Sulfhydryl-based dendritic chain reaction†

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A new dendritic chain reaction probe system was demonstrated to produce exponential signal amplification for the detection of sulfhydryl compounds.

New molecular approaches for signal amplification are frequently applied to improve the sensitivity of chemical sensors.[1–5] A powerful method of signal amplification is exponential generation of a target molecule.[6–10] Recently, we reported a new molecular probe for the detection of hydrogen peroxide that is based on a distinctive dendritic chain reaction (DCR).[11,12] The self-immolative dendritic probe[13–15] was equipped with a triggering group (based on phenylboronic acid) that upon interaction with the analyte, hydrogen peroxide, released a chromogenic molecule and two or more reagent molecules (choline). The free choline was enzymatically oxidized to produce additional hydrogen peroxide that could then activate additional probe molecules. As a result, this chain reaction produces an exponential increase in the concentration of the chromogenic molecule and a strong diagnostic signal is developed from a relatively small starting amount of analyte.[16] The choline/hydrogen-peroxide/phenyboronic acid reactions work beautifully together, but to be broadly useful, the DCR technique must be demonstrated for other analytes. In principle, the DCR technique could be applied for the detection of other analytes since its probe has a modular structural design. Generally, any analyte of interest that has cleavage reactivity toward a specific trigger could be incorporated collectively with the specific triggering group in the dendritic platform. In order to validate this claim, an additional example of a DCR probe that is based on different chemistry than that of hydrogen peroxide as an analyte must be demonstrated. We now report a new dendritic chain reaction for the detection of molecules with ubiquitous sulfhydryl functional group.

Thiols are important biomarkers that participate in several physiological functions and their plasma level can indicate the diagnosis of disease states.[17–19] Therefore, the design of diagnostic probes for identification of thiols is of obvious importance.[20] In order to employ a dendritic chain reaction for the detection of thiols, two major chemical reactivities should be in hand: a "disassembly" chemical pathway that enables the release of a covalently linked thiol and a specific protecting group that is cleaved by reaction with a thiol. These two reactions are illustrated in Fig. 1. The release of a covalently linked thiol was achieved through the design of molecules like compound **1**. The phenylacetamide moiety of **1** is removed by penicillin-G-amidase (PGA) followed by 1,6 elimination and decarboxylation to release amine **1a**. The latter undergoes spontaneous elimination and disassembles to methanimine and free mercaptoacetic acid. Protecting groups cleaved by thiols have been described in the literature before.[21–26] We have used compound **2** with a benzoquinone trigger[24] that upon reaction with a thiol generates intermediate **2a**. This intermediate undergoes rapid elimination to release quinone **2b** and a reporter molecule like 5-amino-2-nitrobenzoic acid.

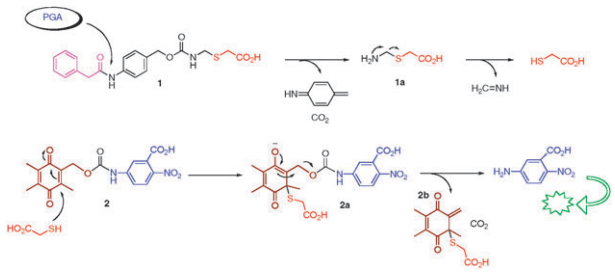

Fig. 1 Disassembly mechanism of compound **1** and probe **2**.

In order to test if we can release a general thiol and use it to activate a chromogenic probe, compounds **1** and **2** (syntheses are described in the ESI†) were incubated together in PBS (pH 7.4) with or without PGA and the release of the reporter 5-amino-2-nitrobenzoic acid was monitored by UV-Vis spectroscopy. Reaction of PGA with compound **1** resulted in the release of free mercaptoacetic acid that then activated probe **2** to release 5-amino-2-nitrobenzoic acid (Fig. 2, blue plot). No release was observed in the absence of PGA (Fig. 2, red plot).

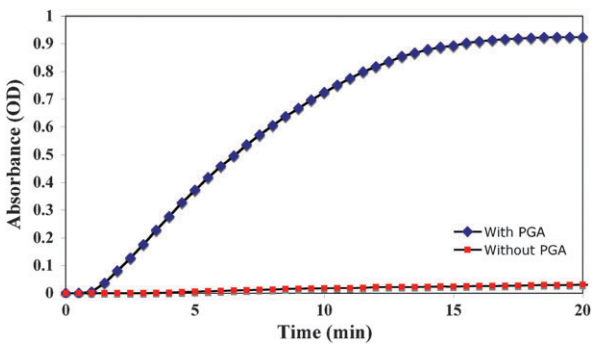

Fig. 2 PGA-catalyzed the release of 5-amino-2-nitrobenzoic acid through sequential disassembly of compounds **1** and **2**. Conditions: wavelength, 405 nm; **1** and **2** [500 μM] in PBS 7.4 and PGA [0.01 mg mL^{-1}].

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With these results in hand, we sought to incorporate the described chemical reactivities into a thiol dendritic chain reaction system. The two-component dendritic chain reaction[27] (2CDCR) is a simple methodology that provides exponential amplification and thus was chosen for evaluation of the thiol analyte. The first component is based on an AB_2 self-immolative dendron[28] equipped with two reagent units and a trigger designed to react with a specific analyte. The second component is a probe composed of the same trigger attached to a reporter. The AB_2 self-immolative dendron acts as an amplifier moiety, and the other component acts as a probe that releases a chromogenic molecule to produce a diagnostic signal. The 2CDCR mode of action for the thiol-based reactions is illustrated in Fig. 3. Dendron **3** was composed of two mercaptoacetic acid units and a benzoquinone moiety as a trigger, which is cleaved upon reaction with any sulfhydryl. Probe **2** was composed of the 5-amino-2-nitrobenzoic acid reporter attached to the benzoquinone trigger. Cleavage of the trigger of dendron **3** by a thiol molecule will initially generate intermediate **3a**. The latter will undergo double eliminations to release two mercaptoacetic acid molecules that will then activate some AB_2 dendrons and some probe molecules. Since the concentration of dendron **3** is at least twice that of probe **2**, the rate of the system disassembly should exponentially increase until all of the reporter molecules have been released. The signal can be detected with a spectrophotometer by monitoring the yellow color of the released 5-amino-2-nitrobenzoic acid.

In order to test the sulfhydryl two-component DCR system, dendron **3** and probe **2** were incubated with various amounts of ethyl-2-mercaptoacetate and the release of 5-amino-2-nitrobenzoic acid reporter was monitored at a wavelength of 405 nm (Fig. 4). When 1.0 equivalent of ethyl-2-mercaptoacetate (*vs.* probe **2**) was used, the system reached complete disassembly within 15 min. As expected, disassembly was slower when less thiol was used, however, the signal level has indicated full release of the 5-amino-2-nitro-benzoic acid reporter (Fig. 4). The exponential progress of the system disassembly is indicated by the sigmoidal shape of the plots of time *vs.* percent conversion obtained for reactions with various equivalents of ethyl-2-mercaptoacetate. The background signal obtained due to some spontaneous hydrolysis is also amplified; therefore, the sensitivity of this detection system for sulfhydryl is limited to the low micromolar range.

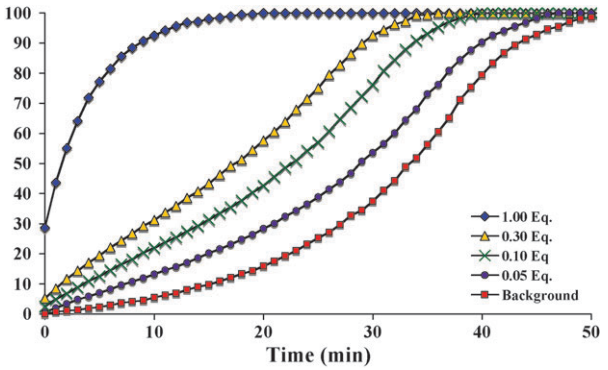

Fig. 4 The release of 5-amino-2-nitrobenzoic acid from probe **2** [500 μM] in the presence of dendron **3** [1000 μM] in Tris buffer (pH 7.2) upon addition of the indicated equivalents of ethyl-2-mercaptoacetate (relative to probe **2**). The reaction progress was monitored at 405 nm for the indicated time period.

The increased sensitivity obtained by the 2CDCR technique is demonstrated in Fig. 5. When three different sulfhydryls were incubated with probe **2** (in the absence of dendron **3**) no amplification was expected and the observed signal was stoichiometrically correlated to the amount of the analyte. However, the net signal (after subtraction of the background signal) measured in the presence of dendron **3** was significantly larger than that obtained from probe **2** alone. In fact, the ratio between the signal with and without dendron **3** was increased as the analyte concentration decreased. When 0.05 equivalent of sulfhydryl *vs.* probe **2** was evaluated, the signal from the 2CDCR system was roughly 6-fold stronger than that obtained by the probe in the absence of dendron **3**.

In addition, the benzoquinone trigger was found to have high selectivity towards addition reaction with thiols in the presence of other nucleophiles.[24]

In the first example of our DCR amplification technique, we designed a probe that had a detection activity for hydrogen

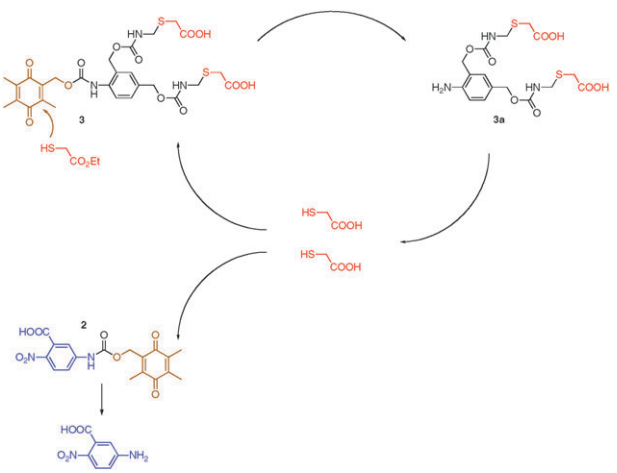

Fig. 3 A two-component DCR system to detect sulfhydryl compounds; mercaptoacetic acid reagent units and 5-amino-2-nitrobenzoic acid reporter are indicated by red and blue color, respectively.

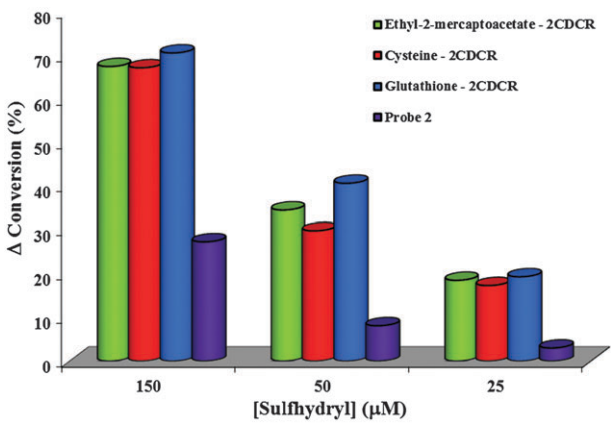

Fig. 5 Comparison of signals measured by the 2CDCR amplification technique (with three different sulfhydryls) *vs.* signals measured using probe **2** without dendron **3** (purple). The background signal present at 22 min was subtracted from the values shown.

peroxide.[12] The generation of the analyte by the release and enzymatic oxidation develops a chain reaction that results in exponential amplification of a diagnostic signal. In this report we showed that the distinctive pathway of the DCR amplification is not limited to one analyte and can be extended to other reactivities. The modular design of DCR probes allows us to introduce detection capabilities for other compounds once the analyte of interest that has cleavage reactivity toward a specific trigger would be incorporated collectively with the specific triggering group. In the sulfhydryl-based DCR system, the dendritic probe directly releases the analyte of interest, a thiol, which can then initiate additional diagnostic cycles. This is the first example of a DCR amplification system that does not require any additional reagents or enzymes in order to produce the chain reaction.

The addition reaction of the thiol-analyte to the benzoquinone moiety of probe **2** or dendron **3** occurs very fast (time-scale of seconds). The rate-determining step of the DCR sequence is the release of the thiol units from dendron **3**. Thus, almost any thiol compound is expected to present similar kinetic behavior upon reaction with the 2CDCR probe system.

It should be noted that although there are more sensitive probes for the detection of thiols, this study represents a general route for achieving exponential signal amplification, which is based merely on reactions of small molecules. The DCR technique is particularly useful in circumstances when a reporter molecule with relatively weak spectroscopic signal is used. In such example the exponential amplification leads to strong observable diagnostic signal despite a low extinction coefficient (in the case of UV-Vis reporter) or a low quantum yield (in the case of a fluorescence reporter).

In summary, a new two-component DCR probe system for the detection of sulfhydryl compounds was developed. The probe is activated by a thiol analyte through a stoichiometric reaction to generate a chain reaction that exponentially amplifies a diagnostic signal. Importantly, this demonstrates the advantage of our modular design of the two-component DCR system and shows that versatile reactivities can be incorporated in order to achieve exponential signal amplification for the detection of various analytes. Additional DCR probes for the detection of other analytes based on different chemistries are currently being developed in our lab.

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