}$ and $[Gd(TTDA\text{-}CHE)]^-$ indicates that the replacement of the middle carboxylate group by a galactopyranose group increases the τ_R value.

The signal intensity of the MR image for $[Gd(TTDA\text{-}CGP)]^-$ solution with and without β-gal were given. The enhancement result of the enzymatic cleavage of $[Gd(TTDA\text{-}CGP)]^-$ indicates that the enhancement (77.6±1.2%) is saliently higher than before the galactopyranose residue is removed.

Conclusion

In summary, a linear poly(aminocarboxylate) ligand, TTDA-CGP, forms a kinetically stable complex with trivalent Gd(III) ion. It does not exchange with divalent Zn(II) to an appreciable extent. The water exchange rate values of $[Gd(TTDA\text{-}CGP)]^-$ in the presence and absence of β-gal are significantly higher than that of $[Gd(DTPA)]^{2-}$. Inner-sphere water increase, spin-lattice relaxation time decrease and MR image enhancement increase are observed for the $[Gd(TTDA\text{-}CGP)]^-$ in the presence of β-gal. Therefore, $[Gd(TTDA\text{-}CGP)]^-$ possesses higher kinetic stability, higher enzymatic cleavage, shorter water residence lifetime, longer rotational correlation time and high relaxivity that might result in a novel type of contrast agent for the visualization of gene expression in living animals by MRI.

References

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