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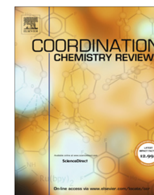

Review

Design and applications of fluorescent detectors for peroxynitrite

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ABSTRACT

Peroxynitrite ($ONOO^-$) is one of the endogenous reactive oxygen species (ROS), which causes damage to a wide array of molecular components in the cells, including DNA and proteins, owing to its high oxidizing as well as nitrating properties. However, the precise pathogenic roles played by this substance in biological systems have not yet been elucidated completely owing to its short lifetime, high reactivity, low concentration and elusive nature in the *in vivo* applications. Thus, the development of more sensitive and selective techniques for detecting $ONOO^-$, with high biocompatibilities, sensitivities, and site-specificities, is a significant goal. This review summarizes the recent advances that have been made in developing fluorescent sensors for $ONOO^-$ and their biological applications in diverse living systems.

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1. Introduction

Intracellular reactive oxygen species (ROS), also referred to as oxygen-containing substances, is a family of molecules such as $^{\cdot}OH$ (hydroxyl radical), $O_2^{\cdot-}$ (Superoxide anion), H_2O_2 (hydrogen peroxide), HClO (hypochlorous acid), ROOH (lipid peroxides), O_3 (ozone), $^1O_2$ (singlet oxygen), and $ONOO^-$ (peroxynitrite) with strong oxidizing character, and are constantly generated, transformed and consumed in living organism. As an important substance, ROS maintains the intracellular oxidation-reduction (redox) balance in both temporal and sequence-specific manner to regulate the structure and functions of biomolecules [1]. These are formed by electron transfer reactions at the oxygen as a major redox equivalents at the cellular and organism level and

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are prevalent in several diseases like cancers, diabetes, and neurodegenerative diseases. $ONOO^-$ was not considered to be a ROS earlier, until a study conducted by Beckman in 1990 [2]. Till now, it is well-recognized as a highly reactive species, which is endogenously generated by diffusion-controlled ($k = 0.4–1.9 \times 10^{10}\ M^{-1}\ s^{-1}$) reaction of nitric oxide (NO) with $O_2^{\cdot-}$ ion [3]. Although $ONOO^-$, generated under basic conditions (pH = 7.4), is more stable than its acidic form ONOOH ($pK_a = 6.8$), it has an extremely short biological half-life of about <10 ms [4]. Thus, the intracellular steady-state concentrations of this ROS species are in the nanomolar range.

In living cells, $ONOO^-$ plays vital roles in signal transduction and it exhibits antimicrobial and antibacterial activities as well. However, owing to its high oxidizing and nitrating capacities, excess $ONOO^-$ can damage a wide variety of biomacromolecules, including DNA and proteins [5–7]. Furthermore, abnormally high levels of $ONOO^-$ can result in several pathogenic effects, such as neurodegenerative, cardiovascular and inflammatory diseases and cancers [8–11]. In 2014, $ONOO^-$ was reported to be generated during drug-induced hepatotoxicity and can be used as a biomarker for the development of new therapeutics and drug-safety evaluations [12].

Comprehending the roles played by $ONOO^-$ in biological systems requires the availability of reliable and effective techniques for its selective detection over other ROS species. Until now, several approaches have been developed for sensing $ONOO^-$, which include those based on electron spin resonance, UV/Vis spectroscopy, immunohistochemistry and electrochemical analysis [13–16]. In 2017, Pu et al. developed the first activatable photoacoustic nanoprobe for *in vivo* imaging of $ONOO^-$ levels [17]. Nevertheless, these methods have significant limitations in bio-imaging applications owing to their low sensitivities, insufficient spatial and temporal resolutions, and invasive nature. In contrast, development of new fluorescent methods for $ONOO^-$ detection have received increasing attention due to their high sensitivities, non-invasive nature and excellent spatial and temporal resolution [18–20].

Since the first $ONOO^-$-specific fluorescent probe was described by Yang group in 2006 [21], the development of fluorometric methods for sensing $ONOO^-$ progressed only slowly until 2015 (Fig. 1). However, the number of published reports were dramatically increased thereafter (past 2015), an indicator of increased research activity in this particular area. Nonetheless, no review article has been published that specifically describes the investigations aimed at the development of $ONOO^-$-targeted fluorescent probes. For instance, even though Chen and Yoon summarized the progress of studies focusing on the development of optical probes for detection of reactive oxygen and nitrogen species in 2016 and 2011 respectively, coverage of $ONOO^-$-specific probes was limited [22,23]. Recently, an article by Vasilescu summarizing the detection and imaging of $ONOO^-$, discussed about the nanomaterial-based electrochemical and optical probes [24]. But most of the small organic molecules based optical probes were not covered in this review article. In light of this deficiency, a comprehensive review describing recent update and progress made in this field is highly desired.

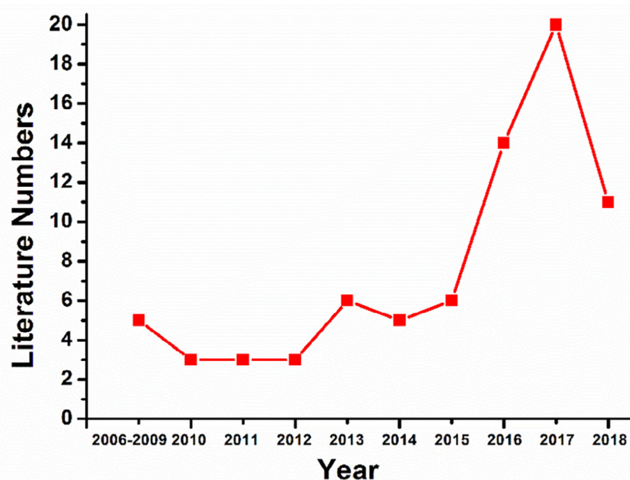

Fig. 1. Statistics for the development of $ONOO^-$-specific fluorescent probes since 2006. (Data collected up to May 4th, 2018).

In the current review, our discussion is primarily focused on $ONOO^-$-specific fluorescent probes reported since 2013 and includes but is not limited to small molecules, proteins, and nanomaterials. Coverage of the probes is organized according to the type of reaction processes operating, which includes oxidation of chalcogenides, oxidation of boronic acids or boronates, oxidation of hydrazides, cleavage of the C=C double bonds, oxidative N-dearylation, and other reported processes till date.

2. Fluorescent detectors for peroxynitrite with various reaction patterns

All of reported $ONOO^-$-specific fluorescent probes are reaction-based, utilizing the oxidizability and nucleophilicity of $ONOO^-$. Considering the similar chemical properties of $ONOO^-$ and other ROS, the reaction type chosen for reporting purpose should avoid the interference from other known ROS, which demands it to be highly specific towards $ONOO^-$. The probes based on reaction types are further subdivided into following categories discussed below.

2.1. Oxidation of chalcogenides

Chalcogenide (S, Se, Te)-containing species are commonly found in biological systems. For example, selenium (Se) is present in a key active site center in the antioxidant enzyme, glutathione peroxidase (GPx) [25]. The electron-rich nature of chalcogenides enables them to react rapidly with reactive oxidizing agents such as $ONOO^-$, leading to the alteration of the fluorescence response of chalcogenide-containing dyes. In the past years, several organo-chalcogenide based fluorescent probes have been developed for selective detection of $ONOO^-$.

In 2013, Han et al. developed a boron dipyrromethene (BODIPY)-based probe containing selenium as the reaction site for $ONOO^-$ (Fig. 2) [26]. Addition of *N*-(ethoxycarbonyl)-3-(4-mor pholino)sydnoneimine (SIN-1), as an $ONOO^-$ source to a phosphate saline buffer (PBS) solution of **1** (pH 7.4, 40 mM, containing 5% CH_3CN as co-solvent), resulted in the formation of the corresponding selenoxide. Spirocyclization promoted by the water molecule led to the cleavage of selenoxide to form an amino-styrene based product over a period of 5 h. This was accompanied with the corresponding color change from red to blue (naked eye) and an obvious fluorescence quenching owing to a strong intramolecular charge transfer phenomenon (ICT). The formation of product was confirmed by using ^1H NMR, mass spectrometry, and HPLC analysis. Further studies demonstrated that **1** operates well under physiological conditions with a calculated detection limit (DL) of 5 μM toward $ONOO^-$. The selectivity of probe **1** was shown to be excellent as observed by interference studies in presence of other ROS, including **.**OH, *t*-BuOOH, H_2O_2, ClO^-, cumene hydroperoxide, and BrO^-, that cause only small changes in its emission. Further, the intracellular imaging ability of **1** was also demonstrated using the mouse macrophage cell line RAW264.7. Probe **1** displayed

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