ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2013, **49**, 11421

Received 24th August 2013,
Accepted 14th October 2013

DOI: 10.1039/c3cc46471g

www.rsc.org/chemcomm

An adhesive ^{19}F MRI chemical probe allows signal off-to-on-type molecular sensing in a biological environment†

Tomohiro Doura,‡[a] Ryunosuke Hata,‡[a] Hiroshi Nonaka,[a] Fuminori Sugihara,[b] Yoshichika Yoshioka[b] and Shinsuke Sando*[a]

We report a new strategy for designing a signal off-to-on-type ^{19}F MRI chemical probe that operates in biological environments. The present strategy is based on the control of adherence of a ^{19}F MRI chemical probe to certain blood proteins, accompanied by a change in transverse relaxation time of ^{19}F nuclei.

Magnetic resonance imaging (MRI) is a powerful technique for *in situ* biomolecular analysis. Recent work has focused on ^{19}F MRI because of its relatively high sensitivity (83% of ^{1}H) and the absence of background signals.[1] To date, various ^{19}F MRI chemical probes for sensing biomolecules have been reported.[1–12]

In particular, current attention has been focused on signal activatable ^{19}F MRI probes, which turn on ^{19}F MRI signals only when targeted biological events occur. The off-to-on-type ^{19}F MRI signaling allows us to detect the targeted event much more easily and more precisely.

There are two elegant ways of controlling the signal; the switching of paramagnetic relaxation[4] or precise control of supramolecular self-assembly.[5] Both utilize a change in ^{19}F transverse relaxation times (T_2). These ^{19}F chemical probes initially have short ^{19}F T_2 values, which are elongated after the targeted events. The T_2-weighted ^{19}F MRI gives a stronger signal for ^{19}F nuclei having a relatively longer T_2 value. Therefore, in the T_2-weighted ^{19}F MRI, such chemical probes operate to turn on the ^{19}F probe after a targeted biological event. Herein, we report a new option for designing the signal off-to-on-type ^{19}F MRI chemical probes.

The present approach is based on the control of adherence of the chemical probes to endogenous biomaterials.[12] The selected environment in this research is blood. Blood contains abundant serum albumin (*ca.* 50% of blood proteins, typically 35–50 g L^{-1}) to which small compounds, especially hydrophobic compounds including hormones, fatty acids, and synthetic chemical probes, tend to adhere. The adherent small molecules have a shorter T_2 value because of the lower mobility of the complex. Therefore, we hypothesized that if the hydrophobic ^{19}F chemical probe adheres to blood components such as serum albumin (short T_2), and produces a less adhesive hydrophilic ^{19}F product after the targeted event (long T_2), such a chemical probe may function as a turn-on ^{19}F chemical probe in T_2-weighted ^{19}F MRI (Fig. 1a).

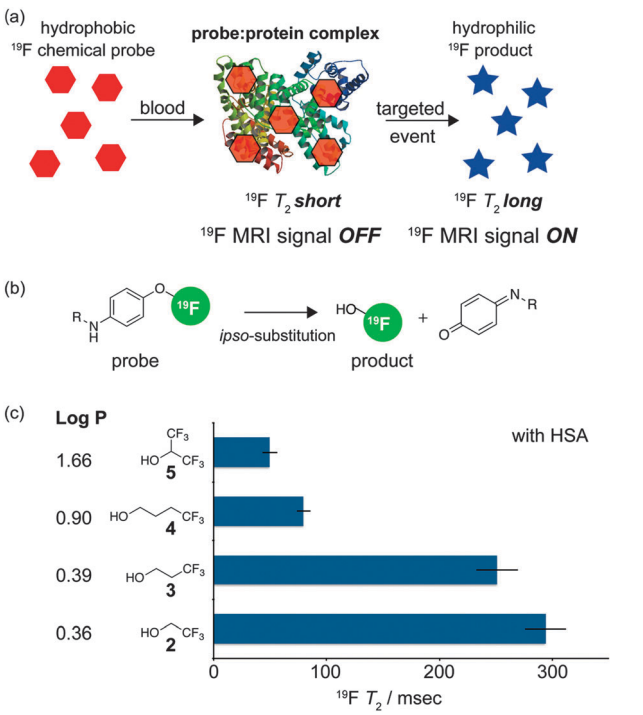

Fig. 1 (a) The proposed mechanism for signal off-to-on sensing of a targeted biological event in blood. (b) Proposed scheme for production of a fluoroalcohol (product) from fluoroalkoxyaniline (probe) *via* an ipso-substitution mechanism. (c) Relationship between log *P* and ^{19}F T_2 of compounds **2–5** dissolved in 100 mM sodium phosphate buffer (pH 7.4) containing 150 mM NaCl and HSA (50 g L^{-1}). Log *P* values are taken from LOGKOW database.

[a] *INAMORI Frontier Research Center, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka, Japan. E-mail: ssando@ifrc.kyushu-u.ac.jp; Fax: +81 92 802 6961; Tel: +81 92 802 6960*

[b] *Immunology Frontier Research Center, Osaka University, 3-1 Yamadaoka, Suita, Osaka, Japan*

† Electronic supplementary information (ESI) available: Experimental procedures of synthesis, ^{19}F NMR measurements and ^{19}F MRI measurements. See DOI: 10.1039/c3cc46471g

‡ These authors contributed equally to this work.

Along this concept, we designed ^{19}F chemical probes, shown in Fig. 1b, for detection of hypochlorous acid (HOCl) that is an important biomarker for inflammatory diseases. We have found that the fluoroalkoxyaniline scaffold (probe) can react with HOCl expeditiously to produce a relatively hydrophilic fluoroalcohol (product) *via* an ipso-substitution mechanism (Fig. 1b).[8] This hydrophobic-to-hydrophilic conversion is thought to be suitable for the present concept.

Initially, we explored an appropriate ^{19}F alcohol (product) that is sufficiently hydrophilic and thus does not suffer from substantial shortening of ^{19}F T_2 in blood. We measured ^{19}F T_2 values of four types of ^{19}F alcohols, **2–5,** in the presence of human serum albumin (HSA, 50 g L^{-1}) (Fig. 1c). The observed ^{19}F T_2 values are in proportion to log*P* values for each compound, suggesting that lipophilicity is an important factor for determining the ^{19}F T_2 values in blood.[13] Based on the result, we selected trifluoroethanol CF_3CH_2OH (compound **2** in Fig. 1c) as an appropriate ^{19}F product for this purpose, because compound **2** has relatively long ^{19}F T_2 (293.8 ms) in the presence of HSA and, in a practical sense, such a simple chemical structure makes it easier to design and synthesize ^{19}F chemical probes.

Based on the above discussion, compound **1**, having a CF_3CH_2O-leaving moiety, was synthesized as a candidate probe, which produces trifluoroethanol (compound **2**) upon reaction with HOCl (Fig. 2a).

Binding of **1** and nonbinding of trifluoroethanol to HSA were demonstrated by an ultrafiltration assay (Fig. S1, ESI†). Only 29% of **1** was recovered in the presence of HSA (1.9 equivalent) compared with that in the absence of HSA, while as much as 94% was recovered in the case of trifluoroethanol.

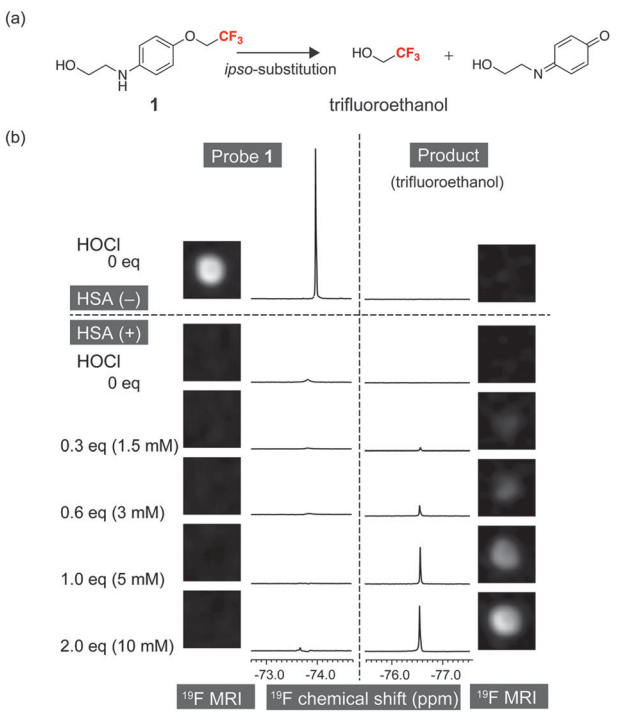

Fig. 2 (a) Probe **1** and a proposed reaction scheme *via* ipso-substitution. (b) ^{19}F NMR (long spin echo time, TE = 20 ms) and T_2-weighted ^{19}F MRI (chemical shift selective) of probe **1** solution (5 mM) in 100 mM sodium phosphate buffer (pH 7.4) containing 150 mM NaCl in the presence or absence of HSA (50 g L^{-1}), incubated with HOCl (0–2 eq.).

Table 1 T_2 values of ^{19}F nuclei of **1** and trifluoroethanol **2** in phosphate buffer, in phosphate buffer containing HSA, or in blood plasma

Compounds	Conditions[a]	T_2[b] ms^{-1}
1	Phosphate buffer	873.6 ± 184.9
1	Phosphate buffer, HSA	14.7 ± 1.6
1	Blood plasma	15.0 ± 3.2
Trifluoroethanol (**2**)	Phosphate buffer	1744.6 ± 324.0
Trifluoroethanol (**2**)	Phosphate buffer, HSA	293.8 ± 18.3
Trifluoroethanol (**2**)	Blood plasma	216.0 ± 2.9

[a] Concentrations of compounds = 5 mM, phosphate buffer; 100 mM sodium phosphate (pH 7.4) containing 150 mM NaCl with or without HSA (50 g L^{-1}). [b] T_2 values were measured using a spin-echo method.

Table 1 summarizes the T_2 values of ^{19}F nuclei of **1** (probe) and trifluoroethanol (product) in phosphate buffer and in blood plasma. The ^{19}F T_2 value for **1** in blood plasma (15.0 ms) was much shorter than that in phosphate buffer (873.6 ms). In addition, the ^{19}F T_2 value for **1** was also shortened to the same range by adding HSA to phosphate buffer (14.7 ms). By contrast, the ^{19}F T_2 value for trifluoroethanol was 216.0 ms in blood plasma and 293.8 ms in phosphate buffer with HSA, which are shorter than the value in phosphate buffer (1744.6 ms), but still >10 times longer than the ^{19}F T_2 value for **1** in blood plasma.

From these results, it is feasible that **1** tends to adhere to blood plasma proteins, resulting in the remarkable shortening of ^{19}F T_2, whereas the product trifluoroethanol is less adherent and has a relatively long ^{19}F T_2 value in blood, suggesting that probe **1** has appropriate chemical properties for off-to-on ^{19}F MRI sensing.

In fact, probe **1** operates as a signal on-type ^{19}F NMR/MRI agent. We measured ^{19}F NMR using a long echo time (TE). Probe **1** (5 mM) showed a sharp ^{19}F NMR signal at *ca.* −74.0 ppm in a phosphate buffer (top-left in Fig. 2b). The signal was highly attenuated in the presence of HSA (second from the top in the left column, Fig. 2b) in a HSA concentration-dependent manner (Fig. S2, ESI†). The signal attenuation reflects the shortening of T_2 of ^{19}F nuclei because of binding to HSA (the same signal attenuation of **1** was observed also in blood plasma, data not shown). On the other hand, after addition of HOCl, the ^{19}F signal at −76.6 ppm, corresponding to the product trifluoroethanol, appeared in a HOCl concentration-dependent manner (from top to bottom in the right column, Fig. 2b).

Similar results were observed for T_2-weighted ^{19}F MRI (Fig. 2b). To analyze signal off-to-on behavior of the probe and the product more precisely, chemical-shift-selective ^{19}F imaging was applied, where the probe and the product were imaged separately according to each chemical shift. Without HOCl, no ^{19}F MRI signals for **1** and the product (trifluoroethanol) were observed. By contrast, with HOCl, clear contrast images were observed only for product-selective ^{19}F MRI. These results demonstrated that **1** operates as a signal on-type ^{19}F NMR/MRI agent responsive to HOCl.

Finally, we applied probe **1** in the detection of enzyme myeloperoxidase (MPO), abundant in neutrophils. MPO is an important factor in various cardiovascular and neurodegenerative diseases and is also a biomarker for future acute coronary

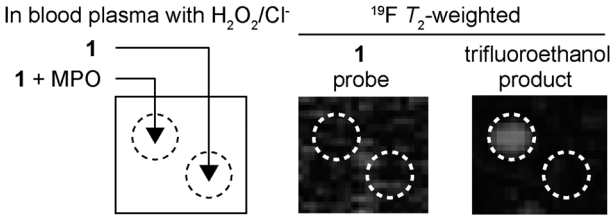

Fig. 3 T_2-weighted ^{19}F MRI images of probe **1** (5 mM) incubated with or without 470 nM MPO in 60 mM sodium phosphate buffer (pH 7.4) containing 90 mM NaCl, 4 mM H_2O_2 and 40% blood plasma.

disease. Accordingly, imaging of MPO activity in blood would be useful for medical applications.[14] It is known that MPO oxidizes the alkoxyaniline group directly or *via* the produced HOCl.[15,16] Therefore, probe **1** might also operate as a ^{19}F chemical probe for MPO activity.

We used T_2-weighted ^{19}F MRI to visualize the MPO activity in blood plasma (Fig. 3). Without MPO (dotted circle, bottom-right), no ^{19}F MRI signals of **1** (probe) and trifluoroethanol (product) were observed. By contrast, with MPO (dotted circle, top-left), a detectable signal appeared in the product-selective imaging, while no signal for **1** was detected. Although the MPO concentrations used are much higher than that of the physiological MPO level[17] and the sensitivity needs to be improved further, these results demonstrated that probe **1** operates as a turn-on ^{19}F MRI probe for sensing MPO activity in blood.

In conclusion, we showed a proof-of-concept study for designing a signal off-to-on-type ^{19}F MRI probe that operates in blood. This strategy allows us to develop a turn-on ^{19}F MRI probe, responding to HOCl and MPO, in T_2-weighted ^{19}F MRI. The key concept of this off-to-on ^{19}F MRI is control of the adhesive and nonadhesive properties of the ^{19}F probe and the product to endogenous biomaterials. In blood, hydrophobic-to-hydrophilic conversion, *i.e.*, adhesive and nonadhesive properties to HSA, was demonstrated to operate as a switch. The adhesive probe keeps the signal off state in blood and turns on its ^{19}F signal after targeted events. This concept is not limited to this case. More generally, other basic biochemical events, *e.g.*, simple binding or anchoring to cells and release, could operate as a switch to modulate ^{19}F signals. This approach is based on a simple concept, and thus could be a strategy for the design of a variety of signal off-to-on-type ^{19}F MRI probes.

This work was supported by the Funding Program for Next Generation World-Leading Researchers (NEXT), JSPS. RH thanks JSPS for the fellowship.

Notes and references

1 For reviews on ^{19}F MRI probes, see for example ref. 1 and 2: J.-X. Yu, V. D. Kodibagkar, W. Cui and R. P. Mason, *Curr. Med. Chem.*, 2005, **12**, 819.
2 S. L. Cobb and C. D. Murphy, *J. Fluorine Chem.*, 2009, **130**, 132.
3 For recent examples of ^{19}F MRI probes, see 3–12: B. J. Stockman, *J. Am. Chem. Soc.*, 2008, **130**, 5870.
4 S. Mizukami, R. Takikawa, F. Sugihara, Y. Hori, H. Tochio, M. Wälchli, M. Shirakawa and K. Kikuchi, *J. Am. Chem. Soc.*, 2008, **130**, 794.
5 Y. Takaoka, T. Sakamoto, S. Tsukiji, M. Narazaki, T. Matsuda, H. Tochio, M. Shirakawa and I. Hamachi, *Nat. Chem.*, 2009, **1**, 557.
6 K. Yamaguchi, R. Ueki, H. Nonaka, F. Sugihara, T. Matsuda and S. Sando, *J. Am. Chem. Soc.*, 2011, **133**, 14208.
7 K. Tanaka, N. Kitamura and Y. Chujo, *Bioconjugate Chem.*, 2011, **22**, 1484.
8 T. Doura, A. Qi, F. Sugihara, T. Matsuda and S. Sando, *Chem. Lett.*, 2011, 1357.
9 J.-X. Yu, V. D. Kodibagkar, R. R. Hallac, L. Liu and R. P. Mason, *Bioconjugate Chem.*, 2012, **23**, 596.
10 P. Harvey, K. H. Chalmers, E. D. Luca, A. Mishra and D. Parker, *Chem.–Eur. J.*, 2012, **18**, 8748.
11 X. Huang, G. Huang, S. Zhang, K. Sagiyama, O. Togao, X. Ma, Y. Wang, Y. Li, T. C. Soesbe, B. D. Sumer, M. Takahashi, A. D. Sherry and J. Gao, *Angew. Chem., Int. Ed.*, 2013, **52**, 8074.
12 Recent attempt at ^{19}F signal control by molecular recognition, see: Y. Sun, Y. Takaoka, S. Tsukiji, M. Narazaki, T. Matsuda and I. Hamachi, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 4393.
13 G. Colmenarejo, A. Alvarez-Pedraglio and J.-L. Lavandera, *J. Med. Chem.*, 2001, **44**, 4370.
14 For a recent review, see for example: J. A. Ronald, *Curr. Cardiovasc. Imaging Rep.*, 2011, **4**, 24.
15 K.-I. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima and T. Nagano, *J. Biol. Chem.*, 2003, **278**, 3170.
16 J. Shepherd, S. A. Hilderbrand, P. Waterman, J. W. Heinecke, R. Weissleder and P. Libby, *Chem. Biol.*, 2007, **14**, 1221.
17 MPO under physiological conditions, see: S. Baldus, C. Heeschen, T. Meinertz, A. M. Zeiher, J. P. Eiserich, T. Münzel, M. L. Simoons and C. W. Hamm, *Circulation*, 2003, **108**, 1440.