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RESEARCH ARTICLE

A conjugated polymer–Gd (III) complex as pH sensitive contrast agent in magnetic resonance imaging

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A novel pH-responsive contrast agent (PFP-aa/Gd) for magnetic resonance imaging (MRI) was prepared by binding Gd(III) to a water-soluble conjugated polyfluorene with pendant carboxylate and amine moieties. The PFP-aa is a good chelator for Gd^{3+} and the PFP-aa/Gd complex has good stability. As the pH changes from 10.0 to 4.0, both the carboxylate and amine are protonated, thus PFP-aa exhibits positive charges and forms tight aggregation, which reduces molecular tumbling and accelerates the exchange of bound water leading to the increase of relaxivity R_1. More importantly, the R_1 increases by about eight fold as the pH changes from 8.0 to 6.0, which makes PFP-aa/Gd suitable as a potential marker of the pH below physiological level. In comparison to other contrast agents, the unique sensitivity of the water relaxivity of PFP-aa/Gd indicates that this complex could be used in MRI experiments to monitor physiological pH change.

Keywords magnetic resonance imaging, pH-sensitive, conjugated polymer, Gd (III) chelate

1 Introduction

In recent years, magnetic resonance imaging (MRI) has become a powerful tool in clinical diagnosis [1,2]. A key advantage of MRI is that it provides the ability to diagnose abnormalities, especially tumors, *in vivo* in a non-invasive fashion. MRI is based on the NMR signal from the protons of water, where the signal intensity depends on the water concentration and relaxation times (T_1 and T_2). The contrast-enhancing ability of contrast agents is evaluated by relaxivity ($R_1 = 1/T_1$) and is usually realized by reducing the longitudinal relaxation time (T_1) of water protons. In other words, the higher the R_1, the larger the signal enhancement detected in the corresponding T_1-weighted images. Paramagnetic Gd (III) chelates are widely used as contrast agents for MRI to enhance image contrast due to their abilities to shorten the relaxation times and enhance R_1 [3–5].

It's well known that the pH in human tumors is lower than that of normal tissues; the fact provides clues to diagnosing tumors by monitoring pH changes by the MRI technique [6–9]. Usually it is necessary to use contrast agents to enhance signal differences between normal and diseased tissues or cells. By linking Gd (III) chelates to liposomes [7,10,11], dendrimers [12] and polyion complexes [13], a variety of contrast agents responsive to pH have been reported. Although these systems exhibit enhanced images as the solution is changed from basic to acidic, there is usually only a two or three times increase in R_1 as the pH changes in solution.

Very recently, we prepared a new MRI contrast agent by covalently linking Gd (III) chelates to the side chain of water-soluble conjugated polyfluorene (PF-Gd) that exhibits a higher relaxivity and a pronounced enhancement in contrast, compared to that of commercial $(NMG)_2$-Gd-DTPA [14]. The rigidity of the conjugated polymer backbone may result in an increase in the rotational correlation time and, subsequently, an increase in relaxivity. In addition, the formation of aggregation due to the amphiphilic characteristic of water-soluble conjugated polyfluorene could also play a role in the increase of relaxivity [15,16]. Herein, we present a novel pH-responsive MRI contrast agent by binding Gd (III) to conjugated polyfluorene (PFP-aa/Gd). The chemical structure of PFP-aa, as shown in Figure 1, has two amino and four

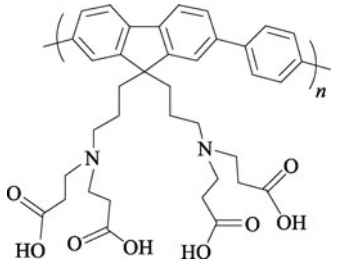

Figure 1 The chemical structure of PFP-aa.

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carboxylic acid groups in each repeat unit (RU). Our previous studies showed that the protonation and deprotonation of both the carboxylate and amine were controlled by medium pH values, and different fluorescent responses of the PFP-aa was demonstrated as environmental pH changed from 3 to 12 [17]. In this paper, we show that the pendant structure of PFP-aa is a good chelator for Gd^{3+}. The R_1 of PFP-aa/Gd exhibits large enhancement as the pH changes from basic to acidic. Especially, the R_1 increases by about eight fold as the pH changes from 8.0 to 6.0, thus the PFP-aa/Gd complex shows good potential as an MRI contrast agent for pH detection.

2 Experimental methods

2.1 Materials and measurements

PFP-aa was synthesized according to our reported procedure [17]. The concentration of Gd (III) was determined by inductive coupled plasma emission spectrometry (ICP). All the measurements were performed in 50 mmol·L^{-1} 2-(N-morpholino)ethanesulfonic acid buffer (MES). The pH value of solutions were adjusted with NaOH or HCl. Fluorescence measurements were obtained in 3 mL quartz cuvettes at room temperature using a Hitachi F-4500 fluorimeter equipped with a Xenon lamp excitation source. MRI was performed on a Siemens 3T scanner (MAGNETOM Trio, A Tim System) with a TxRx Head coil at the BNU Imaging Center for Brain Research. T_1 values were measured using an Inversion Recovery TSE (IR-TSE) imaging sequence with varying IR times.

2.2 Preparation of PFP-aa/Gd complex

PFP-aa (6.4 mg) was dissolved in 10 mL of methanol with K_2CO_3 (6.4 mg). 10 mL of $GdCl_3$ solution in water (1 mmol·L^{-1}) was added into this solution and the mixture was stirred for 1 day. The precipitation was collected by centrifugation and washed with water to yield PFP-aa/Gd. It was dispersed in 5 mL of water for further use and determination of Gd (III) concentration.

2.3 Fluorescence quenching experiment of PFP-aa by Gd^{3+}

To the solution of PFP-aa ([PFP-aa] = 5.0×10^{-6} mol·L^{-1} in RUs) in MES buffer (50 mmol·L^{-1}) with varying pH (pH = 4.0, 5.0, 7.0, and 10.0), Gd^{3+} solution (0 ~ 1.0×10^{-6} mol·L^{-1}) was successively added at room temperature. The fluorescence spectra were measured with the excitation wavelength of 375 nm.

3 Results and discussion

The ability of PFP-aa to chelate Gd^{3+} at different pH has been studied by fluorescence quenching experiments in aqueous solution. The binding constant is related to the Stern-Volmer constant (K_{sv}) [18]. In these experiments, the PFP-aa was first dissolved in MES buffer (50 mmol·L^{-1}) with the concentration of 5×10^{-6} mol·L^{-1} in repeat units (RUs), and then the solution pH was adjusted from 4.0 to 10.0 with HCl or NaOH. At each pH value, the Gd^{3+} was successively added at room temperature, and the fluorescence spectra were measured with the excitation wavelength of 375 nm. As shown in Figure 2(a),

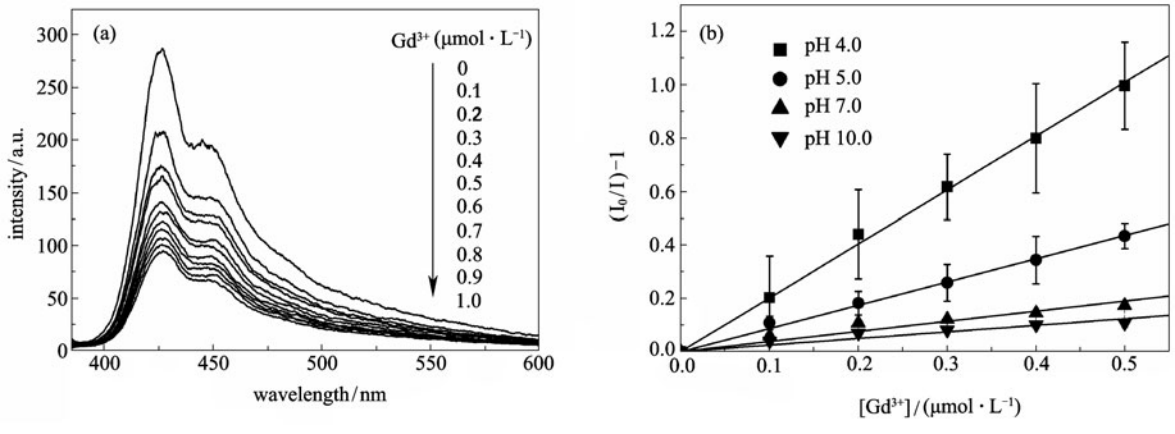

Figure 2 (a) Fluorescence emission spectra of PFP-aa in MES buffer (50 mmol·L^{-1}) as a function of Gd^{3+} concentration at pH of 4.0. (b) The Stern-Volmer plots of PFP-aa in the presence of Gd^{3+} (0 ~ 0.5 μmol·L^{-1}) at pH 4.0, 5.0, 7.0 and 10.0. [PFP-aa] = 5.0×10^{-6} mol·L^{-1} in RUs. The excitation wavelength is 375 nm.

upon the addition of Gd^{3+} from 0 to 1.0 μmol·L^{-1}, the fluorescence emission of PFP-aa is efficiently quenched at pH 4.0. The quenching efficiency is related to the K_{sv} and is determined by monitoring fluorescence changes of PFP-aa at 425 nm according to the Stern-Volmer equation [18]:

$$I_0/I = 1 + K_{sv}[Q],$$

where I_0 is the emission intensity of PFP-aa at 425 nm in the absence of the Gd^{3+} quencher, I is the emission intensity of PFP-aa in the presence of Gd^{3+}, and [Q] is the Gd^{3+} concentration.

Figure 2(b) shows the linear Stern-Volmer plots of PFP-aa under pH 4.0, 5.0, 7.0, 10.0 at low concentrations of Gd^{3+} (0 ~ 0.5 μmol·L^{-1}). The K_{sv} values of 2.02×10^6, 8.73×10^5, 3.80×10^5 and 2.48×10^5 L·mol^{-1} were obtained for pH 4.0, 5.0, 7.0 and 10.0, respectively. These results not only demonstrate the good chelating ability of PFP-aa to Gd^{3+}, but also show that the chelating ability of PFP-aa to Gd^{3+} is responsive to medium pH.

To further confirm the stability of the PFP-aa/Gd complex, we studied its fluorescence recovery upon addition of excess chelating EDTA at pH 7.0. As shown in Figure 3, the fluorescence of PFP-aa was efficiently quenched by three equivalent Gd^{3+} (relative to PFP-aa concentration in RUs). After six equivalent EDTA was added to the PFP-aa/Gd complex, there was only a little increase in the emission intensity of PFP-aa. This observation shows that the PFP-aa/Gd complex has good stability and the PFP-aa is a good chelator for Gd^{3+}.

The pH response of the PFP-aa/Gd contrast signal in MRI images was studied by measuring T_1 of water protons as functions of Gd (III) concentration and medium pH in aqueous solution. Figure 4(a) shows the T_1-weighted MRI of PFP-aa/Gd under pH 4.0 to 10.0 with varying Gd^{3+} concentrations. At each concentration of Gd^{3+}, the imaging contrast decreases as pH increases from pH 4.0 to 10.0. The R_1 values were obtained by taking the slope of a plot of T_1^{-1} versus Gd (III) concentration (Figure 4(b)). Figure 4(c) shows the relaxivity R_1 as a function of medium pH. The R_1 of PFP-aa/Gd exhibits large enhancement as the pH changes from 10.0 to 4.0. More importantly, the R_1 increases by about eight fold as the pH changes from 8.0 to 6.0, which is suitable for triggering the pH change from normal tissues or cells to tumors *in vivo*. At low pH, both the carboxylate and amine are protonated, thus PFP-aa exhibits positive charges and forms tight aggregation [17]. The aggregated morphology in acidic solution helps PFP-aa/Gd to reduce molecular tumbling, leading to the increase of R_1. Furthermore, for PFP-aa/Gd, the exchange of bound water may be accelerated by the protonation of adjacent carboxylic acid [19], also leading to the increase of R_1. The above results show that the MRI contrast of PFP-aa/Gd exhibits highly pH-responsive characteristics over a wide pH range as expected. Thus, the PFP-aa/Gd complex can be used as an MRI contrast agent for monitoring the pH change of the medium.

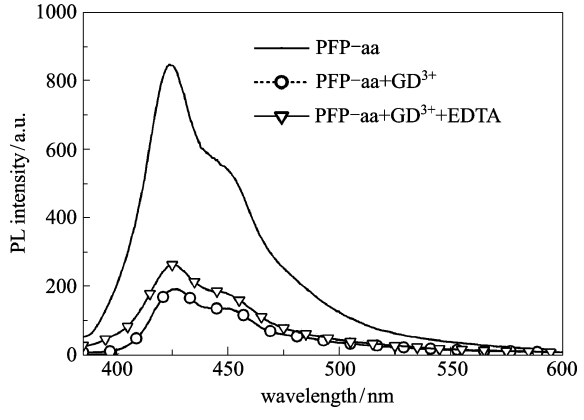

Figure 3 Fluorescence emission spectra of PFP-aa in MES buffer (50 mmol·L^{-1}, pH 7.0) before and after the addition of Gd^{3+}, followed by six fold EDTA. [PFP-aa] = 5.0×10^{-6} mol·L^{-1} in RUs, [Gd^{3+}] = 1.5×10^{-5} mol·L^{-1}, [EDTA] = 3.0×10^{-5} mol·L^{-1}. The excitation wavelength is 375 nm.

4 Conclusions

In summary, we prepared a novel pH-responsive magnetic resonance imaging (MRI) contrast agent by binding Gd (III) to water-soluble conjugated polyfluorene containing carboxylate and amine moieties (PFP-aa/Gd). The PFP-aa is a good chelator for Gd^{3+} and the PFP-aa/Gd complex has good stability. As the pH changes from 10.0 to 4.0, both the carboxylate and amine are protonated, thus PFP-aa exhibits positive charges and forms tight aggregation, which reduces molecular tumbling and accelerates the exchange of bound water leading to the increase of relaxivity R_1. More importantly, the R_1 increases by about eight fold as the pH changes from 8.0 to 6.0, which makes PFP-aa/Gd suitable as a potential marker of the pH below physiological level. In comparison with other contrast agents, the unique sensitivity of water relaxivity of PFP-aa/Gd indicates that this complex could be used in MRI experiments to monitor changes in pH. The present results demonstrate that it may be possible to modulate molecular tumbling and prototropic exchange by pendant arms in ligands to design new contrast agents with differing pH sensitivities. The potential drawback of PFP-aa/Gd is its toxicity, which may limit its real-world application. The design of pH-responsive MRI contrast agents based on conjugated polymers with good biocompatibility is underway in our group.

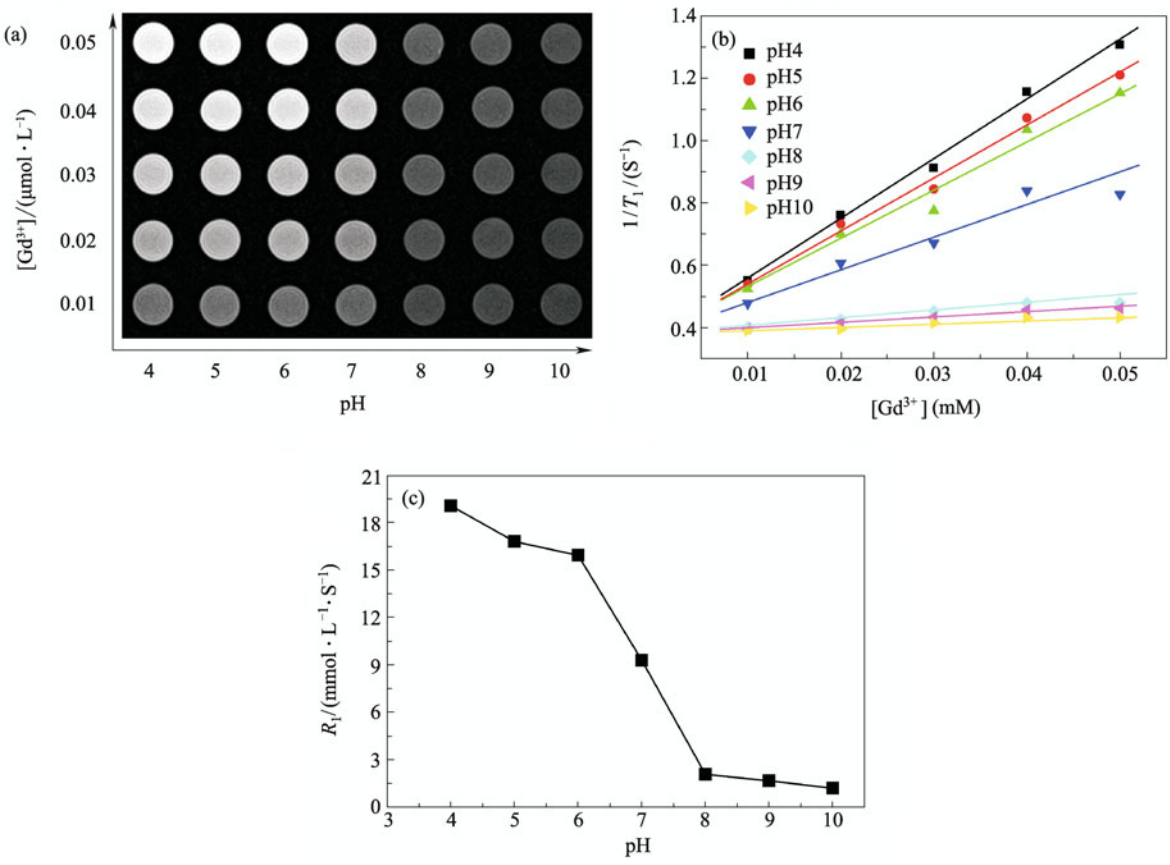

Figure 4 (a) T_1-weighted magnetic resonance images of PFP-aa/Gd in MES buffer (50 mmol·L^{-1}) at various Gd^{3+} concentrations and pH values. (b) Water proton longitudinal relaxation rate ($1/T_1$) of PFP-aa/Gd as a function of Gd^{3+} concentration. (c) Relaxivity R_1 of PFP-aa/Gd as a function of medium pH.

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