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Mini Review

Strategies in Developing Fluorescent Probes for Live Cell Imaging and Quantitation of Hydrogen Sulfide

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Abstract

The involvement of hydrogen sulfide (H2S) in a number of cellular signal transduction pathways has triggered great interest in the development of selective and sensitive detection methods for H2S. This mini-review summarizes three current strategies used in the design of hydrogen sulfide-reactive fluorescent probes, including copper complexation, redox reaction, and nucleophilic cyclization. Examples from these three categories of fluorescent probes are also compared and discussed briefly.

Hydrogen sulfide (H_2S), known as a toxic gas with unpleasant rotten egg smell, is the most recently discovered gasotransmitter, joining nitric oxide (NO) and carbon monoxide (CO) [1]. H_2S is found to participate in a number of cell signaling pathways [2]. Its concentration is closely related to cardiovascular diseases [3], Down syndrome [4] and inflammation [5,6]. It shows protective effects in multiple systems including cardiovascular (CV) [7] and central nervous systems (CNS) [8].

Although the significance of H_2S has been revealed in a variety of systems, the accurate determination of its concentration in biological samples remains unsolved. Real-time imaging of H_2S in live cell is challenging, too. Concentration of hydrogen sulfide in blood and tissues has been reported to be 50-150 μM [9,10], while some studies indicated a much lower (nanomolar) concentration [11]. This discrepancy could be attributed to differences in sampling techniques and detection methods as well as the actual analyte (free H_2S or total sulfide, including HS^- and S^{2-}). One of the difficulties in accurate detection is due to its volatility and reactive nature. Current detection methods of hydrogen sulfide include chromatographic, electrochemical and colorimetric/fluorometric methods. Chromatographic methods include gas chromatography (GC) [10] and high performance liquid chromatography (HPLC) [12]. Electrochemical methods include sulfide ion selective electrode [13] and polarographic methods [14]. Colorimetric method include the methylene blue method [15,16], which has been used as one of the standard methods for sulfide quantitation in water samples. Fluorescent probes form an attractive new method for hydrogen sulfide detection due to the convenient low-cost measurement and perfect compatibility with live cell imaging. Sulfide reactive fluorescent probes are based on metal-sulfide interaction, redox reaction and nucleophilic cyclization. Mechanisms and examples of these probes will be briefly introduced in this mini-review. For detailed mechanisms and discussion on development of H_2S reactive fluorescent probes, readers are referred to previously published reviews [17-21].

The sulfide anion shows strong intrinsic affinity for transition metals such as copper (K_{sp} (CuS) = 6×10^{-36}) and mercury (K_{sp} (HgS) = 2×10^{-53}). The formation of copper sulfide complex has been utilized in the design of fluorescent probes for H_2S. This was first published in 2009 by Chang and co-workers (Compound **1**) for the sensing of sulfide in aqueous media [22]. Later a number of other probes are published. These probes are composed of a fluorophore attached to heterocyclic ligands such as di-(2-picolyl)amine (DPA) (Compound **2**) [22,23], cyclen (Compound **3**) [24] and others [25]. Chelation of Cu^{2+} to the ligands quenches the fluorescence. When exposed to trace amount of sulfide, Cu^{2+} is extracted and the fluorescence is restored. Although these metal containing sulfide reactive probes have disadvantages such as irreversible reaction and heavy metal toxicity, they are especially useful to quantitatively determine real-time H_2S concentration due to fast response and high sensitivity (LOD< 1 μM).

The reduction of azido group by sulfide [26] was found to be a useful strategy in the development of fluorescent probes for H_2S. In 2011, the Chang [27] and Wang [28] groups reported rhodamine (SF1 and SF2) and dansyl-based (DNS-Az) fluorescent probes, respectively. Both probes are consisted of a fluorophore attached to an azido group. The fluorescence was found to be efficiently quenched. When the azido group is reduced by sulfide anion to the corresponding amino group, the fluorescence is

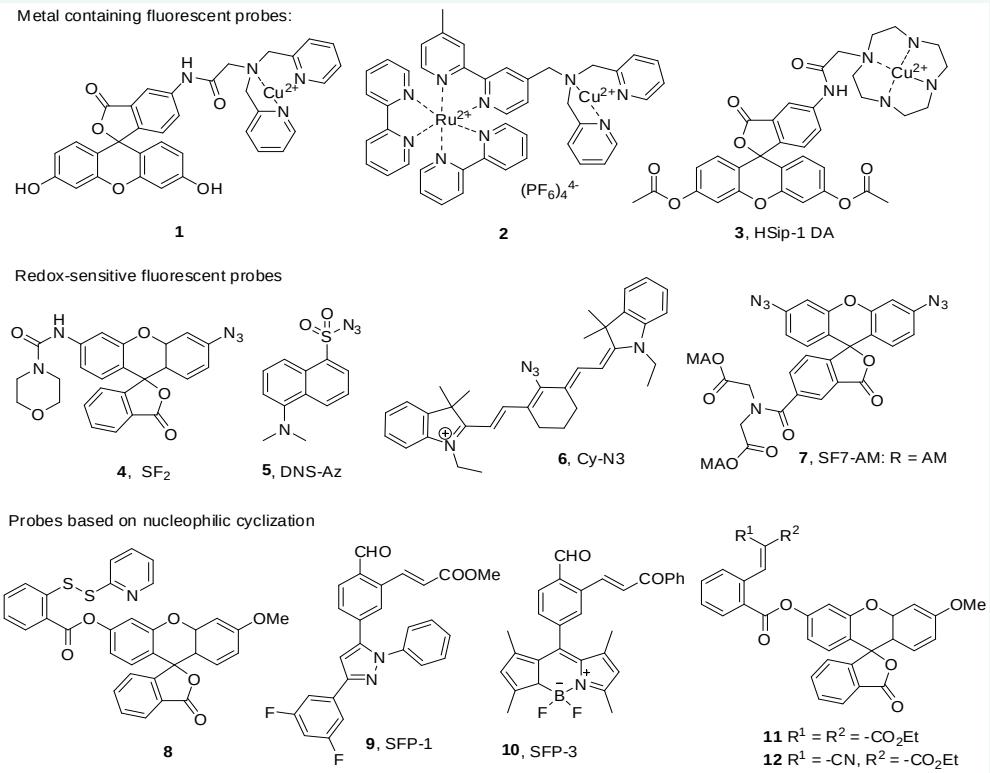

Figure 1 Sulfide reactive fluorescent probes.

recovered to generate highly fluorescent products, which can be used for quantitation of H_2S. DNS-Az was used for determination of endogenous H_2S concentration in blood. Its second-generation probe 2,6-DNS-Az shows much higher fluorescence quantum yield [29]. SF1 and SF2 were used for live cell imaging. Later the Chang group reported a series of bis-azido analogues (SF4-SF7) with improved sensitivity and cell trapping ability [30]. Among them SF7-AM was used for imaging of endogenous H_2S generation in HUVEC cells. Other azido-based H_2S fluorescent probes have also been reported showing various features such as near infrared (NIR) [31] ratiometric detection (Cy-N_3) and two-photon spectroscopic properties [32]. In addition, other redox-sensitive moieties such as nitro [33], hydroxylamine [34] and selenium [35] have also proven successful for H_2S sensing. Due to the unique redox-reactivity feature, these fluorescent probes show exclusive selectivity for H_2S over other none-reducing species. Fast reaction rates (e.g. DNS-Az) also allow accurate detection H_2S.

Sulfur as exist in thiols represents one of the strongest nucleophiles in biological molecules. Unlike other biological thiols such as cysteine and glutathione, H_2S could be deprotonated twice, thus exhibits the "dual-nucleophilicity" and can be used for nucleophilic cyclization. This feature distinguishes H_2S from thiols and has been utilized for selective H_2S sensing in a number of probes. The first example was published in 2003 by the Martinez-Manez group, taking advantage of the pyrylium cycle reaction for sensing of sulfide anion [36]. However, this probe only showed color change and limited sensitivity. In 2011, the Xian [37] and Qian [38] group reported two nucleophilic cyclization-based fluorescent probes **8** and **9** (SFP-1), respectively. They were both successfully used in live cell imaging for H_2S. SFP-1 was also used to monitor H_2S production by recombinant CBS. Xian group reported in 2012 the second generation probe **11** and **12** with improved selectivity over thiols due to the reversible Micheal addition site. A cyanine-based probe was also reported by Tang and co-workers showing NIR fluorescent property [39]. The cyclization-based probes are showing good biocompatibility and exclusive selectivity for sulfide. On the other hand, due to the two-step reaction process, their reaction rates are generally low, thus are unable to accurately quantitate H_2S. However, they are still very useful in the live cell imaging.

In summary, due to the biological significance of H_2S, a great deal of research interest has been invested in developing sensitive and accurate detection methods for this small molecule. A number of fluorescent probes have been reported for live cell imaging and blood serum quantitation of H_2S. These probes are designed based on different strategies including CuS formation, redox reactions, and nucleophilic cyclization. The molecular mechanisms involving cellular functions of H_2S remains to be revealed. It is believed that these probes will serve as useful tools in H_2S detection in both research and clinical practice.

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