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Activity-based fluorescent probes for monitoring sulfatase activity

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ABSTRACT

A small-molecule probe for sulfatase is developed that shows a significant change in fluorescence upon reaction with sulfatase in an activity-based manner. As this probe is free from interference from background fluorescence caused by an unreacted probe, it could be a simple and efficient tool for the study of sulfatase activity.

Sulfation, targeted to various biomolecules, plays an important role in regulating a diverse range of fundamental cellular functions, including cellular degradation and developmental cell signaling.[1] The level of modification is precisely regulated by sulfotransferases and sulfatases which install and hydrolyze a sulfate ester group, respectively. More particularly, sulfatases are associated with the commencement of many pathophysiological situations, such as developmental disorders, bacterial pathogenesis,[2,3] and hormone-dependent cancers.[4] Therefore, sulfatases have been targets of intensive research for several years.[2]

Sulfatases catalyze the hydrolysis of a sulfate ester group in physiological molecules such as small steroid molecules and complex glycans.[1] For example, aryl sulfatase C (ARSC), also known as steroid sulfatase (STS; EC 3.1.6.2), produces active steroid hormones from inactive sulfate conjugates. STS has been extensively studied due to its relevance to estrogen-dependent cancers.[5] Since most estrogens in breast and endometrial tumors are provided by a steroid sulfatase pathway,[6] steroid sulfatase inhibitors are considered to have potential as therapeutic agents for estrogen-dependent tumors.[7]

To explore sulfatase function, the use of a chemical probe that can report on its enzymatic activity should become a popular and versatile strategy.[8] Activity-based probes (ABPs)[9,10] are chemical probes designed to selectively react with target enzymes and which are covalently labeled to the active site of the corresponding target enzyme. To date, a couple of ABPs for sulfatases have been reported.[11,12] However, these probes require further modification to acquire detectable signals, for example, through reaction between an external enzyme and their reporter group or by use of an additional western blotting procedure. These additional steps for analysis require extra time, which is a concern for assaying enzyme activity, making those probes less ideal for high-throughput screening of potent inhibitors. Herein, we report on a simple ABP that undergoes covalent labeling of the enzyme and which leads to selective fluorescence turn-on by sulfatase.

We designed a probe (**1**) that is comprised of a fluorescent dansyl group and a carbamate-linked nitrophenyl group as a reporter, and a phenyl sulfate as a binding unit. **1** exhibits little fluorescence due to fluorescence quenching of the dansyl group by the nitrophenyl group. Recently, a carboxylesterase targeted activity probe which utilizes a carbamate-linked quenching group coupled with a pro-quinone methide reactive core was developed by Steel and coworkers.[13] **1** can be selectively activated when sulfatase hydrolyzes the reactive sulfate moiety. The following fragmentation of the carbamate bond releases 4-nitrobenzylamine and carbon dioxide, thereby producing an unstable quinone methide intermediate. This intermediate should be attacked by nucleophiles that are nearby the active site of sulfatase and covalently labeled to the enzyme (Scheme 1).

The synthesis of **1** and control probe **2**, was carried out using a neopentyl group to protect the sulfate group, which can be removed by a simple and mild nucleophilic displacement (see Supplementary data). [14] **1** possesses a dansyl moiety as a fluorescent reporter and a nitrophenyl moiety as an effective fluorescent quencher which can be easily removed from the enzymatic reaction.

To explore the utility of **1** as a probe for sulfatase activity, its fluorescent response was measured. We incubated **1** (10 μM) in Tris buffer (50 mM, pH 7.5) with commercially available arylsulfatase (1 mg/mL) at 37 °C for 1.5 h.

As shown in Figure 1(a), before the addition of sulfatase, the fluorescence of **1** was quenched by the nitrophenyl moiety compared to that of **2** which has no quencher on it. Upon incubation

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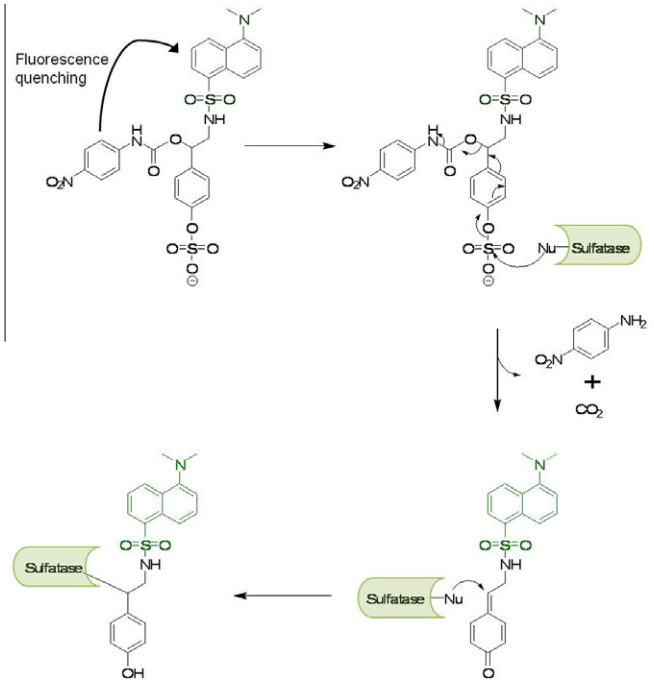

Scheme 1. Selective activation of a fluorescence quenched probe.

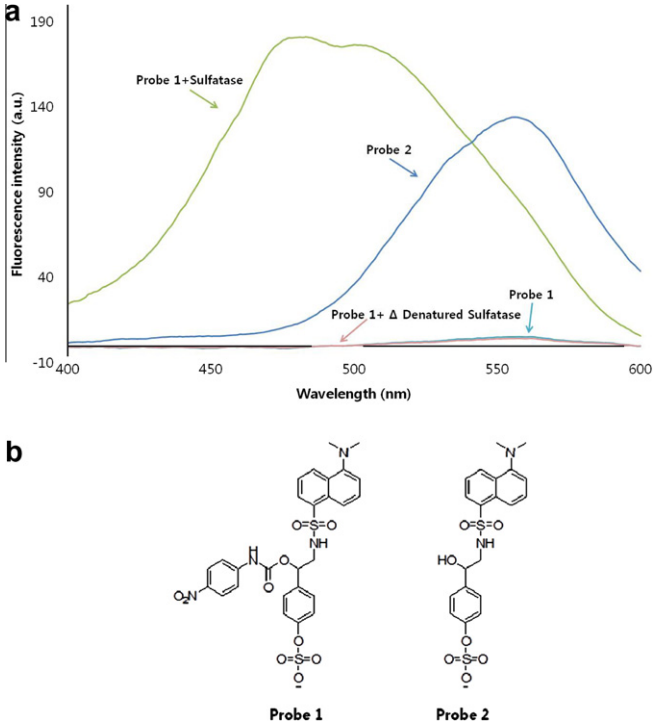

Figure 1. (a) Fluorescence spectra (ex. 328 nm) of probes **1** and **2** (10 μM each) and after incubation of **1** with sulfatase at 37 °C; (b) chemical structures of probe **1** and control probe **2**.

with sulfatase, the fluorescence emission intensity of **1** at 480 nm significantly increased. This indicates that sulfatase removed the sulfate residue from profluorescent probe **1**, causing fragmentation of the carbamate bond and the release of 4-nitrophenylamine and carbon dioxide, and production of a quencher-free probe. Moreover, there was no fluorescence change when **1** was incubated with

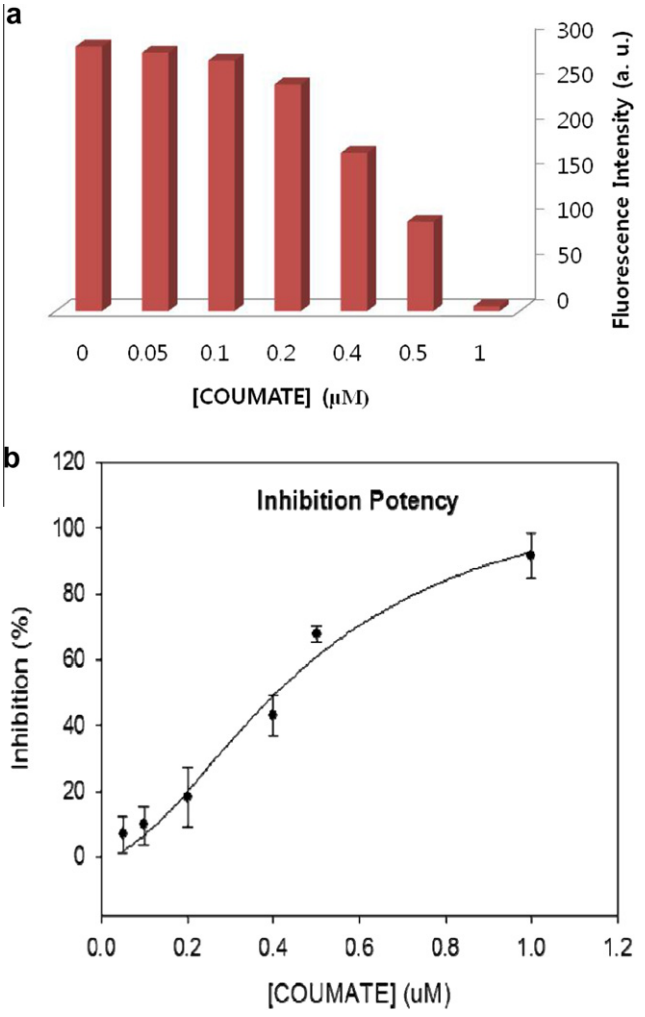

Figure 2. (a) Fluorescence change of probe **1** with various concentrations of 667 COUMATE; (b) inhibition potency.

heat-denatured sulfatase (90 °C, 10 min). **1** is therefore an efficient probe that is capable of measuring sulfatase activity by fluorescent changes (see Fig. 2).

It is interesting that the fluorescence emission (λ_{max} = 480 nm) of **1** is blue-shifted and the intensity is increased compared to that of free **2** (λ_{max} = 531 nm) in solution. This phenomenon can be explained by the microenvironmentally sensitive fluorescence property of the dansyl fluorophore (see Supplementary data). The change in fluorescence emission suggests that the microenvironmental polarity of the fluorophore is decreased by hydrophobic residues in the enzyme active site.[15,16]

To evaluate whether **1** can detect loss of enzyme activity, we performed competitive labeling experiments with 4-methylcoumarin-7-*O*-sulfamate (667COUMATE), which is known to be a potent inhibitor of STS.[17] 667COUMATE inhibits sulfatase by involving the conserved formylglycine (FGly) residue in the enzyme active site. A constant amount of sulfatase (0.5 mg/mL) was pre-incubated with various concentrations of 667COUMATE for 30 min at 37 °C, followed by addition of **1** (5 μM) and incubation for an additional 2 h at 37 °C. The maximum fluorescence intensity at 480 nm was lower than for that described.

The IC_{50} obtained in this experiment is 0.22 ± 0.02 μM, which is comparatively larger than data reported previously (8 nm).[17] However, considering the concentration of enzyme (0.5 mg/mL) used in this experiment, this result can be considered acceptable.

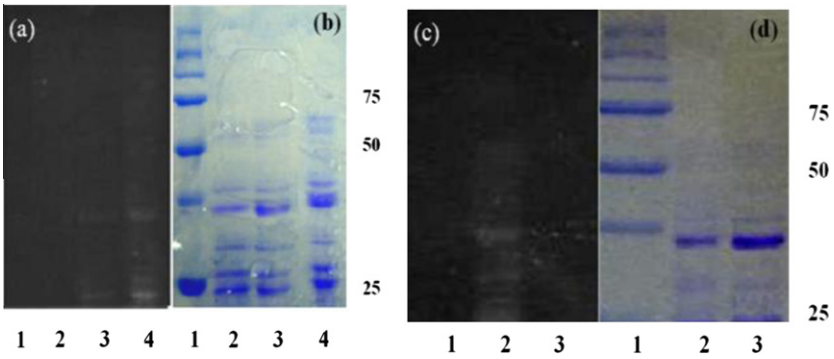

Figure 3. In-gel fluorescence (10% SDS–PAGE) detection of sulfatase activity. (a) and (c) Fluorescence; (b) and (d) Coomassie staining. Sizes in kDa. (a) and (b): 1. Size marker; 2. sulfatase only; 3. sulfatase + 0.5 mM probe **1**; 4. sulfatase + 1 mM probe **1**; and (c) and (d): 1. size marker; 2. sulfatase + 1 mM probe **1**; 3. inhibited sulfatase + 1 mM probe **1**.

Our results reveal that **1** can detect and screen compounds with high inhibition potency for sulfatase.

Having confirmed that **1** can be activated by sulfatase, we sought to establish whether it was labeling the enzyme as expected. Consequently, **1** was incubated with steroid sulfatase at room temperature for 3 h and analyzed by 10% SDS–PAGE gel, with visualization using both UV light and Coomassie staining.

Although SDS–PAGE analysis did not show highly efficient probe labeling to the enzyme,[18,19] observation of a fluorescently labeled polypeptide demonstrated not only that sulfatase turns on the fluorescence of **1**, but also that this is accompanied by covalent labeling of the enzyme. Further, as shown by Figure. 3(c) and (d), when sulfatase was inhibited by estrone-3-*O*-sulfamate (EMATE; a potent inhibitor of steroid sulfatase) no fluorescent bands were observed. This clearly demonstrates that the labeling is a result of sulfatase activity.

In conclusion, we have successfully developed a profluorescent probe, **1**, that is capable of monitoring sulfatase activity. **1** showed a significant fluorescent change upon reaction with sulfatase in solution, without a background fluorescent signal from any unreacted probe. **1** can also label sulfatase in an activity-based manner. In addition, it was illustrated that use of **1** is an efficient means of measuring the inhibitory potency of sulfatase inhibitors. This could serve as a simple and efficient platform for the screening of inhibitors for sulfatase.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.045.

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