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Reactive Oxygen Species-Responsive Thin Films that Display Distinct Color and Pattern Changes

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Summary:

Novel methodologies for the preparation of reactive oxygen species (ROS)-responsive thin-film sensors that display distinct color and pattern changes were developed. The ROS-responsive thin films presented herein are advantageous for the development of ROS sensors because of their relatively simple preparation and non-complex organic synthesis. In addition, there is virtually no limitation to choosing dyes, and any kind of visual color and pattern changes can be realized.

Keywords: reactive oxygen species, hydrogen peroxide, hypochlorous acid, boronic acid, thin film

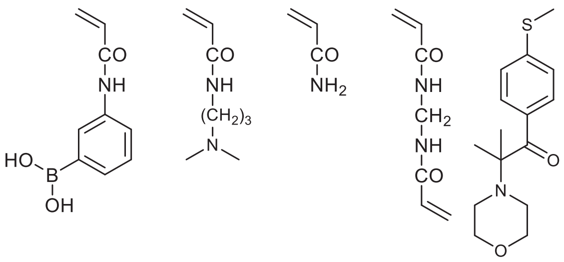
Introduction

Boronic acids are known to form boronate esters with polyhydroxy compounds such as saccharides through reversible boronate-diol interactions. Moreover, boronic acids give rise to their phenol derivatives after reaction with reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and hypochlorous acid. The latter reaction has been utilized to develop colorimetric and fluorescent ROS sensing probes through a strategy in which a boronic acid moiety is directly connected to a chromophoric or fluorophoric group. Boronic acid-based ROS probes are advantageous over conventional enzyme-based sensors in terms of durability and reproducibility because of absence of unstable bioorganic substances such as enzymes. However, one of the drawbacks of the boronic acid-based probes is the necessity of complicated organic synthesis. Moreover, limited color change could be an obstacle for developing user-friendly sensors. Thus, to realize a widely used ROS sensor at a low cost, it is necessary to introduce a novel methodology.

Our research group has developed thin-film-based colorimetric sensing systems that exhibit unprecedentedly large color changes [1,2]. The preparation of these sensors is relatively simple and does not require complex organic syntheses. Moreover, this methodology is the most advantageous as compared to other methodologies as no limitation in selecting dyes for the coloring of the thin films exists. We hypothesized that this mechanism would be applicable for ROS sensing by utilizing the interaction of boronic acids with ROS. Accordingly, thin films containing ROS-responsive boronic acid units in addition to positively charged tertiary amine units with different compositions were prepared. The thin films were immersed in aqueous dye solutions before or after reaction with ROS in an aqueous solution. As a result, the color and/or pattern of the thin films changed distinctly in response to ROS concentration [3].

Experimental

The ROS-responsive thin films were prepared on a glass slide by the radical copolymerization of boronic acid monomer **1**, tertiary amine monomer **2**, acrylamide **3**, and crosslinker **4** in the presence of an initiator **5** (Fig. 1). The monomer solutions were poured onto the glass surface of the slides using a micropipette and covered with an acrylic plate. Polymerization was conducted by irradiating the sandwiched monomer solution with UV light (365 nm) using a UV lamp under nitrogen atmosphere at 20–25 °C for 3 h. After removing the acrylic plate from the glass slide, the resulting thin films were washed with water and air-dried. Then, the sensor was immersed in aqueous solutions containing ROS at 25 °C for 1–60 min. Subsequently, the sensor was immersed in an aqueous dye solution at 25 °C for 1–60 min. Polyols such as fructose or sorbitol were sometimes added to the ROS or dye solutions to enhance the change in charge state of the boronic acid moiety. In the case where colored samples were used for ROS response, the experimental order was reversed: the thin film was immersed in a dye solution, then reacted with ROS.



1 **2** **3** **4** **5**

Fig. 1. Chemical structures of monomers.

Results and Discussion

The dependency of H_2O_2 concentration on the color of thin films at different reaction times at pH 7.4 was measured (Fig. 2). The sensor displayed a visible color change from colorless to blue at 4 µM. The color deepened with increasing reaction time and H_2O_2 concentration. This behavior can be attributed to the change in the charge state of the thin film. Before reacting with H_2O_2, the cationic charges on the thin film should be electrostatically neutralized by boronate groups, resulting in lack of adsorption ability for anionic dyes. After reacting with H_2O_2, the thin film was colored with an anionic dye because the boronate groups were converted into neutral phenol groups and thus the thin film became positively-charged. A plausible response mechanism is illustrated in Fig. 3.

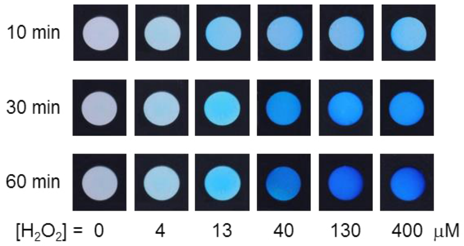


Fig. 2. H_2O_2 concentration-dependent color changes in the thin film colored by Fast Green FCF in the presence of fructose at pH 7.4.

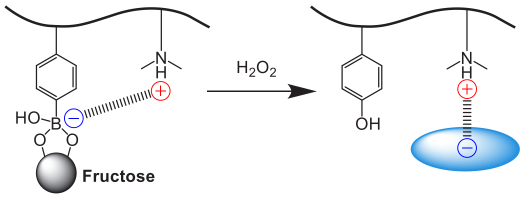


Fig. 3. Mechanism of hydrogen peroxide-responsive color change in the thin film in the presence of fructose at pH 7.4.H_2O_2 concentration-dependent color changes in the thin film.

Instead of an anionic dye, a monovalent cationic red dye (Safranin T) was used to color the thin film after reacting with H_2O_2 (Fig. 4). The thin film was colored red when the H_2O_2 concentration was lower than 13 µM. A further increase in H_2O_2 concentration resulted in a decrease in the adsorption of the dye, which became almost colorless when the H_2O_2 concentration was above 130 µM. In addition, experiments were conducted by simultaneously using anionic and cationic dyes for coloring (Fig. 5). When the H_2O_2 concentration was lower than 40 µM, the thin film became red due to the adsorption of the cationic red dye. By increasing the H_2O_2 concentration from 40 µM to 100 µM, the color of the thin film gradually changed from red to blue. The thin film finally became blue when the H_2O_2 concentration was higher than 100 µM.

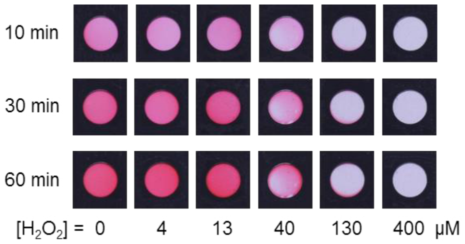


Fig. 4. H_2O_2 concentration-dependent color changes in the thin film colored by Safranin T in the presence of fructose at pH 7.4.

Fig. 5. H_2O_2 concentration-dependent color changes in the thin film colored by Fast Green FCF and Safranin T in the presence of fructose at pH 7.4.

Because the response mechanism of the present method is based on changes in the charge state of the thin films, principally, any kind of anionic or cationic dyes can be used. To confirm this experimentally, various dyes were used to color the thin films. Accordingly, I successfully established a novel methodology for creating H_2O_2 sensors that exhibit diverse color changes. In the conference, I will also talk about sensors with changing patterns and hypochlorous acid sensors.

References

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