A pH-Sensitive Contrast Agent for Functional Magnetic Resonance Imaging (MRI)

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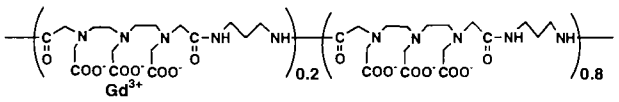
A pH-sensitive MRI contrast agent whose signal intensity is activated only at the lower pH was newly designed to establish a novel imaging concept which depict microenvironment differences in pathophysiological features of damaged tissues.

Now a days, great attention is given on MRI contrast agent to improve reliability and resolution of MRI.[1] The MRI with the conventional MRI contrast agents has depended on the agent distribution differences between target and non-target tissues.[2] Though many efforts have been exerted for agent targeting, true targeting have been rarely achieved.[3]

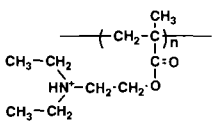
MRI contrast agents which depict microenvironment differences in pathophysiological states between normal and damaged tissues can open a novel imaging concept different from aforementioned conventional agents. The damaged tissue with an abnormal microenvironment state, such as lower pH and/or pO_2, can be depicted by the contrast agent of which MRI signal intensity is activated only at the lower pH and/or pO_2, even though the agent is distributed evenly between normal and damaged tissues. Paramagnetic metals such as gadolinium, Gd, ion enhance MRI signal by shortening the longitudinal relaxation time, T1, of protons of its surrounding water molecules.[4] Thus, one can modify the signal enhancement activity of Gd ions by regulating its hydration behavior. In order to regulate hydration behavior of Gd ions in response to pH changes, we have focused on pH-dependent behavior of polyion complex composed of a couple of weakly acidic and basic polymers. Because it is well understood that these oppositely charged polyions form stable polyion complex accompanying with release of water molecules when the charge in the complex is electrostatically equivalent. This complex formation and hydration behavior are significantly affected by pH, since pH change disorders the charge balance in the complex.[5] It is, therefore, expected that Gd ions chelated in the polyion complex will exhibit pH-dependent change in imaging activity. On the basis of above hypothesis, we have prepared polyion complex system containing Gd ions.

Poly(diethylenetriamine-N, N, N', N'', N''-pentaaceto)(1, 3-propanediamide), **1a**, was prepared by polyaddition of diethylenetriamine-N, N, N', N'', N''-pentaacetic acid (DTPA) dianhydride with 1, 3-propanediamine in DMF.[6] **1a** was impregnated with Gd ions at [Gd]/[DTPA unit] ratio of 0.2 to form a polyanionic MRI contrast agent, poly[(Gd-DTPA)(1, 3-propanedimamide)], **1b**, whose number-average molecular weight ($\overline{Mn}$) was estimated to be 2.4×10^4 with $\overline{Mw}/\overline{Mn}$ of 1.05 by GPC (Figure 1). Poly[2-(dimethylamino)ethyl methacrylate], **2**, (Figure 1) was prepared by a radical polymerization. $\overline{Mn}$ of **2** thus obtained was estimated to be 8.6×10^4 with $\overline{Mw}/\overline{Mn}$ of 1.53.

At first, we assessed ionic characters of the polymers in physiological saline by acid-base titration. Carboxyl groups of **1b** were gradually dissociated within pH 4 to pH 7, indicating polyanionic properties of **1b** varied in this pH range. Deprotonation of the amino groups of **2** was occurred from pH 7 to pH 8 (data not shown). We expected that the polyion complex from these polymers will change its properties as pH change around neutral pH. For further confirmation, an unimolar mixture of **1b** and **2** was titrated with 1N NaOH and turbidity change (OD 500 nm) of the mixture was examined. The turbidity occurs above pH 5, indicating the formation of polyion complexes (PICs) between **1b** and **2**. The PICs are in the form of complex coacervates and are stably suspended in saline below pH 8 as assessed by visual and microscopic inspections (data not shown).



Poly[(Gd-DTPA)(1, 3-propanediamide)], **1b**



Poly[2-(diethylamino)ethyl methacrylate], **2**

Figure 1. Polymer structures of **1b** and **2**.

We then measured the relaxivity values (R1) of **1b** solution and its mixture with **2** using a magnetic resonance spectrometer (Bruker NMS 120 Minispec) at 0.47 T and 40℃. As shown in Figure 2, R1 relaxivities of **1b** are unchanged from pH 5 to pH 9, being 7.6 L/mmol/sec. Although the mixture solution of polymers (**1b** and **2**) show a similar R1 value to that of **1b** at pH 5, R1 value is drastically reduced at pH 7, being less than half of that observed at pH 5. The observed R1 relaxivity change is considered to be closely related with Gd ion/water interaction which is probably affected by properties, in particular, by hydration behavior, of the polyion complex. Further increase in

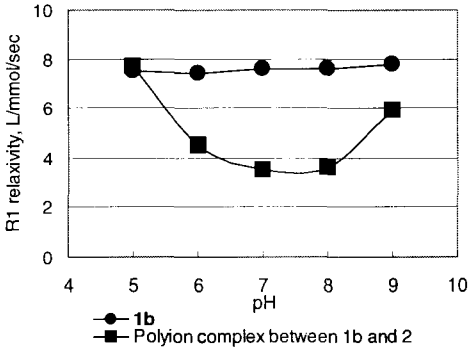


Figure 2. R1 relaxivity changes in **1b** (●) and the polyion complex (■) between **1b** and **2** at various pHs. R1 relaxivities were observed from the T1 relaxation times determined with a magnetic resonance spectrometer (Bruker NMS 120 Minispec) at 0.47 T and 40℃.

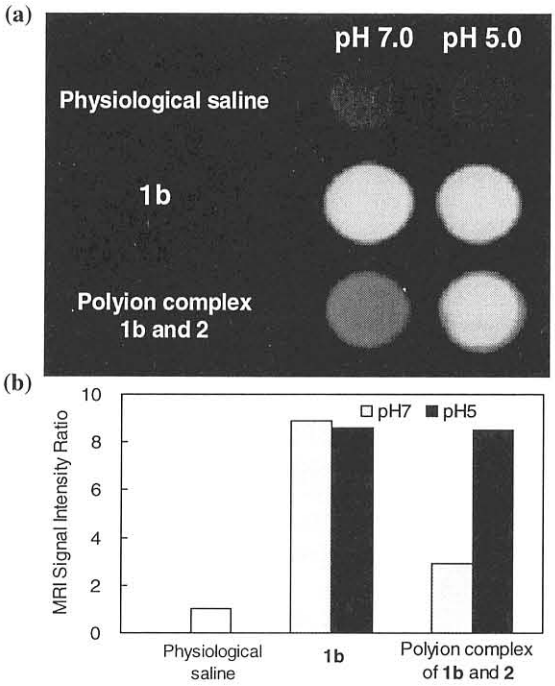


Figure 3. **(a)** MRI T1 weighted imaging pictures and **(b)** MRI signal intensity ratios of **1b** and the polyion complex between **1b** and **2** at pH 5 and 7. The MRI signal intensity was presented as relative value to that of water. MRI phantom study was carried out under the following conditions. Saline solution (final [Gd^{3+}] concentration was 1.0 mmol/l) of polymers at pH 5 or pH 7 was filled in a 1 ml disposable syringe (5 mm ϕ). These two syringes were placed in the magnet bore of the 4.7 T animal imager (Omega CDI-2, GE-Bruker). T1 Weighted (TR/TE = 300/12 ms) MRI pictures were obtained at 60 mm field of view with 128 x 128 dot matrix.

pH to 9 recovers R1 value to 5.9 L/mmol/sec, indicating dissociation of the polyion complex caused by thorough deprotonation of **2.**

The observed pH dependent behavior of the complex prompted us to carry out MRI phantom study. Saline solution (final [Gd^{3+}] concentration was 1.0 mmol/l) at pH 5 or pH 7 was filled in an 1 ml disposable syringe (5 mm ϕ). These two syringes were placed in the magnet bore of the 4.7 T animal imager (Omega CDI-2, GE-Bruker). T1 Weighted (TR/TE = 300/12 ms) MRI pictures were obtained at 60 mm field of view with 128 x 128 dot matrix. As shown in Figure 3a, the MRI signals of **1b** solution at both pH 5 and pH 7 are strongly enhanced. The relative signal intensity ratios compared with water are 8.6 and 8.9 at pH 5 and pH 7, respectively (center bars, Figure 3b). Of interest is that the MRI signals of the complex solution between **1b** and **2** at pH 7 is considerably lower than that at pH 5, where signal intensity ratio relative to that of water is 8.5 and 2.9 at pH 5 and pH 7, respectively (right bars, Figure 3b). These results demonstrate successful pH-driven switching of the signal enhancing activity of Gd ions combined with pH-responsive polyion complex.

Change in microstructure and microenvironment in the polyion complex may play a role on the observed pH dependency. Microscopic observation indicated the coacervates of **1b** and **2** gradually dehydrated with increasing pH from pH 5 to 7 (data not shown). Although the further study is needed to clarify the mechanism in the pH-dependent signal switching, the combination of polyion complex and paramagnetic metal such as Gd is shown to be a potential strategy to design a pH-sensitive MRI agent. This pH-dependent switching behavior of our newly designed MRI contrast agent will certainly be an excellent tool for cancer diagnosis, since the extracellular fluid of tumor is more acidic than normal tissue and blood stream.[7]

A "smart" MRI agent of whose signal intensity is changed by the β-galactosidase activity have been reported.[8] This agent is, however, irreversible and magnitude of relaxivity change is only about 20 %. In contrast, our "intelligent" polyion complex system offers reversible change in signal intensity and shows drastic change in a signal-on to a signal-off state (i.e. <50% in signal intensity) depending on the pH. Our preliminary examination to the tumor-bearing mouse indicated specific enhancement of MRI signal at the tumor site.[9] This novel agents described here can be further strengthened by synergy with efficient targeting technologies and will shed light on macromolecular MRI reagents consist of protein ligands. It may be possible to enhance target-to-background signal ratio by pH-sensitive MRI reagents, because the signal enhancement could be turned on only at acidic endosome of target cells.

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References and Notes

1 H. Degani, V. Gusis, D. Weinstein, S. Fields, and S. Strano, *Nature Medicine*, **3**, 780 (1997).
2 E. C. Unger, K. Ugurbil, and R. E. Latchaw, *J. Magn. Reson. Imag.*, **4**, 235 (1994) ; G. Schuhmann-Giampieri, *Investigative Radiol.*, **28**, 753 (1993).
3 R. Weissleder, A. Bogdanov, and M. Papisov, *Magn. Reson. Quarterly*, **8**, 55 (1992) ; A. Bogdanov, R. Weissleder, and T.J. Brady, *Advanced Drug Delivery Reviews*, **16**, 355 (1995).
4 R. B. Lauffer. *Chem. Rev.*, **87**, 901 (1987); J. Kowalewski, L. Nordenkioeld, N. Benetis, and P.O. Westlund, *Progress in NMR Spectroscopy*, **17**, 141 (1985) ; R.C. Brasch.
5 K. Abe, H. Ohno, and E. Tsuchida. *Makromol. Chem.*, **178**, 2285 (1977) ; Logan M, Cannon G., and McCormick C.L. *Biopolymer*, **41**, 521 (1997)
6 D. B. Love, W. C. Dow, R. J. Himmelsbach, A. D. Watson, and S. M. Rocklage, U.S. Patent. No.5446145 (1995).
7 P. Vaupel, F. Kallinowski, and P. Okunieff, *Cancer Res.*, **49**, 6449 (1989) ; D. K. Kelleher, and P. W. Vaupel, *Experimental Oncology*, **17**, 253 (1995) ; L. E. Gerweck and K. Seetharaman, *Cancer Res.*, **56**, 1194 (1996).
8 R. Moats, S. Fraser and T. Meade, *Angew. Chem., Int. Ed. Engl.*, **36**, 726 (1997).
9 M. Mikawa, N. Miwa, M. Bräutigam, T. Akaike, and A. Maruyama, *in preparation.*