Polyhedron 38 (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

A new potential contrast agent for magnetic resonance imaging: Synthesis and relaxivity studies of a gadolinium(III) complex of glucose-6-phosphate conjugated 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid

S. James Ratnakar, N. Arockia Samy, V. Alexander *

Department of Chemistry, Loyola College, Chennai 600034, India

ARTICLE INFO

Article history:
Received 12 December 2011
Accepted 2 February 2012
Available online 8 March 2012

Keywords:
MRI
Gadolinium complexes
Contrast agents
Relaxivity
Glucose-6-phosphate
Polyazacarboxylate ligands
Lanthanide complexes

ABSTRACT

Synthesis and longitudinal and transverse relaxivities of a gadolinium(III) complex, [Gd(DO3A-Pr-Glu-6-phos)$(H_2O)_2$] (**4**), of glucose-6-phosphate conjugated DO3A (DO3A-Pr-Glu-6-phos = 10-(3-(glucose-6-phosphate)oxypropyl)-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane and DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) are reported. DO3A-Pr-Glu-6-phos (**L**[1]) is synthesized by the reaction of bromopropane appended DO3A (**3**) with glucose-6-phosphate at room temperature. The magnetic moment of **4** is 7.49 BM, which is close to that of the free gadolinium(III) ion. The X- and Q-band epr spectra of **4** at LNT show a broad band with *g*-values of 2.167 and 2.033, respectively. The higher longitudinal relaxivity of **4** (r_{1p} = 6.99 mM^{-1} s^{-1}, 24 MHz, 35 °C ± 0.1) than that of $[Gd(DOTA)(H_2O)]^-$ (r_{1p} = 3.56 mM^{-1} s^{-1}, 20 MHz, 39 °C, pH 7.3) and [Gd(DO3A)$(H_2O)_2$] (r_{1p} = 4.8 mM^{-1} s^{-1}, 20 MHz, 40 °C) is attributed to the nature of the glucose-6-phosphate pendant arm. The longitudinal relaxivity of the complex in the presence of β-cyclodextrin increases to 9.62 mM^{-1} s^{-1} due to the formation of the inclusion complex. The transverse relaxivity of **4** is 7.02 mM^{-1} s^{-1} and the r_{2p}/r_{1p} ratio of 1.01 indicates that it is a T_1-weighted contrast agent.

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1. Introduction

The development of magnetic resonance imaging (MRI) as a clinical modality has provoked an explosive growth of interest in the study of gadolinium(III) complexes as contrast enhancing agents (CAs) for MRI [1–3]. This technique relies upon the acquisition of the images of soft tissues which are the topological representation of in vivo water protons. The image contrast is based on the differences in the proton relaxation rates of water molecules in different tissues. Contrast agents enhance the intrinsic contrast of the magnetic resonance images by accelerating the relaxation rates of water protons. Gadolinium(III) complexes of polyazacarboxylate ligands have been extensively studied as CAs and the topic has been reviewed [4–10]. New classes of MRI contrast agents [11,12] such as blood pool agents [13–17], targeting CAs [11] and smart contrast agents such as those that are pH sensitive [18–22], oxygen pressure (pO_2) responsive [23,24], enzyme responsive [25–27] and metal ion sensitive [28–31] have been reported. Receptor induced magnetization enhancement (RIME) CAs involving targeting Gd(III) chelates to protein binding sites [32,33], chemical exchange saturation transfer (CEST) CAs [34–37] and LIPOCEST agents for improved sensitivity [38] have also been reported.

The FDA approved CAs are low molecular weight gadolinium(III) chelates which exhibit poor bioselectivity for tissues and organs. They are cleared rapidly from the blood stream and a higher dosage is required for prolonged clinical examinations. There is an ongoing demand for the development of highly efficient pathology specific and organ specific CAs. In general, in the case of gadolinium(III)-based CAs, higher relaxivity may be achieved by improving the inner-sphere relaxivity which, according to SBM theory [39], depends primarily on the number of coordinated water molecules (q) and the correlation time (τ_c). The correlation time τ_c, which modulates the dipole–dipole relaxation mechanism, depends on the molecular rotational correlation time (τ_R) of the complex, the exchange rate ($k_{ex} = 1/\tau_m$) of the coordinated water molecules and the electronic relaxation time ($T_{1e,2e}$) of the metal ion. One important strategy to attain high relaxivity is by slowing down the molecular rotation by increasing the molecular weight and dimension by binding gadolinium(III) chelates to systems of different dimensions [1,2].

Biomolecules-conjugated gadolinium(III) chelates are expected to be biocompatible and increase relaxivity by slowing down the molecular rotation in solution. Examples of biomolecule- and biomacromolecule conjugated gadolinium(III) chelates include a gadolinium(III) chelate of galactopyranose functionalized DO3A, which

* Corresponding author. Tel.: +91 44 28175351; fax: +91 44 28175566.
E-mail address: valexander@rediffmail.com (V. Alexander).

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http://dx.doi.org/10.1016/j.poly.2012.02.022

exhibits selective activation from low to high relaxivity (r_{1p} = 0.903–2.72 mM^{-1} s^{-1}) with respect to the enzymatic response by Meade and co-workers [25], a Gd(DO3A) chelate appended onto a polysaccharide backbone [starch-(Gd-DO3A)] (r_{1p} = 14.1 ± 0.1 mM^{-1} s^{-1} and r_{2p} = 17.8 ± 0.9 mM^{-1} s^{-1}, 20 MHz, 39 °C) by Brasch and co-workers [40], a Gd(DO3A)-functionalized macromolecular conjugate of dextran [CMD-A_2-Gd-(DO3A)] (r_{1p} = 10.59 mM^{-1} s^{-1}, 37 °C) as an intravascular CA for myocardial perfusion by Corot et al. [41], DO3A conjugated cholesterol for adrenal imaging by Muhler et al. [42], a gadolinium(III) chelate of glucitol conjugated DO3A (r_{1p} = 5.19 mM^{-1} s^{-1}) by Aime et al. [43], and the gadolinium(III) complex of ATP-conjugated DO3A [Gd(DO3A-Pr-ATP)(H_2O)$_2$] (r_{1p} = 6.51 and 5.64 mM^{-1} s^{-1} at pH 5.6 and 8.4, respectively, and r_{2p} = 7.48 mM^{-1} s^{-1}, 24 MHz, 35 ± 0.1 °C) [44]. Gadolinium(III) complexes of phosphonic acid and phosphinate based pendant arms have also been reported [45–51]. We report herein the synthesis and relaxivity studies of a gadolinium(III) chelate of glucose-6-phosphate conjugated DO3A (**L**[1]) (Chart 1).

2. Experimental

2.1. Chemicals

Triethylenetetraamine (99%), *p*-toluenesulfonyl chloride, 1,2-dibromoethane, 1,3-dibromopropane, chloroacetic acid, xylenol orange, β-cyclodextrin, Celite, potassium carbonate anhydrous and Tris buffer (E. Merk, India) were used as received. Glucose-6-phosphate monosodium salt (Aldrich) was converted into the free form by passing it through Amberlite IR120 (H^+, strongly acidic) (Aldrich) cation exchange resin and eluting with triply distilled water. The resin was washed with distilled water five times before use. Triethylamine and perchloric acid (Merck, India); sodium hydroxide and potassium hydroxide pellets (Rankem, India); gadolinium(III) carbonate (Indian Rare Earths Ltd.) were used as received. Gadolinium(III) perchlorate hydrate was prepared from gadolinium(III) carbonate and perchloric acid in water and recrystallized from triply distilled water prior to use. Cyclen was synthesized by the method of Chavez and Sherry [52]. The solvents were purified by standard procedures [53]. HPLC grade water was used for the relaxivity studies. DO3A and bromopropane appended DO3A were synthesized by the method reported by us [44].

2.2. Physical measurements

Infrared spectra were recorded on a Perkin-Elmer Spectrum RX-I FT-IR spectrometer in the range 4000–400 cm^{-1} using KBr pellets. Potassium bromide (FT IR grade, Aldrich) was used to make the pellets. FAB mass spectra were recorded on a Jeol SX-102/DA 6000 mass spectrometer/data system using argon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzyl alcohol as the matrix. Electrospray ionization mass spectra were recorded using a Micromass Quattro-II Triple Quadrupole mass spectrometer. The sample was dissolved in water and introduced into the ESI capillary using a 5 μL syringe pump. The ESI capillary was set at 3.5 kV with a cone voltage of 40 V. CHN microanalyses were carried out using a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer interfaced with a Perkin-Elmer AD 6 Autobalance. Helium (analytical grade) was used as the carrier gas. Analytical and preparative HPLC analyses were carried out using a Varian PrepStar 218 (Varian Instruments Inc., USA) binary gradient solvent delivery module with an inline three channel degasser Model 2000 for solvent delivery. A Rheodyne injector valve (20 μL) was used for the sample injection. HPLC column (250 × 4.6 × 1/4″ Valco, Microsorb-MV 100-5 C18, analytical) was used for the HPLC analysis of the free ligand and its gadolinium(III) complex. A UV–Vis detector (Model 345) operating in the range 190–1100 nm was used. The fraction was collected using a Model 704 fraction collector. EPR spectra were recorded on a Jeol instrument at the Q-band (34.5 MHz) and the X-band (9.4 MHz) with a scan range of 8 kG and the field was set at 12 500 T. Magnetic susceptibility measurements were carried out on an EG&G PAR Model 155 vibrating sample magnetometer at 25 °C. ^1H and ^{13}C NMR spectra were recorded in D_2O and $CDCl_3$ (99.95 atom % D, Aldrich) on a Jeol GSX-400 multinuclear NMR spectrometer working at 400 MHz (for ^1H) and at 100 MHz (for ^{13}C) at 25 °C.

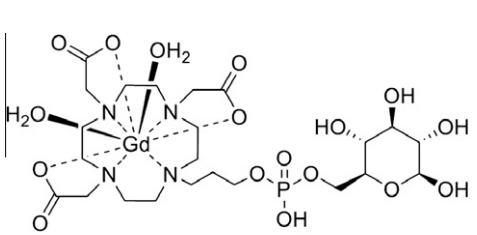


Chart 1. Structure of the gadolinium(III) complex of 10-(3-(glucose-6-phosphate)oxypropyl)-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane.

2.3. Longitudinal relaxivity (r_{1p}) measurements

The longitudinal relaxivity of the gadolinium(III) complex was determined from the spin lattice relaxation time (T_1). The T_1 measurements were carried out on a Maran wide line NMR (Resonance Instruments Ltd., UK) operating at 24 MHz and 35 ± 0.1 °C. The temperature was controlled using a temperature console. The solutions of the complex were taken in a 10 mm stoppered quartz tube and the instrument parameters were optimized for each T_1 measurement. Solutions of five concentrations of the complex were prepared in HPLC grade water (Merck, India) in a 5 mL standard measuring flask (Vensil, Class "A"). The presence of the free gadolinium(III) ion in the solution has been checked by the xylenol orange test. The T_1 measurements were made using the standard inversion recovery pulse sequence (180°-τ-90°) with phase sensitive detection [54], with τ values ranging from 50 μs to 6 s for each concentration of the complex. The computer program WINFIT was used to plot the time versus signal intensity to get an exponential plot, and the T_1 values were calculated from the plot. A plot of $1/T_1$ versus concentration of the complex gave a straight line and the slope was taken as the longitudinal relaxivity (r_{1p}), which was normalized to 1 mM concentration of the complex. The longitudinal relaxivity at pH 8.5 was measured by adding Tris buffer. The longitudinal relaxivity in the presence of β-cyclodextrin was determined by adding an aqueous solution of β-cyclodextrin and keeping the solution overnight at ambient temperature to attain equilibrium. The concentration of β-cyclodextrin was maintained 50 times higher than that of the complex to ensure the complete formation of the inclusion complex.

2.4. Transverse relaxivity (r_{2p}) measurements

The transverse relaxivity was determined from the spin–spin relaxation time (T_2) under the same experimental conditions used to determine longitudinal relaxivity. A standard CPMG (Carr–Purcell–Meiboom–Gill) pulse sequence (90°-τ-180°) [55] with a τ value of 50 μs was used to determine T_2. The computer program WINFIT was used to plot the time versus signal intensity to get an exponential curve, and T_2 was calculated from the graph. The T_2 values for five different concentrations of the complex

were measured. The transverse relaxivity was calculated from the slope of the regression line, obtained by the plot of $1/T_2$ versus concentration of the complex by least squares fitting method. Five different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mmol) of the complex were prepared in HPLC grade water.

2.5. 10-(3-(Glucose-6-phosphate)oxypropyl)-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A-Pr-Glu-6-phos) (**L¹**)

Glucose-6-phosphate monosodium salt (0.28 g, 1 mmol) in 10 mL water was passed through a column packed with Amberlite IR 120 (H^+, strongly acidic) cation exchange resin and eluted with deionized water. To the eluent was added bromopropane appended DO3A (**3**) (0.46 g, 1 mmol) in 25 mL water and the resulting mixture was stirred for 20 h at room temperature. The solvent was removed in a rotavapor. The resulting white solid was washed with acetone, recrystallized in water, dried in a vacuum desiccator and then stored in a refrigerator: yield 56%, mp 135 °C (dec). The purity of **L¹** was checked by HPLC (analytical, C18 column, H_2O, UV–Vis detector: 234 nm, flow rate 1 mL per min, R_f = 4.75). IR (KBr, cm^{-1}): 3366 ν_s(OH), 2903 ν_s(CH), 1454 ν(CH), 1391 δ_s(OH), 1080 ν_s(CN), 1191 ν_s(CO), 1271 ν_s(P=O), 910 ν_s(P–O). FAB MS: *m*/*z* 645 $[M-H]^+$ $(C_{23}H_{42}N_4O_{15}P)^+$, 492 $[M-C_5H_{13}O_5]^+$ $(C_{18}H_{29}N_4O_{10}P)^+$, 460 $[M-C_6H_{17}O_6]^+$ $(C_{17}H_{25}N_4O_9P)^+$, 154 $[M-C_{15}H_{26}NO_{15}P]^+$ $(C_8H_{16}N_3)^+$. ^{1}H NMR (400 MHz, D_2O, TMS) δ: 1.28 (2H, CH_2), 2.72 (m, 2H, CH_2), 3.55 (m, 12H, NCH_2CH_2N), 3.27 (m, 2H, OCH_2), 3.72 (m, 2H, OCH_2) 4.04 (m, 4H, CHOH), 5.23 (s, 6H, CH_2COOH). ^{13}C NMR (100 MHz, D_2O) δ: 37.4 (CH_2), 52.6 (CH_2), 66.0 (CH_2COOH), 73.0–78.0 (CH_2OH), 95.0 (OCH_2), 98.9 (OCH_2), 185.7 (COOH). *Anal.* Calc. for $C_{23}H_{43}N_4O_{15}P$ (M_r = 646.05): C, 42.25; H, 6.46; N, 8.63. Found: C, 42.15; H, 6.35; N, 8.56%.

2.6. {10-(3-(Glucose-6-phosphate)oxypropyl)-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane}(diaqua)gadolinium(III) [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] (**4**)

To a solution of DO3A-Pr-Glu-6-phos (**L¹**) (0.16 g, 0.25 mmol) in 30 mL water was added gadolinium(III) perchlorate (0.11 g, 0.25 mmol) and the resulting mixture was stirred at room temperature (30 °C, pH 5) for 36 h. The unreacted gadolinium(III) ions were removed by treating with Dowex cation exchange resin and the absence of free gadolinium(III) ion was tested using xylenol orange indicator [56]. The complex was isolated as white flakes by distilling off the solvent to dryness in a rotavapor, recrystallized in water and then stored at 4 °C: yield 53%, mp 170 °C (dec). The purity of **4** was checked by HPLC (analytical, C18 column, H_2O, UV–Vis detector: flow rate 1 mL per min, R_f = 4.58). IR (KBr, cm^{-1}): 3427 ν_s(OH), 2942 ν_s(CH), 1653 ν_s(C=O), 1617 δ_s(OH), 1024 ν_s(CN), 1462 ν_s(CH), 1403 ν_s(C–O). ESI MS: *m*/*z* 834 $[M-2H]^+$ $(C_{23}H_{44}GdN_4O_{17}P)^+$, 818 $[M-H_2O]^+$ $(C_{23}H_{42}GdN_4O_{16}P)^+$, 653 $[M-C_5H_{11}O_7]^+$ $(C_{18}H_{33}GdN_4O_{10}P)^+$, 623 $[M-C_6H_{13}O_8]^+$ $(C_{17}H_{31}GdN_4O_9P)^+$, 563 $[M-C_8H_{17}O_{10}]^+$ $(C_{15}H_{27}GdN_4O_7P)^+$, 427 $[M-C_{11}H_{24}O_{13}NP]^+$ $(C_{12}H_{20}GdN_3O_4)^+$. *Anal.* Calc. for $(C_{23}H_{40}GdN_4O_{15}P)(H_2O)_2$ (M_r = 836.05): C, 32.84; H, 5.02; N, 5.98. Found: C, 32.80; H, 5.01; N, 5.79%.

3. Results and discussion

3.1. Synthesis of the ligand and the complex

The glucose-6-phosphate conjugated DO3A (DO3A-Pr-Glu-6-phos) (**L¹**) was synthesized by the reaction of bromopropane appended DO3A (**3**) [44] with glucose-6-phosphate in a 1:1 mole ratio in water at room temperature (Scheme 1). The commercially available glucose-6-phosphate monosodium salt was converted into the free form by passing an aqueous solution through Amberlite (IR 120) cation exchange resin and eluting with water. The ligand **L¹** was isolated as a white solid in 56% yield in the pure form and the purity was checked by HPLC. It undergoes decomposition at room temperature when stored over long period of time, but is stable when stored below 4 °C. The direct reaction of the glucose-6-phosphate monosodium salt with the bromopropane-appended DO3A forms sodium bromide as a side product which is difficult to separate from **L¹**. The complex [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] was synthesized by the reaction of the preformed **L¹** with gadolinium(III) perchlorate in water at 30 °C (pH 5). The ligand **L¹** coordinates to the metal ion through the four amine nitrogen donors of the macrocycle and the three carboxylate oxygens of the acetate pendant arms. Two inner-sphere water molecules complete the coordination sphere.

3.2. NMR spectra of DO3A-Pr-Glu-6-phos

3.2.1. ^{1}H NMR spectrum of **L¹**

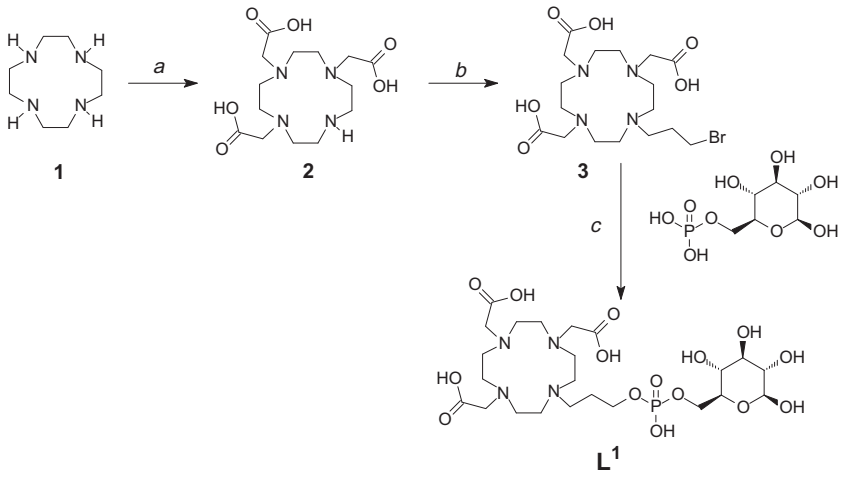
The ^{1}H NMR spectrum of DO3A-Pr-Glu-6-phos in D_2O shows a triplet at 1.28 ppm (t, 2H, CH_2) assignable to the middle methylene protons of the propyl spacer (N–CH_2–CH_2–CH_2–O–). The resonance at 2.72 ppm is assignable to the protons of the methylene group of the propyl spacer which is bonded to the cyclen framework (N–CH_2–CH_2–CH_2–O–). The resonances centered at 3.55 ppm (m, 12H) are assignable to the methylene protons N–CH_2–CH_2–N of the cyclen framework. The multiplet at 3.72 ppm (m, 2H) is assigned to the propyl CH_2 protons adjacent to the glucose-6-phosphate moiety (N–CH_2–CH_2–CH_2–O–). The resonance at 3.27 ppm (m, 2H, OCH_2) is assigned to the OCH_2 protons of glucose-6-phosphate. The resonance at 4.04 ppm (*m*, 4H) is assigned to the CHOH proton of the glucose moiety and the signal at 5.23 ppm is assigned to the methylene protons of the acetate pendant arms. The OH protons of the glucose moiety are in fast chemical exchange with D_2O and hence no detectable signal was observed.

3.2.2. ^{13}C NMR spectrum of DO3A-Pr-Glu-6-phos (**L¹**)

The ^{13}C NMR spectrum of DO3A-Pr-Glu-6-phos in D_2O shows six resonances. The resonance at 37.4 ppm is assigned to the methylene carbon (C_2) of the propyl linker. The signal at 95.0 ppm is assignable to the methylene carbon (C_3) of the propyl linker that bridges the glucose-6-phosphate and the macrocyclic ring. The signal at 52.6 ppm is assignable to the methylene carbon of the cyclen framework. The resonance at 66.0 ppm is assignable to the methylene carbon of the acetate arm. The signal at 185.7 ppm is assignable to the carbon of the carboxylic acid. The signals at 73.0–78.0 ppm are assignable to the carbon atoms of the glucose unit. The resonance at 98.9 ppm is assignable to the methylene carbon that bridges the phosphate group and the glucose moiety.

3.3. EPR spectra and magnetic moment of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$]

The X- and Q-band EPR powder spectra of the complex show a broad band with no hyperfine splitting at room temperature (g = 2.167 and 2.033 for the X- and Q-band, respectively). It shows a broad band with no hyperfine splitting at liquid nitrogen temperature (X-band, g = 2.022). The linewidth of the Q-band spectrum is 1250 G. The broad linewidth and the absence of hyperfine splitting indicate a weak crystal field effect and weak Zeeman interactions. The magnetic moment of [Gd(DO3A-Glu-6-phos)(H_2O)$_2$] is 7.49 BM, which is close to the theoretical value of the free gadolinium(III) ion. The trivalent gadolinium ion, with a half filled 4*f* shell, has an $^8S_{7/2}$ ground state; since L = 0, the orbital contribution is



Scheme 1. Synthesis of DO3A-Pr-Glu-6-phos (yield 56%). *Reagents and conditions:* (a) chloroacetic acid, −4 °C, water, NaOH, pH 10; (b) 1,3-dibromopropane, DMF–water, triethylamine; (c) water, RT, 36 h.

almost entirely quenched and an isotropic *g*-value of 1.99 is universal. Due to the long spin lattice relaxation times, EPR spectra are observed at room temperature. Its magnetochemistry is, therefore, straightforward and perhaps the best known of all the rare earths. Furthermore, the first excited state is located at some 30 000 cm^{-1} above the ground state. The zero field splitting within this ground state is very weak, of the order of 10^{-2} cm^{-1}, and not detectable in magnetism except in the temperature range of fractions of Kelvin. The magnetic susceptibility is almost perfectly isotropic and follows the Curie law [57,58].

3.4. Relaxivity of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] (**4**)

3.4.1. Longitudinal relaxivity

The complex [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] (**4**) exhibits a longitudinal relaxivity (r_{1p}) of 6.99 mM^{-1} s^{-1} (24 MHz, 35 °C ± 0.1, pH 5.6), which is higher than that of other systems with $q = 2$. In the case of gadolinium(III) chelates characterized by $q \geqslant 1$, a large contribution to the longitudinal relaxivity of water protons arises from the exchange of the bound water molecules with the bulk solvent. This contribution (r_{1p}^{is}) is determined by the relaxation time T_{1M} and the exchange lifetime (τ_m) of the protons of the water molecules in the inner-coordination sphere: $r_{1p}^{is} = 1.8 \times 10^{-5}\, q/(T_{1M} + \tau_m)$ [59,60]. Thus, r_{1p} is maximized when $T_{1M} > \tau_m$ (fast exchange condition) and T_{1M} is as short as possible. The coordination sphere of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] is expected to be similar to that of the parent complex [Gd(DO3A)(H_2O)$_2$], and the higher relaxivity of **4** than the latter indicates that the glucose-6-phosphate pendant arm plays a significant role in accelerating the relaxivity of the complex. The longitudinal relaxivity of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] at pH 8.4 (Tris buffer) is 3.84 mM^{-1} s^{-1}. The gadolinium(III) chelate of the ATP-conjugated DO3A also exhibits a similar behavior at pH 8.4 (r_{1p} = 5.64 mM^{-1} s^{-1}) [44].

3.4.2. Longitudinal relaxivity in the presence of β-cyclodextrin

The longitudinal relaxivity of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] in the presence of a known excess of β-cyclodextrin is found to be 9.62 mM^{-1} s^{-1}. The higher relaxivity of the complex in the presence of β-cyclodextrin indicates the formation of an inclusion complex between the glucose-6-phosphate pendant arm and the hydrophobic cavity of β-cyclodextrin. The complex is allowed to stand in the presence of a 50-fold excess of β-cyclodextrin for 12 h to attain equilibrium, to ensure the complete formation of the inclusion complex. The higher molecular dimension of the inclusion complex leads to an increase in the rotational correlation time (τ_R), leading to an enhancement of relaxivity. Aime et al. [61] have reported the formation of ternary inclusion complexes by gadolinium(III) chelates by the interaction with the hydrophobic cavity of β-cyclodextrin. Unsubstituted monomeric gadolinium(III) complexes do not show a significant increase in relaxivity in the presence of β-cyclodextrin, whereas there is a marked increase in the longitudinal relaxivity for the substituted derivatives since the host-guest interaction is enhanced through the non-covalent interaction of the substituent with the β-cyclodextrin [62,63]. The plot of concentration of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] versus $1/T_1$ in the presence and in the absence of β-cyclodextrin is given in Fig. 1.

3.4.3. Transverse relaxivity

The transverse relaxivity of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] is 7.02 mM^{-1} s^{-1} (24 MHz, 35 ± 0.1 °C), which is higher than that of $[Gd(DOTA)(H_2O)]^-$ (r_{2p} = 4.75 mM^{-1} s^{-1}, 20 MHz, 39 °C, pH 7.3) [43]. The r_{2p}/r_{1p} ratio of 1.01 indicates that the complex is a T_1-weighted contrast agent. This value determines the signal intensity attainable by CAs for MRI, with the highest signal intensity occurring when the ratio is unity. The transverse relaxivity of the Gd(III)

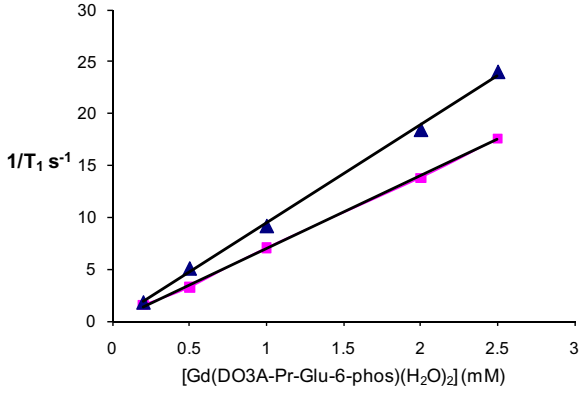


Fig. 1. The plot of the concentration of [Gd(DO3A-Pr-Glu-6-phos)(H_2O)$_2$] versus $1/T_1$ in the presence (◄) and in the absence (■) of β-cyclodextrin.

Table 1
Longitudinal and transverse relaxivities of gadolinium(III) complexes with $q = 2$.

Complex	r_{1p} ($mM^{-1}\ s^{-1}$)	r_{2p} ($mM^{-1}\ s^{-1}$)	q	Ref.
$[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$[a]	6.51, 5.64[b], 8.97[c]	7.48[d]	2	[44]
$[Gd(DO3A\text{-}Pr\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]$[a]	6.99, 3.84[b], 9.62[c]	7.02[d]	2	This work
$[Gd(DO3A)(H_2O)_2]$[e]	4.80		2	[64]
$[Gd(PCTA)(H_2O)_2]$[f]	5.42	6.52	2	[65]
$[Gd(PC2A)(H_2O)_2]^+$[f]	7.50	8.30	2	[65]
$[Gd(BP2A)(H_2O)_2]^+$[f]	8.27	8.63	2	[65]

DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid.
PCTA = 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetate.
PC2A = 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,9-diacetate.
BP2A = *N,N′*-diacetic acid-2,11-diaza[3.3]-(2,16)pyridinophane.
[a] 39 °C, 24 MHz.
[b] pH 8.4 (Tris buffer).
[c] In the presence of β-cyclodextrin.
[d] 24 MHz, 35 ± 0.1 °C.
[e] 40 °C, 20 MHz.
[f] 25 °C, 40 MHz, pH 7.0.

complex of the ATP-conjugated DO3A under the same experimental conditions is 7.48 $mM^{-1}\ s^{-1}$ [44].

3.5. Gd(III) chelates of ATP- and glucose-6-phosphate conjugated DO3A: a comparison

The gadolinium(III) chelates of ATP- and glucose-6-phosphate conjugated DO3A, $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ and $[Gd(DO3A\text{-}Pr\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]$, respectively, exhibit higher longitudinal and transverse relaxivities due to the presence of two inner-sphere water molecules and an increase in the molecular weight and dimension of the complexes compared to the parent complex $[Gd(DO3A)(H_2O)_2]$. The higher relaxivity of these complexes compared to other systems with $q = 2$ is explained by the role of τ_R in determining the relaxation behavior. An increase in the rotational correlation time results in a larger correlation time τ_c, which modulates the dipole–dipole interaction between the proton spin of water molecules and the electron spin of the metal ion. The relaxivity values of these two complexes are of the same order of magnitude indicating the presence of the same number of coordinated water molecules in the inner-coordination sphere. The relaxivities of other $q = 2$ systems are presented in Table 1. The glucose-6-phosphate and ATP pendant arms, covalently appended to the DO3A through the propyl spacer, have no significant influence on the coordination of these ligands to gadolinium(III).

The ratio of the r_{1p} values of the two complexes ($r_{1p[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]}/r_{1p[Gd(DO3A\text{-}Pr\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]}$) is 0.93 (neat solution) and 1.47 (pH 8.4). The $r_{2p[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]}/r_{2p[Gd(DO3A\text{-}Pr\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]}$ ratio is 1.07. Both complexes exhibit a decrease in proton relaxivity at pH 8.4: $[Gd(DO3A\text{-}Pr\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]$ exhibits a reduction of 45% while $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ shows a reduction of 13%. The longitudinal relaxivities of both complexes for the neat solution and at pH 8.4 indicate that these complexes exhibit similar behavior. Nevertheless, the substantial reduction in the r_{1p} value (45%) for the glucose-6-phosphate conjugated chelate at higher pH (8.4) indicates the importance of the pendant arm functionality in modulating the relaxation behavior. The enhancement in the relaxivity of $[Gd(DO3A\text{-}Pr\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]$ in the presence of β-cyclodextrin is higher than that of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$, probably due to the difference in the interaction of the pendant arms with β-cyclodextrin. The interaction of the complex with β-cyclodextrin is stronger and the formation of the inclusion complex is more pronounced for $[Gd(DO3A\text{-}Pr\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]$ than for $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ due to less steric hindrance of the glucose-6-phosphate pendant arm.

4. Conclusion

The advent of attaching metal complexes to biological molecules has created a new realm of research in the field of bioconjugate chemistry. The gadolinium(III) chelate of the glucose-6-phosphate conjugated DO3A, $[Gd(DO3A\text{-}Glu\text{-}6\text{-}phos)(H_2O)_2]$ (**4**), exhibits higher longitudinal and transverse relaxivities than that of the parent $[Gd(DO3A)(H_2O)_2]$ chelate due to the nature of the pendant arm. The covalent conjugation of glucose-6-phosphate makes the complex to remain intact under different experimental conditions, and the complex is expected to be biocompatible. There is a wide scope for studying the relaxivity of this complex in the presence of blood serum albumin to evaluate its suitability as a blood pool agent. Furthermore, biomolecule-conjugated Gd(III) chelates that have affinity for specific tissues and biomolecules could be employed to study biological processes and physiological functions.

Acknowledgments

This research was carried out with the financial support from the Department of Biotechnology (DBT), Government of India. We thank Dr. Moni, Sophisticated Analytical Instrumentation Facility (SAIF), IIT-M, Chennai, for his help in recording NMR spectra.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.poly.2012.02.022.

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