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Development of a Potassium Ion Sensor for ^{19}F Magnetic Resonance Chemical Shift Imaging Based on Fluorine-labeled Thrombin Aptamer

Takashi Sakamoto,[1] Hikaru Hayakawa,[1] and Kenzo Fujimoto*[1,2]
[1]*School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292*
[2]*Research Center for Bio-Architecture, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292*

(Received April 11, 2011; CL-110302; E-mail: kenzo@jaist.ac.jp)

To develop a molecular imaging probe for potassium cation based on the chemical shift change of ^{19}F magnetic resonance (MR), thrombin aptamer, whose structure was changed with the selective binding of potassium cation, was labeled with a fluorine compound. We demonstrated that the probe could detect potassium cation by chemical shift change of the ^{19}F MR signal, and that the chemical shift change was caused by potassium cation selective G-quadruplex formation.

In vivo imaging technologies, such as X-ray computed tomography, positron emission tomography, and nuclear magnetic resonance imaging, are promising technologies for the diagnosis of various disorders and follow up after surgical treatments. To image biological events in greater detail in vivo, molecular probes targeting biomolecules, such as disease marker enzymes[1] or receptors,[2] have been developed. Imaging probes that can image enzyme activity,[3–5] protein,[6] or protein aggregate[7] by ^{19}F nuclear magnetic resonance (MR) imaging technology have been reported. Due to the low background signal of ^{19}F MR in vivo, these ^{19}F MR-based imaging probes have potential for high contrast and specific imaging of target biomolecules. However, no molecular probes that can image specifically metal cation in vivo have been reported. On the other hand, various fluorescent probes that can fluorescently detect the potassium cation have been reported,[8,9] however, the low permeability of the fluorescence in bioorgans prevents the application of these probes for in vivo imaging of potassium cation.

In this study, we attempted to develop a novel potassium cation detection probe based on ^{19}F MR imaging technology. We designed and synthesized a thrombin aptamer (TBA)-based probe having a fluorine compound at the 5′ termini of the TBA (Figure 1). As the structure of the thrombin aptamer is changed by potassium cation dependent intramolecular G-quadruplex formation,[10] it is expected that the environment around the fluorine compound at the 5′ termini of the probe could be detected as chemical shift change in the ^{19}F MR signal of the fluorine nuclei. To attach the fluorine compound to the thrombin aptamer, we synthesized a phosphoramidite derivative containing 3,5-bis(trifluoromethyl)benzene moiety (Scheme 1), and labeled the 5′ termini of the TBA by conventional phosphoramidite chemistry using an automated DNA synthesizer. The coupling yield of **2** to the 5′ termini of the TBA was 97%. The ^{19}F-TBA was then easily obtained by HPLC purification after the cleavage and deprotection with 28% ammonium hydroxide. Details of the methods for synthesis of the probe are described in Supporting Information.[11]

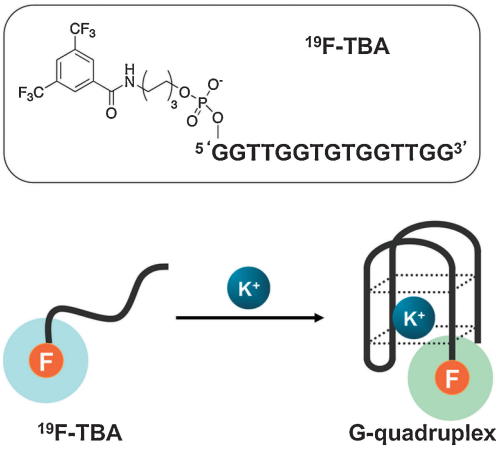
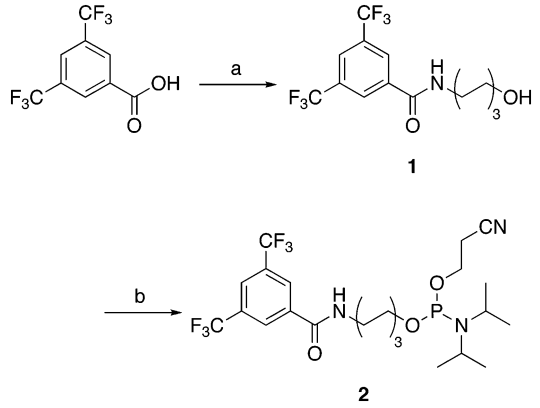


Figure 1. Schematic drawing of the ^{19}F-TBA-based potassium cation detection. The chemical shift of the ^{19}F MR signal was changed with the microenvironmental change around ^{19}F nuclei tethered on the 5′ termini of TBA caused by potassium cation dependent G-quadruplex formation.



Scheme 1. a: 6-Amino-1-hexanol, EDC, HOBt, *N*,*N*-diisopropylethylamine, DMF, b: 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite, benzylthio-1*H*-tetrazole, acetonitrile.

As shown in Figure 2, the chemical shift of the ^{19}F MR signal of 3,5-bis(trifluoromethyl)benzene moiety at the 5′ termini of the ^{19}F-TBA was clearly shifted toward low magnetic field by the addition of KCl in a concentration-dependent manner. Since the UV melting profile and CD spectrum in the presence of potassium cation (Figure 3) showed characteristic behavior of the G-quadruplex structure, i.e., a decrease of the absorbance with the increase of temperature and a positive and

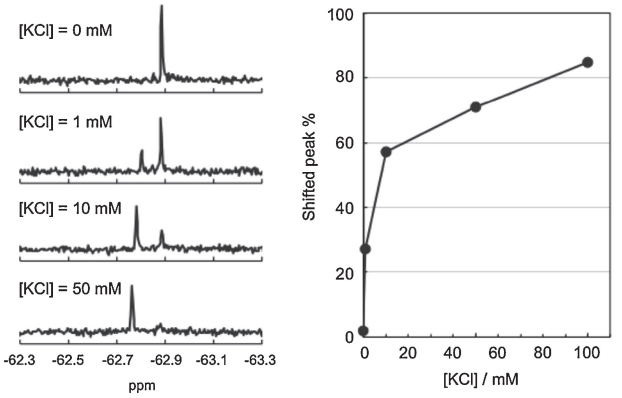


Figure 2. ^{19}F NMR spectra and chemical shift change of ^{19}F-TBA with the addition of KCl. [^{19}F-TBA] = 10 μM in 10 mM Tris-HCl (pH 7.4) containing 10% (v/v) D_2O and 5 μM trifluoroacetic acid as an internal standard (−75.6 ppm). Measurements were carried out at 25 °C.

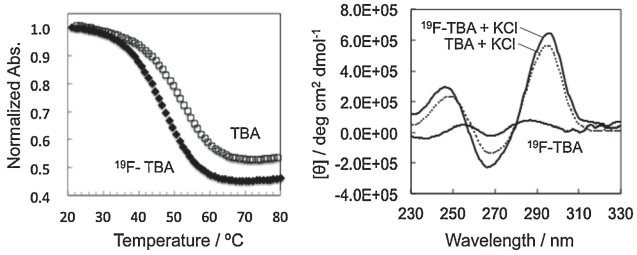


Figure 3. UV melting behavior monitored at 297 nm (left) and CD spectra (right) of ^{19}F-TBA. Condition for UV melting measurement: [^{19}F-TBA] = 10 μM, [KCl] = 200 mM in 10 mM Tris-HCl (pH 7.4). Condition for CD measurement: [^{19}F-TBA] = 10 μM, [KCl] = 500 mM in 10 mM Tris-HCl (pH 7.4) containing 5 μM trifluoroacetic acid. Measurements were carried out at 25 °C.

a negative band around 295 and 270 nm, respectively, it is suggested that the change in chemical shift was caused by the change of environment around the fluorine atom at the 5′ termini of 3,5-bis(trifluoromethyl)benzene moiety with the potassium cation-dependent G-quadruplex formation of ^{19}F-TBA. The detectable range of potassium cation using ^{19}F-TBA was in the millimolars range, and the dissociation constant (K_D) of the interaction was 1.3 ± 0.14 mM. As the reported K_D of the TBA and potassium cation is approximately 5 μM,[10] this indicates that the binding of potassium cation was inhibited by the 5′ labeling of 3,5-bis(trifluoromethyl)benzene moiety. This was also supported by the T_m value estimated from a UV melting experiment (Figure 3, left). The T_m of the ^{19}F-TBA (45 °C) was lower than that of TBA (52 °C), indicating that the G-quadruplex structure was destabilized by 5′ labeling of 3,5-bis(trifluoromethyl)benzene moiety. The difference between the CD spectra of ^{19}F-TBA and TBA in the presence of potassium cation (Figure 3, right) indicates the possibility that the labeling of 3,5-bis(trifluoromethyl)benzene moiety causes the change in the G-quadruplex structure of the TBA. It seems that further optimization of the fluorine compound or the linker between the TBA and fluorine label is required for the detection of potassium cation with a lower concentration range. The K_D of ^{19}F-TBA was not so

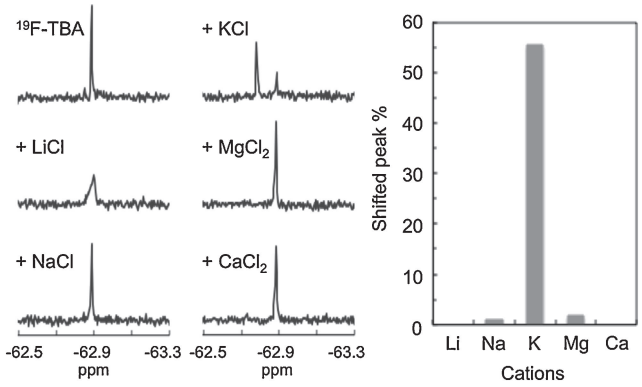


Figure 4. ^{19}F NMR spectra of the ^{19}F-TBA in the presence of various metal cations. [^{19}F-TBA] = 10 μM, [cation] = 10 mM in 10 mM Tris-HCl (pH 7.4) containing 10% (v/v) D_2O and 5 μM trifluoroacetic acid as an internal standard (−75.6 ppm). Measurements were carried out at 25 °C.

different from other reported potassium cation sensors, such as PBFI (K_D = 8 mM)[12] and CD222 (K_D = 0.8 mM),[13] suggesting that ^{19}F-TBA is also useful as potassium cation sensor.

To evaluate the selectivity of the ^{19}F-TBA-based detection of potassium cation, ^{19}F NMR spectra were measured in the presence of various metal cations (Figure 4). As the chemical shift of the ^{19}F-TBA was shifted only in the case of the addition of potassium cation, it was demonstrated that the selective detection of potassium cation was achieved by this method.

In summary, we demonstrated that the chemical shift of ^{19}F-TBA was clearly shifted by G-quadruplex formation caused by the selective binding of potassium cation to the ^{19}F-TBA. It was suggested that the ^{19}F-TBA, whose chemical shift of the ^{19}F MR signal was ratiometrically changed in a potassium cation concentration dependent manner, has potential for ratio imaging of the potassium cation in vivo with ^{19}F MR chemical shift imaging technology.

References and Notes

1 R. L. Scherer, J. O. McIntyre, L. M. Matrisian, *Cancer Metastasis Rev.* **2008**, *27*, 679.
2 K. Bouchelouche, J. Capala, P. Oehr, *Curr. Opin. Oncol.* **2009**, *21*, 469.
3 S. Mizukami, R. Takikawa, F. Sugihara, Y. Hori, H. Tochio, M. Wälchli, M. Shirakawa, K. Kikuchi, *J. Am. Chem. Soc.* **2008**, *130*, 794.
4 S. Mizukami, R. Takikawa, F. Sugihara, M. Shirakawa, K. Kikuchi, *Angew. Chem., Int. Ed.* **2009**, *48*, 3641.
5 Y. Takaoka, T. Sakamoto, S. Tsukiji, M. Narazaki, T. Matsuda, H. Tochio, M. Shirakawa, I. Hamachi, *Nat. Chem.* **2009**, *1*, 557.
6 M. Higuchi, N. Iwata, Y. Matsuba, K. Sato, K. Sasamoto, T. C. Saido, *Nat. Neurosci.* **2005**, *8*, 527.
7 L. Helm, *Prog. Nucl. Magn. Reson. Spectrosc.* **2006**, *49*, 45.
8 S. Nagatoishi, T. Nojima, E. Galezowska, B. Juskowiak, S. Takenaka, *ChemBioChem* **2006**, *7*, 1730.
9 S. Nagatoishi, T. Nojima, B. Juskowiak, S. Takenaka, *Angew. Chem.* **2005**, *117*, 5195.
10 J. M. Wilcox, D. L. Rempel, M. L. Gross, *Anal. Chem.* **2008**, *80*, 2365.
11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
12 K. Meuwis, N. Boens, F. C. De Schryver, J. Gallay, M. Vincent, *Biophys. J.* **1995**, *68*, 2469.
13 H. Szmacinski, J. R. Lakowicz, *Sens. Actuators, B* **1999**, *60*, 8.

