A bio-activated paramagnetic Gd(III) complex [Gd(DO3A-FPG)] for MRI

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Abstract

In this study, a novel β-galactopyanose-containing gadolinium(III) complex, [Gd(DO3A-FPG)] (DO3A-FPG = 1-(2-difluoromethyl-4-(1-(4,7,10-triscarboxymethyl-(1,4,7,10-tetraazacyclodecyl))acetamido)phenyl)-β-D-galacopyranose shown in Fig. 1), was designed, synthesized and characterized. The number of inner-sphere water of [Gd(DO3A-FPG)] was obtained by the Dy(III)-induced ^{17}O water NMR shifts (q = 0.92) and Eu(III) luminescence experiment (q = 1.08). The ^{17}O NMR experiments were conducted to estimate the water residence lifetime (τ_M) and rotational correctional time (τ_R). The τ_M value of [Gd(DO3A-FPG)] (339 ns) is higher than that of $[Gd(DOTA)]^-$ (208 ns) (DOTA = 1,4,7,10-tetrakis-(carboxymethyl)-1,4,7,10-tetraazacyclododecane). The τ_R value of [Gd(DO3A-FPG)] (279 ps) is significantly longer than that of $[Gd(DOTA)]^-$ (90 ps). This phenomenon indicates that the replacement of one carboxylate group by a galactopyranose residue increases the τ_R value. The increasing relaxivity (r_1) and enhancement for MRI obtained in the presence of β-galactosidase (β-gal) and bovine serum albumin (BSA) for [Gd(DO3A-FPG)] in PBS buffer solution indicates that the BSA or β-gal binds to Gd(III) chelate after the galactopyranose residue removed.

Introduction

MR Image offers several advantages over other clinical diagnostic techniques for molecular imaging, including high spatial resolution, non-invasiveness, high anatomical contrast, and lack of harmful radiation. However, sensitivity of MRI to depict small molecular is constrained by the ubiquitous protons in the body, resulting in a high background, hence, lower signal-to-noise ratio (SNR). The different signal characteristics upon interaction with the specific target by smart contrast agents could provide the new ways for detecting stages of biological molecules. Other enzyme motif such as β-gal, an often-used gene reporter enzyme in molecular biology, has been explored for smart MR contrasting [1,2]. In this study, we synthesized and characterized an enzymatic contrast agent, which could be activated by β-galactosidase. As an attempt to achieve improvements in enzyme mediated relaxivity enhancement, we decide to link a bio-activated group onto gadolinium chelate in order to achieve conjugation and, therefore, higher molecular weight of the final product. The τ_M and τ_R values of [Gd(DO3A-FPG)] were obtained from the ^{17}O NMR. The kinetics of enzyme-catalyzed hydrolysis of [Gd(DO3A-FPG)] were measured at 20 MHz relaxometer. The MR imaging of the [Gd(DO3A-FPG)] in the absence and presence of β-gal was also investigated.

Methods

Relaxation times T_1 of aqueous solutions of gadolinium complexes were measured to determine relaxivity r_1. All measurements were made using a NMR relaxometer operating at 20 MHz and 37.0 ± 0.1 °C. The number of inner-sphere water was determined by ^{17}O NMR chemical shift of the water as a function of Dy(III) concentration [3]. The Eu(III) luminescence spectroscopy to measure directly the first coordination sphere water of the metal ion [4]. For the variable temperatures ^{17}O NMR measurements, the concentrations and pH values of the solutions used were as follows; [Gd(DO3A-FPG)]: 0.050 mol kg^{-1}, pH 5.2. The reduced ^{17}O NMR transverse and longitudinal relaxation rate and chemical shifts data were analyzed together to determine the τ_M and τ_R values [5].

Results and Discussion

The purity of ligand and Gd(III) complex was performed by HPLC. The r_1 value of [Gd(DO3A-FPG)] is 3.96 mM^{-1} s^{-1}, which is slightly higher than that of $[Gd(DOTA)]^-$ (3.38 mM^{-1} s^{-1})[5]. The τ_M value of [Gd(DO3A-FPG)] (339 ns) is slightly higher than that of $[Gd(DOTA)]^-$ (208 ns)[6]. The substitution of an acetate arm with a amide group caused a meaningful effect in the residence lifetime. The τ_R value of [Gd(DO3A-FPG)] (279 ps) is significantly higher than that of $[Gd(DOTA)]^-$ (90 ps)[6]. The higher τ_R value for [Gd(DO3A-FPG)] compared to $[Gd(DOTA)]^-$ indicates that the replacement of the carboxylate group by a galactopyranose group increases the τ_R value. In Fig. 2, the kinetics of enzyme-catalyzed hydrolysis of [Gd(DO3A-FPG)] were measured, the T_1 value of [Gd(DO3A-FPG)] decreasing about 59 % after enzymatic cleavage in BSA. The signal intensity of the MR image for [Gd(DO3A-FPG)] solution with and without β-gal in BSA solution was shown in Fig. 3. The enhancement result of the enzymatic cleavage of [Gd(DO3A-FPG)] for MRI indicates that the enhancement (27%) is saliently higher than before the galactopyranose residue is removed.

Conclusion

A novel bio-activated MRI contrast agent containing galactopyranose residue, [Gd(DO3A-FPG)], was designed and synthesized successfully. Relaxation time (T_1) decreasing (59 %) of enzymatic cleavage of [Gd(DO3A-FPG)] in BSA indicates that the BSA or β-gal binds to Gd(III) chelate after the galactopyranose residue removed. MR image significant enhancement was observed for [Gd(DO3A-FPG)] solution in the presence of β-gal and BSA. [Gd(DO3A-FPG)] possesses enzymatic cleavage, longer rotational correlation time, higher relaxation rate and higher MR image enhancement in the presence of β-gal that might result in a novel type of contrast agent for visualization of gene expression by MRI.

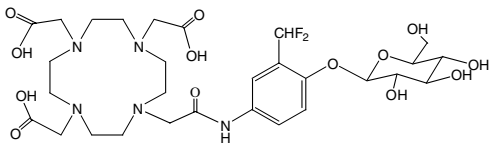

Fig. 1 Structural formula of the ligand DO3A-FPG

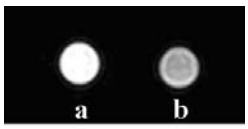

Fig. 3 a) 0.8 mM [Gd(DO3A-FPG)], 0.95mg/ml (2 μM) β-gal, in 100 mM PBS solution and b) 0.8 mM [Gd(DO3A-FPG)] in 100 mM PBS solution.

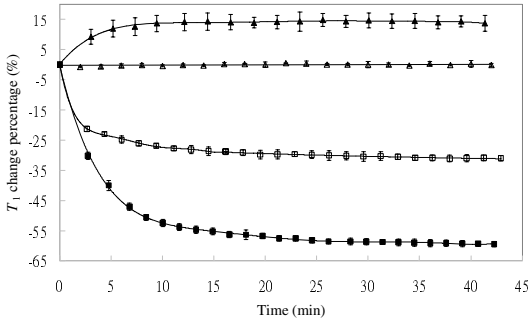

Fig. 2 Kinetics of enzyme-catalyzed hydrolysis of [Gd(DO3A-FPG)] measured by bulk water T_1 relaxation at 20 MHz, 37 ± 0.1 °C. (Δ) 0.5 mM [Gd(DO3A-FPG)] in 100 mM PBS solution; (▲) 0.5 mM [Gd(DO3A-FPG)] and 0.95 mg/mL of β-gal in 100 mM PBS solution; (□) 0.5 mM [Gd (DO3A-FPG)] and 0.5mM BSA in 100 mM PBS solution; (■) 0.5 mM [Gd(DO3A-FPG)], 0.95 mg/mL of β-gal and 0.5 mM BSA in 100 mM PBS solution.

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