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A *N*-(2-Aminophenyl)-5-(dimethylamino)-1-naphthalenesulfonic Amide (Ds-DAB) Based Fluorescent Chemosensor for Peroxynitrite

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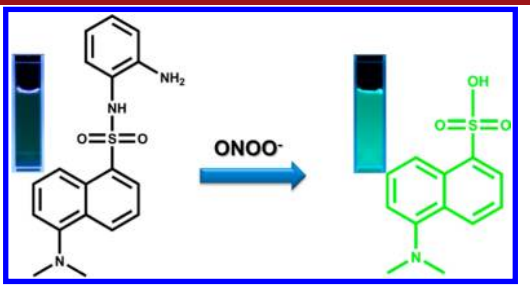
ABSTRACT



A dansyl derivative (Ds-DAB) was prepared and used as a fluorescent probe for peroxynitrite ($ONOO^-$) detection. The results showed that the addition of peroxynitrite to the aqueous solution of Ds-DAB would result in obvious fluorescence enhancement. This probe is highly specific for peroxynitrite in aqueous solution, avoiding interference from other reactive oxygen species (ROS) and nitrogen species (RNS). The advantages of high selectivity, fast reaction rate, and peroxynitrite bioimaging render Ds-DAB suitable for peroxynitrite detection.

Peroxynitrite ($ONOO^-$) is formed in biological systems by direct and rapid combination of nitric oxide (NO) and superoxide (O_2^-), which requires no enzymatic catalysis.[1] It is a potent cytotoxic agent which has attracted much attention over the past decades. Peroxynitrite is relatively stable in comparison to peroxynitrous acid with a half life of ~1 s at pH 7.40,[2] which contributes to signal transduction, homeostasis regulation, and oxidative damage concerning human health and disease.[3]

In order to comprehend the factual mechanisms by which peroxynitrite performs its diverse biological roles, development of sensitive and selective techniques for detecting biological peroxynitrite is important. Because of its high sensitivity, high spatiotemporal resolution, and experimental feasibility, fluorimetry is the most widely used imaging technique.[4]

There have been a few fluorescent peroxynitrite probes, with a different detection mechanism, reported to date. All of them are organic-based sensing modes, such as the application of a phenylselenyl derivative,[5a] a phenyltellanyl derivative,[5b] and phenol-, anisole-, and diarylamine-derived

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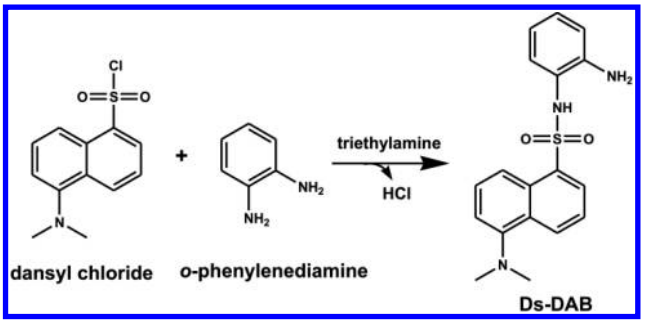
activated ketones;[6] O-dealkylation of aryloxyphenols;[7] utilization of two linked cyanine dyes;[8] and aromatic nitration.[9] However, there are some disadvantages, such as complicated and low-yield synthetic procedures, longer reaction time,[10] short emission wavelengths (< 500 nm), and cross-interference from other ROS species (especially •OH)[11,7a] existing and needing to be promoted.

In this study, we report that Ds-DAB (*N*-(2-aminophenyl)-5-(dimethylamino)-1-naphthalenesulfonic amide) can be used as a peroxynitrite turn-on fluorescent probe. This probe has been prepared by reacting *o*-phenylenediamine with dansyl chloride,[12] as shown in Scheme 1 [for details of the synthesis, see the Supporting Information (SI)].

Scheme 1. Synthetic Route to Ds-DAB



To understand the performance of Ds-DAB for peroxynitrite detection in water media, time- and concentration-dependent fluorescence studies were investigated. As shown in Figure 1a, when 10 μM Ds-DAB (in 10 mM HEPES buffer with 1.0% DMSO at pH 7.4) are reacted with different amounts of $ONOO^-$ at 25.0 ± 0.1 °C under aerobic conditions, the fluorescence intensities at 505 nm increased with time and reached a plateau within about 20 s. Figure 1b shows that the fluorescence enhancement at 505 nm rises with the increasing equivalent of $ONOO^-$. As shown in Figure 1c, there was an excellent linear correlation (R^2 = 0.994) between the fluorescence intensity and the $ONOO^-$ concentration. The detection limit of Ds-DAB for $ONOO^-$ was calculated to be 52.4 nM. These results indicate that Ds-DAB displays the potential for quantitative peroxynitrite measurement.

To estimate the reaction specificity of Ds-DAB for peroxynitrite, the fluorescence intensities of Ds-DAB reacted with a series of possible competitive anions and radicals up to

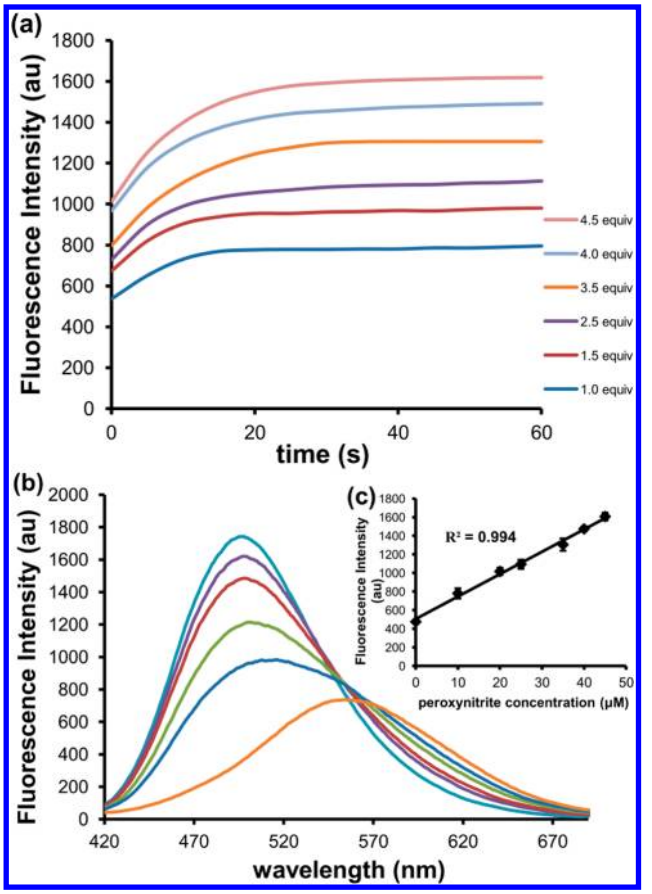


Figure 1. (a) Time-dependent fluorescence spectra obtained when various equivalents of peroxynitrite were added to a 10 μM Ds-DAB (in 10 mM HEPES buffer with 1.0% DMSO at pH 7.4) at 25.0 ± 0.1 °C (λ_{ex} = 350 nm; λ_{em} = 505 nm); (b) concentration-dependent fluorescence spectra with 1.0 to 5.0 equiv of peroxynitrite based on the intensities at 5 min; (c) fluorescence intensities upon various equivalents of $ONOO^-$ based on (b).

a 20-fold excess for HNO, NO, O_2^-, H_2O_2, OCl^-, •OH, NO_2^-, and NO_3^- were measured. As shown in Figure 2, these competitive species did not give any observable enhancement, whereas the fluorescence intensity was significantly enhanced to 6.1-fold after the reaction of Ds-DAB with 5.0 equiv of peroxynitrite. These results demonstrate that the fluorescence response of Ds-DAB is highly specific for peroxynitrite.

Ds-DAB (Φ = 0.004) reacted with $ONOO^-$ yielded dansyl acid (5-(dimethylamino)-1-naphthalenesulfonic acid, Φ = 0.148) and benzotriazol as the major products characterized by ESI-HRMS and ^{1}H NMR. LC-MS analyses also establish that the reaction of Ds-DAB by peroxynitrite indeed generates dansyl acid and benzotriazol (for details of the characterization, see the SI).

It it has been known that the peroxynitrite anion might react with a secondary amine to form nitrosamine by direct

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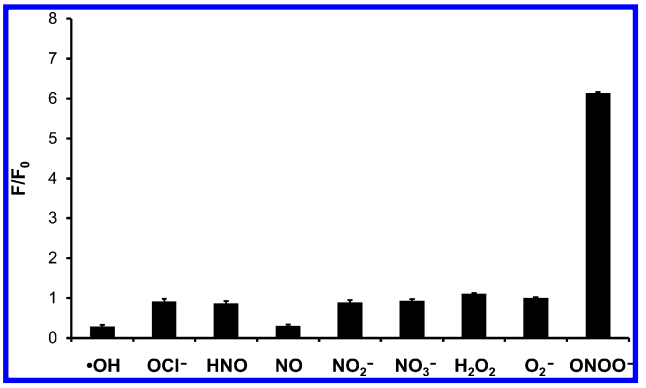


Figure 2. Specificity of 10 μM Ds-DAB for $ONOO^-$ over other reactive nitrogen and oxygen species: fluorescence response of Ds-DAB was determined after addition of 20 equiv of HNO (Angeli's salt $Na_2N_2O_3$), NO, O_2^-, H_2O_2, OCl^-, •OH, NO_2^-, NO_3^-, and 5.0 equiv of $ONOO^-$ for 2.0 h in HEPES/DMSO (10 mM, 99:1, v/v, pH = 7.4); normalized fluorescence response after 2.0 h relative to the emission of the probe (λ_{ex} = 350 nm, λ_{em} = 505 nm).

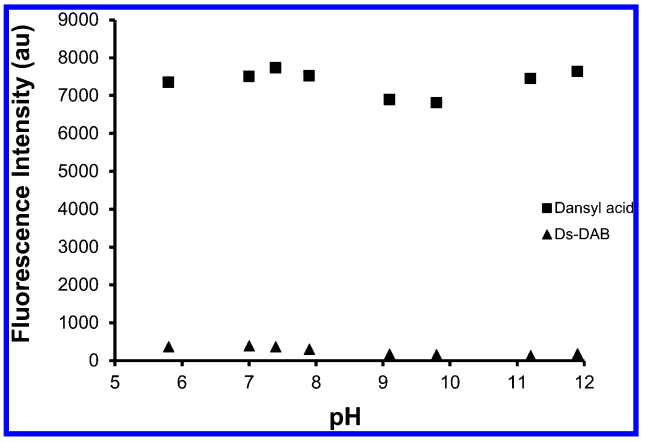


Figure 3. pH dependence of the fluorescence intensities of 10 μM of Ds-DAB (▲) and dansyl acid (■) (in 10 mM HEPES buffer with 1% DMSO) at 25.0 ± 0.1 °C. The fluorescence intensities were detected at 505 nm with excitation at 350 nm.

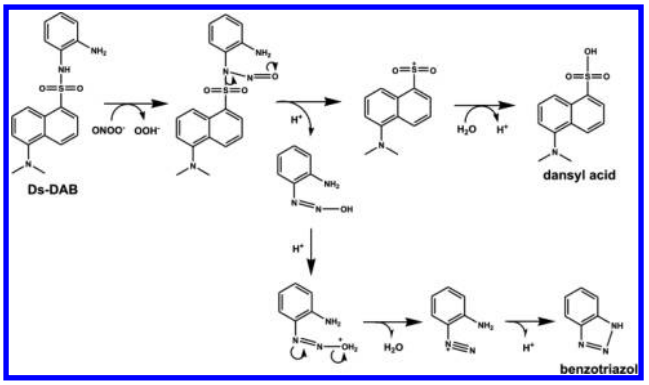
nucleophilic nitrosation (eq 1).[13] Protonation followed by hydrolysis, presumably, yielded dansyl acid along with the formation of benzotriazol. The reaction mechanism for sensing peroxynitrite is proposed as shown in Scheme 2.

$$R_2NH + ONOO^- \rightarrow R_2NNO + HOO^- \qquad (1)$$

To demonstrate the effect of pH, the fluorescence intensity of Ds-DAB and dansyl acid were measured over the pH range from 5.8 to 11.9. However, as shown in Figure 3, there was no significant effect on the fluorescence intensity. Therefore, Ds-DAB and dansyl acid are stable with this pH range.

To evaluate the utility of Ds-DAB for endogenous peroxynitrite detection, confocal fluorescence imaging of

Scheme 2. Proposed Mechanism of Ds-DAB with $ONOO^-$ To Form Dansyl Acid



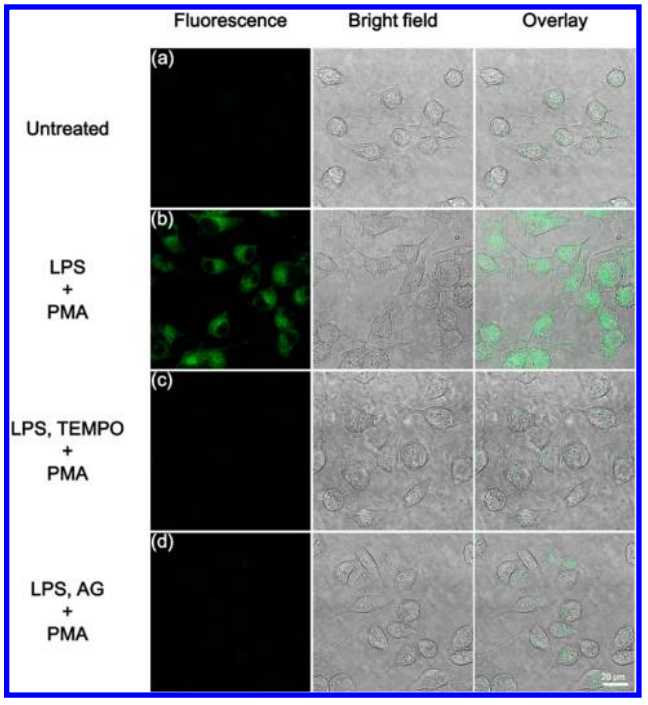


Figure 4. Endogenous peroxynitrite detection in Raw 264.7 murine macrophages by Ds-DAB (10 μM): fluorescence (left); bright field (middle); overlay (right). Ds-DAB incubation with macrophages for 0.5 h (a) without pretreatment of LPS and PMA (control), (b) with pretreatment of LPS (1 μg/mL) for 4.0 h and then PMA (10 nM) for 0.5 h, (c) with pretreatment of TEMPO (100 μM) and LPS (1 μg/mL) for 4.0 h and then PMA (10 nM) for 0.5 h, and (d) with pretreatment of AG (1 mM) and LPS (1 μg/mL) for 4.0 h and then PMA (10 nM) for 0.5 h. The scale bar represents 20 μm.

biologically produced peroxynitrite in Raw 264.7 murine macrophages was performed. In a controlled experiment, the macrophages were incubated for 0.5 h with Ds-DAB (10 μM) in the absence of lipopolysaccharide (LPS, 1 μg/mL), a stimulator of inducible nitric oxide synthase (iNOS), and phorbol 12-myristate 13-acetate (PMA, 10 nM), a stimulant

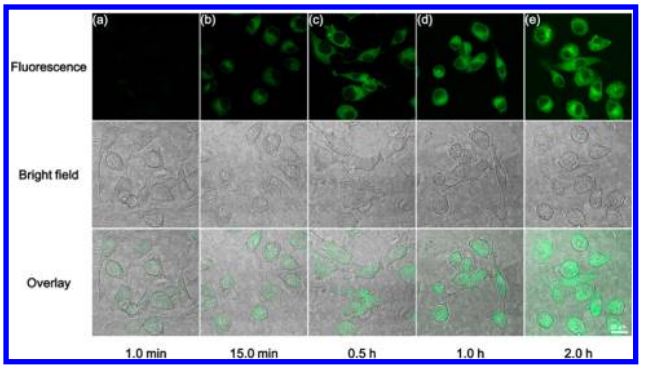


Figure 5. Cells treated with LPS (1 μg/mL) for 4.0 h and then PMA (10 nM) for 0.5 h, and incubated Ds-DAB with different times. The scale bar represents 20 μm.

for superoxide generation. As shown in Figure 4a, no obvious fluorescence in the cytoplasm was observed in the control. By contrast, Figure 4b shows that a visible increase in fluorescence was observed in the macrophages with prestimulation of LPS and PMA.[14] Furthermore, pretreatment of either a superoxide scavenger, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 100 μM), or an NO synthase inhibitor, aminoguanidine (AG, 1 mM), can suppress fluorescence formation in the macrophages,[15] as shown in Figure 4c and 4d, respectively. Therefore, peroxynitrite caused by the combination of endogenous NO and O_2^- can induce the strong fluorescence in the macrophages incubated with Ds-DAB. To test the time of Ds-DAB detection of peroxynitrite in the cell, Raw 264.7 murine macrophages were pretreated with 1 μg/mL LPS for 4.0 h and 10 nM PMA for 0.5 h, incubated with 10 μM Ds-DAB from 1 min to 2.0 h, and then washed and imaged by the fluorescence microscope. As shown in Figure 5b, the fluorescence of the cell image was observed after 15 min. Therefore, Ds-DAB has cell-penetrable and rapid detection characteristics.

Besides, MTT assays revealed that Ds-DAB displayed low cytotoxicity to the macrophages (Figure S1).[16] These results indicate that Ds-DAB can be used as a fluorescent probe for the detection of peroxynitrite formed in living cells.

In conclusion, we have demonstrated a peroxynitrite turn-on fluorescent probe via the dansyl derivative sensing mode. Obvious fluorescence enhancement can be observed within 20 s after addition of peroxynitrite to the aqueous solution of Ds-DAB. Additionally, excellent linearity between the fluorescence intensity and peroxynitrite concentration could provide quantitative measurement through a fluorometric method. Also, Ds-DAB has high specificity for peroxynitrite over other reactive nitrogen species and oxygen species. Moreover, the results of endogenous peroxynitrite bioimaging imply that Ds-DAB could be widely useful for the detection of peroxynitrite in biological systems.

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Supporting Information Available. The details of the experimental details, synthetic procedures, characterization of Ds-DAB and dansyl acid, and cell viability studies. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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