Phenylboronic Acid-based $^{19}$F MRI Probe for the Detection and Imaging of Hydrogen Peroxide Utilizing Its Large Chemical-Shift Change

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Herein, we report on a new $^{19}$F MRI probe for the detection and imaging of H$_2$O$_2$. Our designed 2-fluorophenylboronic acid-based $^{19}$F probe promptly reacted with H$_2$O$_2$ to produce 2-fluorophenol via boronic acid oxidation. The accompanying $^{19}$F chemical-shift change reached 31 ppm under our experimental conditions. Such a large chemical-shift change allowed for the imaging of H$_2$O$_2$ by $^{19}$F chemical-shift-selective MRI.

Keywords $^{19}$F NMR, $^{19}$F MRI, imaging, molecular probe, H$_2$O$_2$

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Introduction

Reactive oxygen species (ROS) play essential roles in our homeostatic regulation.$^{1,2}$ In particular, hydrogen peroxide (H$_2$O$_2$) is one of the most biologically important ROS. H$_2$O$_2$ is related to immune system function and, recently, suggested to be as the function of healthy physiological signaling pathways such as cell proliferation, differentiation, and migration.$^{3-5}$

Conventional H$_2$O$_2$ sensing methods are based on the fluorescence modality. Various fluorescent probes have been developed and these probes have been powerful methods for analyses of H$_2$O$_2$ functions in cells.$^6$ In recent years, some bioluminescent H$_2$O$_2$ probes have been successfully applied to the functional analysis of living organisms beyond cell-level studies.$^7,8$ Although optical-imaging probes have advantages such as high sensitivity, they are not suitable for detection in deep sites of our body because of a low penetration of excitation and emission light. In that respect, there is room to improve the sensing strategy for H$_2$O$_2$.

Magnetic resonance (MR) is one of the promising modalities for molecular analysis in deep sites of an opaque body.$^9$ Indeed, MR chemical probes and contrast agents have been designed for such a purpose.$^{10}$ The MR modality, however, has an intrinsic problem for the selective detection of chemical probes because of large background signals resulting from endogenous MR-detectable nuclei such as $^1$H, $^{13}$C, and $^{31}$P. To overcome this limitation, $^{19}$F MR has received attention in recent years.$^{11}$ $^{19}$F is an MR-detectable nucleus that has a relatively high sensitivity (83% of $^1$H) in spite of the absence of background signals in biological samples. By utilizing these advantages, many $^{19}$F MR probes have been developed and some probes have been applied to in vivo MRI experiments.$^{12}$ With respect to $^3$F ROS probes, nitric oxide (NO)$_3^{13}$ hypochlorous acid (HOCl),$^{14}$ and peroxynitrite ion (ONOOO$^-$$^{15}$) probes have been used for the in vitro detection of each target. However, to our knowledge, no $^{19}$F MRI probe for H$_2$O$_2$ imaging has yet been produced.

Here, we report on a new $^{19}$F MRI probe for the detection of H$_2$O$_2$. The probe reacted promptly with H$_2$O$_2$ with a large $^{19}$F chemical-shift change, allowing the imaging of H$_2$O$_2$ by $^{19}$F chemical-shift-selective MRI.

Experimental

$^{19}$F NMR measurements $^{19}$F NMR spectra were acquired using a Bruker Avance III spectrometer (376 MHz for $^{19}$F NMR) and a JEOL ECS400 spectrometer (376 MHz for $^{19}$F NMR). Chemical shifts are reported in ppm, relative to trifluoroacetic acid (TFA, $\delta = -76.5$ ppm) as an internal standard.

Reaction with H$_2$O$_2$ (Fig. 1) An aqueous H$_2$O$_2$ solution (final concentration 300 μM) was added to a solution of 1 - 5 (final concentration 100 μM) in sodium phosphate buffer (100 mM, pH 7.4) containing NaCl (150 mM) and DMF (0.1%). After 30 min of incubation at 37°C, an aliquot of the solution (450 μL) was mixed with 5 mM TFA in D$_2$O (50 μL) and subjected to NMR analysis (128 scans).

Reaction with H$_2$O$_2$ (Fig. 2) An aqueous H$_2$O$_2$ solution (final concentration 1 mM) was added to a solution of 1 (final concentration 1 mM) in sodium phosphate buffer (100 mM, pH 7.4) containing NaCl (150 mM)
and DMF (0.1%). After 30 min of incubation at 37°C, an aliquot of the solution (450 μL) was mixed with 5 mM TFA in D2O (50 μL) and subjected to NMR analysis (128 scans). The remaining solution was subjected to HPLC analysis.

19F chemical-shift-selective imaging (Figs. 3 and 4)

For the reaction with H2O2 (Fig. 3), probe 1 (5 mM), probe 1 (5 mM) with H2O2 (2 equiv), and 2-fluorophenol (5 mM) were prepared in sodium phosphate buffer (pH 7.4, 100 mM) containing NaCl (150 mM) and 0.1% DMF. After 30 min incubation at 37°C, the reaction samples were used for MR imaging acquisition.

For the reaction with H2O2 produced by enzymes (Fig. 4), probe 1 (10 mM) incubated with glucose, probe 1 (10 mM) incubated with glucose (40 mM) and glucose oxidase (GOX, 300 units/mL), probe 1 (10 mM) incubated with choline, and probe 1 (10 mM) incubated with choline (40 mM) and choline oxidase (COX, 100 units/mL) were prepared in 100 mM sodium
phosphate buffer (pH 7.4) containing NaCl (150 mM) and 1% DMF. After 100 min of incubation at 37°C, the reaction samples were used for MR imaging acquisition.

MR imagings were recorded on a Biospec 117/11 system and processed by using ParaVision software (Bruker Biospin). The 300 μL reaction samples were dispensed into a modified 96-well plate. The RARE (rapid acquisition with refocused echoes) method was used for 1H and 19F imaging. For 19F imaging, the matrix size was 64 × 32 with a field of view of 8.0 cm × 4.0 cm. The repetition time was 1000 ms and effective echo time was 24.0 ms. The number of accumulations was 512.

Results and Discussion

As pioneering work, Chang and coworkers demonstrated that an aryl boronic acid is an efficient reactive moiety for designing fluorescent or bioluminescent probes targeting biologically important H2O2.6 With this in mind, we utilized phenylboronic acid as a reactive moiety to develop a 19F MRI probe for H2O2. We assumed that 19F-labeled phenylboronic acid also reacts with H2O2 to produce the corresponding phenol and this structural change would cause a large 19F chemical-shift change because of a large electron density change around the 19F nucleus (Fig. 1a).

First, we investigated phenylboronic acid derivatives, having fluoro or trifluoromethyl substituents (1–5, Fig. 1b). The reaction of 1–5 (100 μM) with H2O2 (300 μM) in phosphate buffer (pH 7.4, 100 mM) was evaluated using 19F NMR analysis. All of the probes reacted with H2O2. The conversion-induced chemical-shift change is summarized in Fig. 1b. Among the five probes, probe 1 afforded the largest chemical-shift change. The possible product was 2-fluorophenol, which is present mainly as a protonated form at pH 7.4 (pKa of phenolic OH = 8.7316). The observed probe-to-product 19F chemical-shift change reached 31 ppm at pH 7.4, which was sufficient to
clearly discriminate and visualize each compound by \(^{19}\text{F}\) chemical-shift-selective imaging. We speculate that this extremely large \(^{19}\text{F}\) chemical-shift change of probe 1 is based on two factors. The first possible factor is the electron density difference of \(^{19}\text{F}\) between probe 1 and the product. The boronic acid moiety of probe 1 has an electron acceptor property derived from the empty \(p\)-orbital of boron. On the other hand, the hydroxyl moiety of the product 2-fluorophenol has an electron donor property derived from the unshared electron pairs of the oxygen atom. From these tendencies, in addition to the ortho-para orientation of the electron density change, probes 1 and 3 could show a large chemical-shift change for \(^{19}\text{F}\). The second possible factor is the cancellation of the interaction of the boronic acid moiety with the \(^{19}\text{F}\) nuclei of probe 1. An intramolecular OH-F hydrogen bond in 2-fluorophenylboronic acid, which is probe 1, was noted in a previous report.\(^7\) After reaction with \(\text{H}_2\text{O}_2\), this intramolecular hydrogen bond would be cancelled and a \(^{19}\text{F}\) chemical-shift change might be induced. Although the mechanism of this large chemical-shift change is still under investigation, probe 1 was the best candidate in this screening.

To evaluate the detailed sensing properties of probe 1, we performed \(^{19}\text{F}\) NMR (Fig. 2a) and HPLC (Fig. 2b) analyses after incubation with 1 equiv of \(\text{H}_2\text{O}_2\). \(^{19}\text{F}\) NMR measurements showed that probe 1 and the reaction product with \(\text{H}_2\text{O}_2\) gave \(^{19}\text{F}\) signals at \(-107.8\) and \(-138.7\) ppm, respectively. By comparing with a standard sample, this product chemical shift was confirmed to be 2-fluorophenol. HPLC analysis also showed that the reaction product of probe 1 and \(\text{H}_2\text{O}_2\) was 2-fluorophenol. These data showed that the reaction product of probe 1 was 2-fluorophenol, as predicted. A spectrometric analysis indicated a very fast reaction of 1 with \(\text{H}_2\text{O}_2\) (Fig. S1, Supporting Information). The apparent first-order rate constant was determined to be \((7.3 \pm 0.5) \times 10^{\text{-}3}\) s\(^{-1}\). This value was almost of the same order with the reaction rate constant of previously reported phenylboronic acid-based probes \((10^{\text{-}3} - 10^{\text{-}4}\) s\(^{-1}\)).\(^1\) Furthermore, we checked the reaction selectivity of probe 1 for \(\text{H}_2\text{O}_2\) among other ROS and reactive nitrogen species (RNS). HPLC analyses showed that probe 1 had \(\text{H}_2\text{O}_2\) moderate selectivity with the exception of ONOO\(^{-}\) under our experimental conditions (Fig. S2, Supporting Information). In our body, the hydrogen peroxide concentration is much higher than any other ROS and RNS.\(^5,19,20\) Therefore, it may be possible to believe that this reaction selectivity of probe 1 is within a permissible range in this development stage.

Having a promising \(\text{H}_2\text{O}_2\)-reactive-\(^{19}\text{F}\) NMR probe in hand, we moved on to the MR imaging of \(\text{H}_2\text{O}_2\), which was the aim of the present study. Figure 3 shows \(^1\text{H}\) and \(^{19}\text{F}\) MRI phantom images of probe 1 (5 mM), probe 1 and \(\text{H}_2\text{O}_2\) (2 equiv), and 2-fluorophenol (5 mM) as a product standard. In \(^1\text{H}\) MRI (T\(_2\)-weighted), all three samples gave similar images (left lane in Fig. 3). In \(^{19}\text{F}\) MRI, distinct \(^{19}\text{F}\) MR images of probe 1 were observed using \(^{19}\text{F}\) chemical-shift-selective imaging based on the probe 1-selective \(^{19}\text{F}\) pulse frequency (middle lane in Fig. 3). The reactant and 2-fluorophenol were not observed under the same \(^{19}\text{F}\) pulse conditions. In contrast, distinct \(^{19}\text{F}\) MR images were clearly observed from the sample of the reactant (probe 1 reacted with \(\text{H}_2\text{O}_2\)) using the 2-fluorophenol-selective \(^{19}\text{F}\) pulse frequency (right lane in Fig. 3). These results indicate that probe 1 allows us to visualize \(\text{H}_2\text{O}_2\) using \(^{19}\text{F}\) chemical-shift-selective imaging.

Finally, we applied probe 1 to the \(^{19}\text{F}\) MR imaging of \(\text{H}_2\text{O}_2\) produced by an enzymatic reaction. It is well known that \(\text{H}_2\text{O}_2\) is generated in many biological oxidation processes catalyzed by a variety of oxidases. As a proof-of-principle study for the detection of enzyme-mediated \(\text{H}_2\text{O}_2\) production, we checked the utility of probe 1 to visualize \(\text{H}_2\text{O}_2\) production by oxidases, GOX and COX in this case (Figs. 4a and 4b). Samples without GOX and COX were visualized only using the probe 1-selective \(^{19}\text{F}\) MR imaging (middle lane of Fig. 4c). In contrast, samples in the presence of GOX and COX were well visualized using 2-fluorophenol-selective \(^{19}\text{F}\) MR imaging (right lane of Fig. 4c). These data indicate that probe 1 could visualize enzyme-mediated \(\text{H}_2\text{O}_2\) production utilizing \(^{19}\text{F}\) MR imaging.

Conclusions

We designed a \(^{19}\text{F}\) MRI probe that could detect \(\text{H}_2\text{O}_2\). To our knowledge, this is the first \(^{19}\text{F}\) MRI probe for \(\text{H}_2\text{O}_2\) imaging. The advantage of this probe is a large chemical-shift change based on the electron property change from phenylboronic acid to phenol. This large \(^{19}\text{F}\) chemical-shift change allowed us to easily detect and visualize \(\text{H}_2\text{O}_2\) using \(^{19}\text{F}\) chemical-shift-selective imaging. Such a large \(^{19}\text{F}\) chemical-shift change would provide a large advantage for the MR analysis of complicated biological systems such as \textit{in vivo} in the future.

Our next challenge is to improve the reaction selectivity and the biodistribution of probe 1 toward \textit{in vivo} applications by refinement of the probe structure. In addition, \(^{19}\text{F}\) MRI using a much larger voxel could improve the sensitivity of \(\text{H}_2\text{O}_2\) detection. Further work is now under way in our laboratory.

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Supporting Information

The kinetic analysis data of probe 1 and HPLC analysis of ROS and RNS selectivity are contained in Supporting Information. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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