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Turn-on Fluorescence Detection of H_2O_2 and TATP

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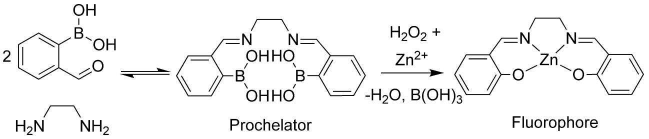
Peroxide-based explosives, like triacetone triperoxide (TATP), are important targets for detection because of their broad use in improvised explosives but pose challenges. We report a highly sensitive turn-on fluorescence detection for H_2O_2 and organic peroxides, including TATP. The detection strategy relies on oxidative deboronation to unmask H_2Salen, which subsequently binds Zn^{2+} to form fluorescent Zn(Salen). Sensitivity is excellent, with detection limits below 10 nM for H_2O_2, TATP, and benzoyl peroxide. In addition, acid treatment is necessary to sense TATP, suggesting the potential to discriminate between H_2O_2 and TATP based upon minimal sample pretreatment.

Peroxide-based explosives, like triacetone triperoxide (TATP), are important targets for detection because of their broad use in homemade explosives, but the absence of nitro or chromophoric groups poses significant challenges for sensing and discrimination.[1,2] The current detection strategies for TATP typically require expensive instrumentation[3−5] and complex sample preparation, including enzymatic treatment.[2,6,7] However, field detection requires more robust and portable optical sensing methods much like technologies available for nitro-organics.[8,9] High-sensitivity optical detection can be realized by turn-on fluorescence strategies, in which analyte binding or reactivity creates a new fluorophore against a dark background. We report such a turn-on fluorescence approach for detecting and discriminating TATP from other peroxides, with limits of detection below 10 nM.

Scheme 1. Formation of Zn(Salen) Fluorophore by Oxidative Deboronation



TATP has extreme sensitivity to detonation upon impact, heating, or friction, leading to the moniker “Mother of Satan”.[10] Difficulties in converting current methods for detecting TATP, which include mass spectrometry, electrochemical methods, and enzyme-linked assays,[2−4] to field-deployable formats prompt the development of optical sensing approaches. While optical methods could include colorimetric or fluorescence-quenching assays, a fluorescence turn-on strategy offers high sensitivity because of the appearance of a new signal on a dark background.[11] Furthermore, minimizing the sample preparation by relying on selective chemical reactivity could further improve the utility of an optical sensor.

Oxidative deboronation is chemically selective for peroxides and involves the oxidative conversion of a C−B bond into a C−O bond by H_2O_2. Such an approach was used to create fluroescein-based probes for H_2O_2 within cells;[12] however, fluorescein tends to photobleach, and its emission is very sensitive to pH, suggesting that other fluorophores would be better suited for explosives detection. Deboronation is ideal to activate prochelators containing masked phenolates,[13] as found in the tight chelator H_2Salen [*N*,*N*′-ethylenebis(salicylaldimine)]. Because Zn(Salen) and related complexes are stable and highly fluorescent,[14] deboronation could create a fluorescent “turn-on” to detect peroxides such as TATP.

The proof of concept was achieved by turn-on fluorescence sensing of H_2O_2 using a mixture of prochelator and Zn(ac-

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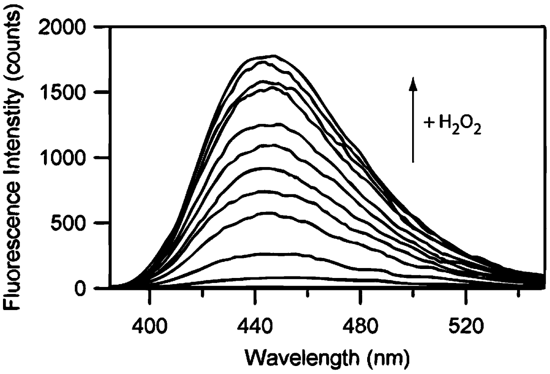

Figure 1. Fluorescence turn-on upon mixing of prochelator (3.13 mM), $Zn(OAc)_2$ (3.13 mM), and H_2O_2 (7.3 mM) in MeOH ($\lambda_{ex} = 350$ nm, $t = 0-20$ min).

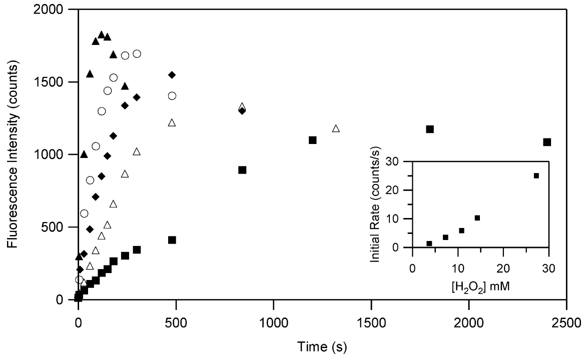

Figure 2. Fluorescence time course for prochelator/$Zn(OAc)_2$ (3 mM in MeOH) after the addition of various concentrations of H_2O_2: 3.7 mM H_2O_2 (■), 7.3 mM H_2O_2 (△), 10.8 mM H_2O_2 (◆), 14.3 mM H_2O_2 (○), 27.3 mM (▲) at 23 °C. Inset: Initial rate dependence on $[H_2O_2]$.

etate)$_2$ in MeOH (Scheme 1). The prochelator was synthesized by a condensation reaction of 2-formylphenylboronic acid and ethylenediamine in the presence of boric acid in a sodium acetate buffer (pH = 4.2). The prochelator precipitated as a white powder, which was collected by filtration and the structure of which was confirmed by ^1H NMR (see the Supporting Information).

Upon addition of H_2O_2 (7.3 mM) to our standard solution of prochelator (3.13 mM) and Zn(acetate)$_2$ (3.13 mM) in MeOH, an increase in the fluorescence intensity ($\lambda_{em} = 440$ nm) was observed (Figure 1). Similar fluorescence turn-on was observed when H_2O_2 was substituted by benzoyl peroxide (BP), a common organic peroxide. Negative controls, in which any single component was omitted, lead to no discernible increase in fluorescence. Zn^{2+} was crucial to the fluorescent nature of this system because substitution of Zn^{2+} with another closed-shell metal (Mg^{2+}), or deprotonation of H_2Salen with triethylamine, resulted in only minor fluorescence signals. Consequently, this strategy can serve to sense organic or hydrogen peroxides in organic solutions.

To test the mechanism of converting the component mixture into the fluorescent Zn(Salen), we evaluated the kinetics of fluorophore formation under various H_2O_2 concentrations (Figure 2). The rate of fluorophore formation was rapid, increasing with elevated concentrations of H_2O_2. The linear dependence of the initial rate on $[H_2O_2]$ indicated that the rate-limiting step required H_2O_2 and was consistent with the reported mechanism of deboronation[15] (Figure 2, inset).

The H_2O_2 concentration affected not only the kinetics of formation but also those of decay, as shown by the fluorescence time courses. The fluorescent signal from Zn(Salen) decreased with time, a process that could arise from photodegradation or oxidation of the prochelator, of H_2Salen generated in situ, or of Zn(Salen). The photostability of isolated Zn(Salen) (3.13 mM) was evaluated in the presence of elevated concentrations of $B(OH)_3$ (0.2 mM) and/or H_2O_2 (30 mM); there was no decrease in the fluorescence intensity over 600 s. To determine the stability of the prochelator toward H_2O_2, prochelator (3.13 mM) was preincubated with H_2O_2 (30 mM) for variable time intervals (0−20 min), followed by the addition of Zn(acetate)$_2$. The fluorescence intensity following Zn^{2+} addition decreased with extended preincubation times. This suggested that the pathway from prochelator to Zn(Salen) was disrupted by H_2O_2 oxidation of the prochelator or H_2Salen. A potential degradation route is Schiff-base hydrolysis, followed by salicylaldehyde oxidation to salicylic acid; however, the fluorescence rise and fall (Figure 2) suggest that the degradation mechanism is more complex than this.

In addition to H_2O_2, BP and TATP were also detected by a 3.13 mM $Zn(OAc)_2$/prochelator solution in MeOH. A stock solution of BP (1 mM) was prepared in a 1:4 solution of acetone/MeOH. As seen for H_2O_2, the fluorescence signal increased rapidly for assays using BP, confirming that organic peroxides could be sensed. TATP was purchased as an analytical standard in an acetonitrile solution (0.5 mM), and assay samples were generated by dilution in MeOH. TATP solutions analyzed with the methodology described above produced no increase in fluorescence, indicating that TATP itself could not deboronate the prochelator. Because of the absence of a response for TATP by the standard methodology, diluted TATP was preincubated for 5 min with acetic acid (1 M) before addition to the component solution. The TATP/H^+ mixture was added directly to the standard prochelator/Zn^{2+} solution, and an 80-fold increase in the fluorescence signal was then observed. It appeared that organic peroxides were sensed only if first converted into H_2O_2; we suspect that BP was hydrolyzed by low levels of H_2O but that TATP required acid to promote H_2O_2 release. Varying the pretreatment by this approach may lead to discrimination between simple peroxides, such as H_2O_2, and TATP.

The largest advantage of turn-on sensing is high sensitivity. The limit of detection was determined for H_2O_2, BP, and TATP, each at concentrations of 10 μM to 10 nM (Figure 3). The fluorescence of a 3.13 mM prochelator/$Zn(OAc)_2$ solution in MeOH was measured 1 h after the addition of peroxide. H_2O_2 or BP was added directly to the prochelator/Zn^{2+} solution, leading to a ~800-fold increase in the fluorescence signal (left axis). Diluted TATP (10 μM to 10 nM) was mixed with acetic acid (0.5 M) in MeOH for 5

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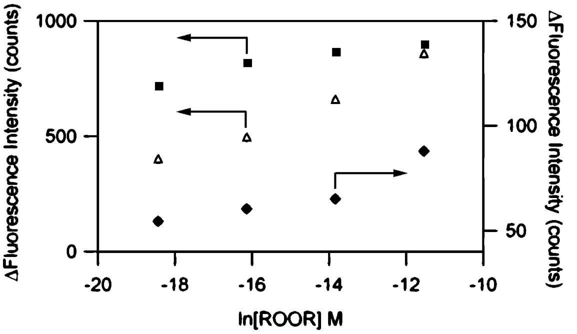

Figure 3. Relative fluorescence increase of prochelator/Zn(OAc)$_2$ (3 mM in MeOH) to peroxide: left axis, H_2O_2 (■), BP (△); right axis, TATP (◆) pretreated with glacial acetic acid. Fluorescence monitored at $\lambda_{em} = 440$ nm ($\lambda_{ex} = 350$ nm) 1 h after the addition of peroxide at room temperature; $\Delta F = F_{60\ min} - F_{t=0}$.

min. Following the addition of each TATP/H^+ solution to a standard prochelator/Zn^{2+} solution, the fluorescence signal at $\lambda_{em} = 440$ nm increased ca. 80-fold. Because significant responses were evident down to 10 nM peroxide, this method was extremely sensitive for inorganic and organic peroxides, including dilute solutions of TATP.

This turn-on fluorescence sensing approach detected simple peroxides, as well as TATP, with a limit of detection below 10 nM. This methodology was effective with minimal sample handling, relying on simple chemistry to form highly fluorescent Zn(Salen), and worked in the presence of low levels of H_2O. In addition, discrimination of organic peroxides may be possible because of differences in the rate of fluorophore formation or various pretreatments. For example, TATP only leads to a fluorescence turn-on response if pretreated with acid. Simple, selective, turn-on fluorescence detection for peroxide-based explosives has not previously been reported, to our knowledge, and suggests the potential for a highly sensitive, portable, and inexpensive field detector for TATP and related compounds.

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Supporting Information Available: Experimental procedures and characterization and limits of detection kinetic traces. This material is available free of charge via the Internet at http://pubs.acs.org.

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