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A boronic acid based intramolecular charge transfer probe for colorimetric detection of hydrogen peroxide

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ABSTRACT

Many oxidative stress related diseases and adverse health conditions have been associated with the negative effects of hydrogen peroxide and other similar reactive oxygen species in human body. Therefore, increasing attention has been attracted to the detection and monitoring of hydrogen peroxide in living organisms and food items. In this work, a simple, inexpensive colorimetric method for the quantitative determination of hydrogen peroxide in aqueous sample is described. This method utilizes the de-protection of aryl boronic acid to yield a strongly colored water-soluble dye, which is capable of absorbing and emitting in the red region of the spectrum. The mechanism is faster in alkaline condition and utilizes the intramolecular charge transfer between strong phenolate donor and TCF acceptor, thus allowing a naked eye detection of hydrogen peroxide within seconds. The method is unaffected by the presence of various salts, metal ions, and other interfering species, and it can provide a limit of detection as low as ~1 ppm in aqueous samples. This unique way of generating a fluorogenic donor-acceptor pair holds a potential of this dye and other related derivatives for understanding the role of hydrogen peroxide in physiology and pathology.

Introduction

Hydrogen peroxide (H_2O_2) is a ubiquitous oxidizing agent. Since the early nineteenth century hydrogen peroxide has been known for its bleaching and antimicrobial properties. In the recent past, it has been suggested as a substitute of pasteurization for its effective bactericidal and bacteriostatic properties [1]. Currently, it is used as germicidal and redox agent for treatment of various food and dairy products [2–5].

Though use of hydrogen peroxide is very common in many areas—including cosmetics and beauty care products—moderate to excessive exposure to hydrogen peroxide could cause health problems such as respiratory and ocular irritation, including loss of consciousness at higher concentrations [6]. Therefore, in the USA, the maximum allowance level of H_2O_2 in food items has been set to 0.4–0.5%, w/w, a level set by the Food and Drug Administration (FDA) [7]. Moreover, hydrogen peroxide has been recognized as a reactive oxygen species (ROS) that is responsible for several oxidative stress related diseases such as cardiovascular disorders, neurodegenerative diseases and cancer [8–10].

Due to various short- and long-term negative effects of H_2O_2 in human and cellular biology, various organic dyes, metal nanoparticles and fluorescent probes have been reported in literature for selective and quantitative estimation of H_2O_2 in samples [11–16]. Other methods such as enzyme assay, high performance liquid chromatography, amperometry, metal nanoparticles and chemiluminescence methods are also well known [17–22]. Although these methods are sensitive to hydrogen peroxide, and some of them are very selective, they are limited by their inherent drawbacks such as cost of specialized apparatus, multi-step synthesis, extensive sample preparation, irreproducibility, poor solubility of the organic compounds, and requirement of large amount of sample(s) for spectrophotometric based method [19,22,23,24]. In this regard, colorimetric analysis—with the aid of a color reagent—is well suited for a convenient, rapid, inexpensive and naked eye detection method for hydrogen peroxide in aqueous and biological samples [16,25].

Working toward the development of a direct, simple, and an inexpensive method for selective detection of hydrogen peroxide, we have reported here a boronic acid based latent probe which, in contact with hydrogen peroxide, undergoes a fast reaction to produce a donor-acceptor dye with concomitant color change from yellow to red. The newly formed donor-π-acceptor dye with a

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strong phenolate (PhO^-) donor and tricyanofuran (TCF) acceptor makes the process conspicuous to the naked eyes.

In the past, several TCF based donor-acceptor fluorophores have been developed for selective detection of chemical and biological analytes and for single molecule imaging of live cells [26–29]. Recently, we have established that phenolate (PhO^-) and its derivatives when conjugated with a strong electron acceptor such as TCF or benz[*e*]indolium produce intense colors in the red/NIR regions of the spectrum [30]. This class of compounds is moderately emissive in water. However, fluorescence can be dramatically enhanced with a viscous liquid or by complexation with a macromolecule. Herein, we have described the development of a simple non-enzyme based colorimetric probe for quantitative determination of hydrogen peroxide in aqueous solution.

Results and discussion

In this study (4-formylphenyl)boronic acid was conjugated with a strong electron acceptor tricyanofuran (TCF) *via* a simple condensation reaction (Scheme 1a). The goal of this design principle was to construct a small molecule probe which would undergo hydrogen peroxide induced electrophilic displacement reaction at the boron center to produce a phenol-π-TCF donor–acceptor fluorophore in aqueous solution (Scheme 1b).

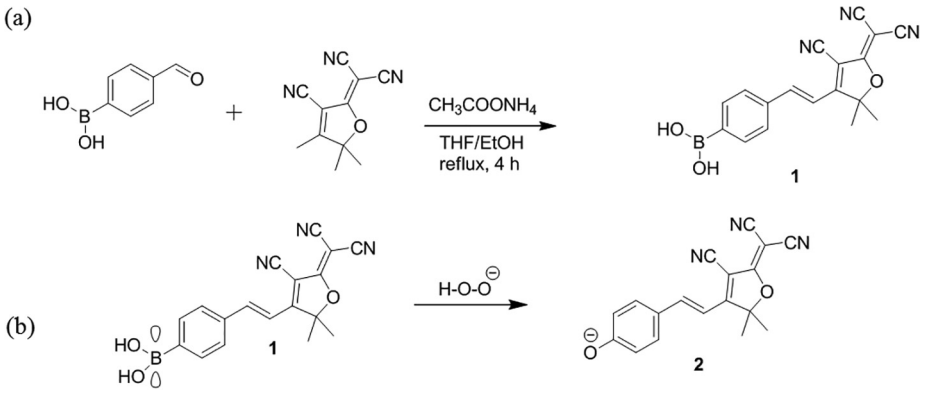
Knoevenagel condensation between (4-formylphenyl)boronic acid and 2-(3-cyano-4,5,5-trimethylfuran-2(5*H*)-ylidene)malononitrile in ethanol resulted **1** in moderate yield (27%) [28]. Details of the synthesis, purification, and characterization are provided in the experimental section (electronic Supplementary materials, Figs. S1–S3). In the ^{1}H NMR spectrum, appearance of a broad signal at 8.25 ppm in DMSO d_6 indicates presence of –OH functional group. Moreover, in the IR spectrum, broad signal at ~3290 cm^{-1} corroborates to the –OH of phenyl boronic acid [31]. As expected, a sharp signal appears at ~2225 cm^{-1}, confirming nitrile groups on **1**. The selectivity to all *trans* isomer of the Knoevenagel condensation was very high, which was confirmed from the high coupling constant (J = 16.2 Hz) of the vinylic hydrogens [32].

We first studied the reaction kinetics of **1** with hydrogen peroxide ($[H_2O_2]$ = 2.94 mM) by UV–vis spectroscopy. The reaction was monitored by recording absorption spectra of the mixtures in a buffer (pH = 9.0; 0.1 M) solution. As shown in Fig. 1a—upon addition of hydrogen peroxide—amount of **1** ([**1**] = 10 μM) rapidly decreased. Two absorption peaks were observed before addition of H_2O_2; after addition of H_2O_2 both signals decreased and a new signal emerged at 570 nm, which leveled-off after ~12 min (Fig. 1b). A clear isobestic point was observed at 465 nm, indicating transformation of **1** into another species. A visible color change from pale yellow to light purple was also observed within minutes (inset, Fig. 1). Formation of an ICT fluorophore was confirmed by comparing the absorption spectra of the mixture after 12 min with an absorption spectrum of **2**. The phenolic form of **2** was previously prepared by our group to investigate the relationship between solution pH and the ICT properties of a donor-acceptor fluorophore [30]. Therefore, with that compound in hand, we readily prepared a standard graph with different amounts of **2** (Fig. S4). Inserting the absorbance value of **1** (at 570 nm) into the standard graph shows ~90% yield for the H_2O_2 induced conversion of **1** into **2**.

Rate constant and instantaneous rate of reaction were then measured. Under pseudo first order condition ([**1**] = 10 μM and 15 μM; $[H_2O_2]$ = 2940 μM) plot of natural logarithm of [**1**] *versus* time showed linear relationships (Fig. 2). The observed rate constant for H_2O_2 induced demasking of boronic acid to the corresponding phenol was $k_{obs} = 1.8 \times 10^{-3}\ s^{-1}$. We measured the instantaneous rate of the reaction for both concentrations, and the first four minutes of the reactions were monitored (Fig. S5). As expected, under similar experimental conditions, the instantaneous rate of reaction for 15 μM of **1** ($r = 1.82 \times 10^{-6}$ M min^{-1}) was 1.5 times faster than 10 μM ($r = 0.82 \times 10^{-6}$ M min^{-1}). Therefore, all these kinetics studies indicate that a rapid colorimetric assay for hydrogen peroxide can be developed with as low as 10 μM of **1**.

Hydrogen peroxide induced conversion of arylboronic acids to phenols is faster at alkaline pH [33,34]. Therefore, the effect of pH on the rate of the reaction (**1->2**) was examined at pH 7.4. As shown in Fig. 3, absorbance at 410 nm slowly decreased with time; after ~38 min a saturation reached. Comparison of the absorbance at 570 nm with the standard graph shows ~46% conversion yield after 38 min. Moreover, an isobestic point was recorded at 435 nm, which is distinctly different from that of the pH 9.0 solution. In pH 7.4 solution, the isobestic point was at shorter wavelength, indicating an equilibrium transformation of **1** to the phenolic form of **2**. We have previously established that the maximum absorbance at 463 nm originates from the protonated (phenolic) form of **2**, and a high percentage of this form exists in equilibrium at pH 7.4 [30]. The rate constant under pseudo first order condition was $k_{obs} = 2.2 \times 10^{-4}\ s^{-1}$, which is one order of magnitude lower than that of the pH 9.0 solution (Fig. S6). The instantaneous rate was also relatively slower ($r = 0.28 \times 10^{-6}$ M min^{-1}), as obtained from the first eight minutes of the reaction (Fig. S6).

From the pH dependent kinetics study it was established that **1** responds rapidly at alkaline pH. Next, to gain an insight into sensitivity of **1**, limit of detection (LOD) was calculated by titrating different amounts of hydrogen peroxide with **1** at pH 9.0 [35]. As shown in Fig. 4a, a good linear correlation (between 0 and



Scheme 1. (a) Synthesis of probe **1**. (b) Demasking of **1** by hydrogen peroxide to a new Intramolecular Charge Transfer (ICT) dye.

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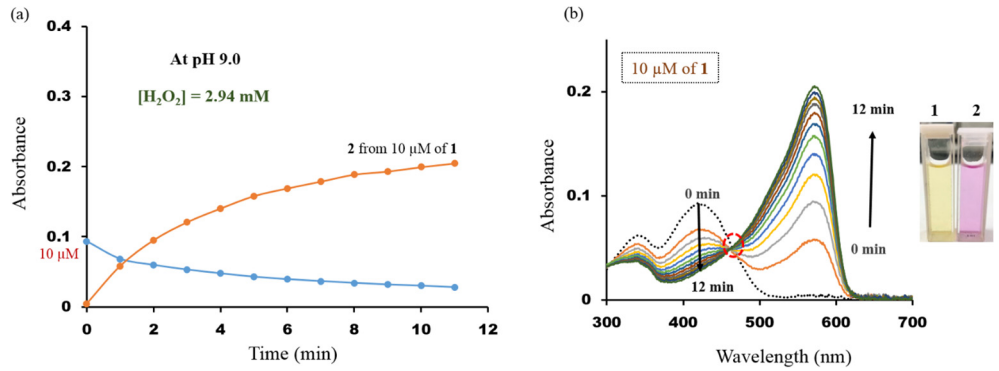

Fig. 1. Time-course of UV–vis spectra of the reaction of **1** (10 μM) with H_2O_2 (2.94 mM) at pH 9.0. (a) Time dependent absorbance change of **1** and **2** recorded over a period of 12 min. (b) The absorbance change of **1** at 410 and 570 nm over 12 min.

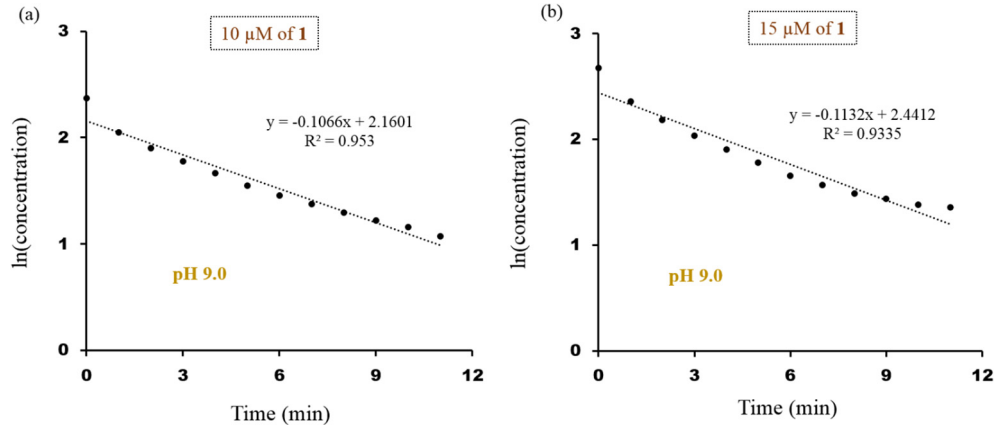

Fig. 2. Determination of rate constant under pseudo first order condition in buffered solution (pH 9.0). (a) 10 μM of **1**, (b) 15 μM of **1**.

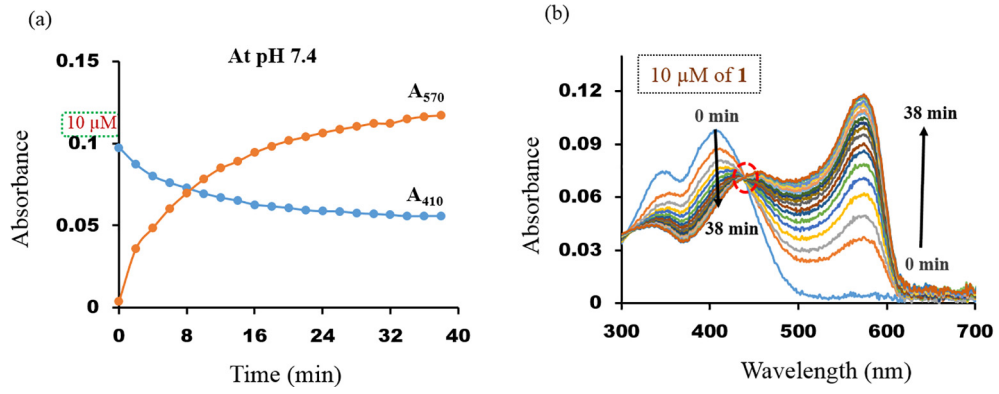

Fig. 3. Time-course of UV–vis spectra of the reaction of **1** (10 μM) with H_2O_2 (2.94 mM) at pH 7.4. (a) Time dependent absorbance change of **1** and **2** recorded over a period of 38 min. (b) The absorbance change of **1** at 410 and 570 nm over 38 min.

300 μM; $r^2 = 0.9959$) was obtained with a LOD value of 74 μM (1.26 ppm; *s.d.* = 0.0030) with as low as 10 μM of **1**. Therefore, the probe can be used to detect hydrogen peroxide in food and dairy products well below the allowance level (0.05%; w/w), a standard set by the US Food and Drug Administration [7]. Moreover, the color change from yellow to purple was visible to the naked eyes—within two minutes—at H_2O_2 concentration as low as 25 ppm (Fig. 4b). For 1.26 ppm H_2O_2, the discernible response time was longer; after five minutes the purple color developed (Fig. S7a). Therefore, these results indicate that **1** is highly sensitive colorimetric probe for hydrogen peroxide. In this aspect we would like to highlight that **1** has reached to a very low detection limit range reported by several research groups (detection limit 1–400 μM) [36–40].

We then investigated the effect of common interfering agents on the selectivity of hydrogen peroxide to **1**. As shown in Fig. S7b, ascorbic acid, BHT, EDTA, potassium bromate, cysteine, *t*-BuOO·, and *t*-BuOOH, were selected, and they were allowed to react with **1** ([**1**] = 10 μM; [Interfering agent] = 100 μM) in pH 9.0 buffer. After five minutes the UV–vis spectra were recorded and compared with a solution of **1** in absence of any interfering species. No significant change was noticed, except for EDTA. This interfer-

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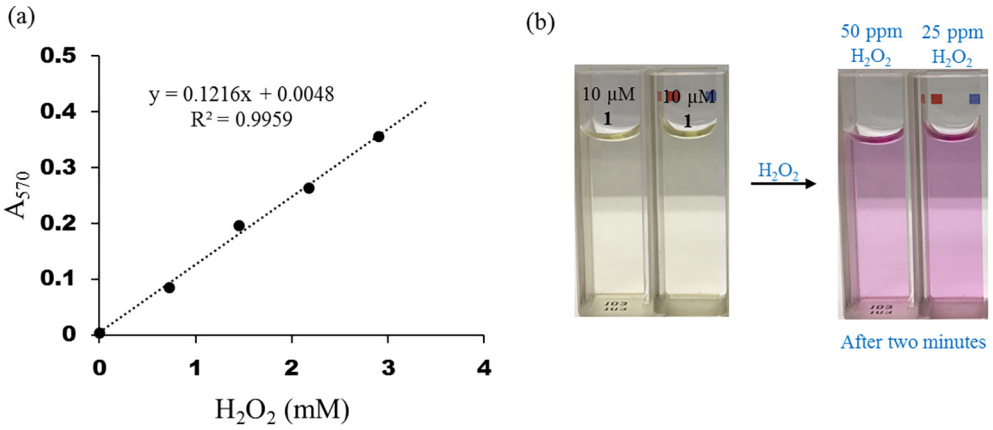
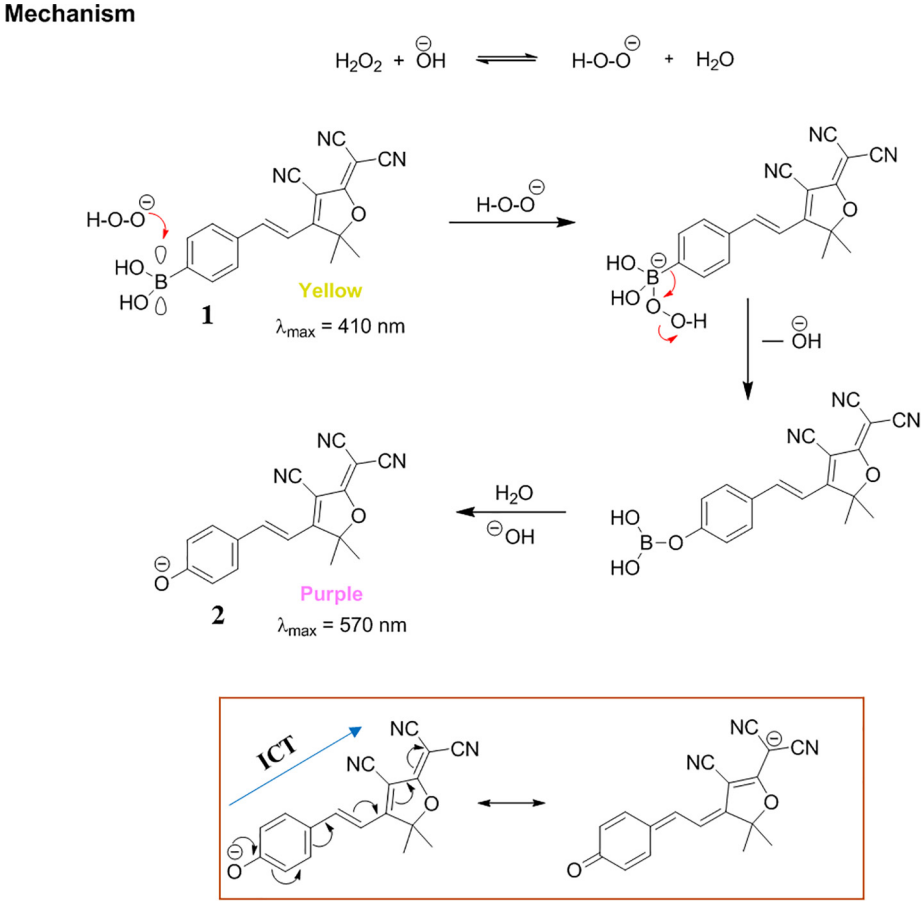


Fig. 4. (a) Linear correlation of released **2** in reaction of **1** with different amounts of H_2O_2. (b) Color change of **1** upon addition of different amounts of H_2O_2 in pH 9.0 buffer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Scheme 2. Mechanism of the reaction of **1** with H_2O_2 under alkaline condition to generate the ICT fluorophore (**2**).

ence can be suppressed by starting with higher concentration of **1**. For hypochlorite ([OCl^- = 230 μM], only 4% conversion was noticed after ~12 min, which is probably due to the similar nucleophilic addition mechanism to the electron deficient boron (Fig. S7c). Overall, all these investigations indicate that **1** selectively responds to H_2O_2 with a distinct color change.

Upon addition of H_2O_2 in **1**, the distinct color change from yellow to purple suggests that the ΔE of electronic transition for **2** is significantly lower than that of **1**. In **1**, boron is sp^2 hybridized with an empty p orbital. Therefore, an efficient charge transfer relation is not established with the TCF acceptor. However, addition of peroxide produces **2**, where $-O^-$ is a very good electron donor which generates a donor-π-acceptor conjugated system with significant intramolecular charge transfer (Scheme 2). The purple color and high molar extinction coefficient ([**1**] = 10 μM, ε = 20470 L mol^{-1} cm^{-1}) reflect this phenomenon.

Conclusions

We have developed a new derivative of phenyl boronic acid for detecting H_2O_2 in aqueous solution. Our design was based on a

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latent donor-π-acceptor system that was able to undergo an analyte selective reaction to form a new dye compound. In alkaline condition, the fast reaction between H_2O_2 and the probe triggered a fast color change in the visible region which was selective over a variety of interfering agents. A detection limit as low as ~1 ppm was obtained from the linear relationship between the concentration of H_2O_2 and the absorbance of the phenolate product. This is very impressive as this method requires simple instrumentation and preparation, and the reaction between the probe and H_2O_2 is a direct measurement of H_2O_2 in samples. Moreover, due to high molar extinction coefficient of the ICT dye, naked eye visualization of the color change was possible near the machine readable detection limit range.

In this work boronic acid was used as masking agent which was demasked by H_2O_2 to generate a simple yet very sensitive ICT fluorophore. ICT fluorophores are known to be strongly colored and emissive in aqueous solution. Thus, dual colorimetric fluorimetric sensors can be in our reach via conjugation of appropriate donors and acceptors. Moreover, absorption and emission can be pushed to the near infrared-I (NIR-I) region by extending π-conjugation and/or by substituting strong electron donor(s) on the phenyl ring [30]. With numerous available phenyl boronic acids and esters a library of ICT fluorophores can be developed for various chemical, environmental, and biological applications. Ongoing efforts are focused on manipulating the electron density on the aromatic ring with different substituents to lower the pKa of the resulting phenols. Successful generation of phenolates at lower pH values—with measurable color and fluorescence change—at faster rate would prove useful for giving specificity for free H_2O_2 in cellular environments in presence of other competitive ROS and alkyl peroxides.

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Appendix A. Supplementary data

Supplementary data (Description of the synthesis of **1**, NMR (^1H and ^{13}C), and kinetics data) to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.151258. These data include MOL files and InChiKeys of the most important compounds described in this article.

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