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Review

Reaction-based BODIPY probes for selective bio-imaging

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ABSTRACT

Complex intracellular environment of cells, which involves interaction of a large variety of bio-molecules, is a dynamic medium with full of important information that can be recovered as well as many unanswered questions. It is highly critical to image and track biologically relevant molecules in their native media without interfering with the regular cellular processes in order to gather as much data as possible to illuminate intricacies of the biological mechanisms. To that end, small-molecule fluorescent probes have been extensively developed during the last few decades with the help of current advances in imaging technologies. Although conventional probes utilizing non-covalent supramolecular interactions with the analyte of interest are successful, significant effort has been also put into the design of reaction-based probes (chemodosimeters). Chemodosimeters exploit selective reactions of analytes with fluorophores in attempt to improve the selectivity of the probes, address the limitations of former sensors and broaden the palette of useful probes. Various types of fluorophore scaffolds can be used in the design of chemodosimeters for visualization of different analytes. In this review, we highlight the 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) based chemodosimeters which have been used to image bio-thiols, reactive oxygen/nitrogen species, and gaseous molecules in living cells.

Contents

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1. Introduction

Cytoplasm of the cells contains myriad of ions, small molecules, and bio-molecules that are continuously interacting with each other in a dynamic environment [1]. These complex and time-dependent interactions are vital for all living organisms and they are tightly regulated by the cells. However, any mismanagement in this regard can cause critical malfunctions and generally triggers the formation of various disease states. Consequently, it is highly important to track ongoing cellular processes at molecular-level in living cells in order to understand and clarify the biological roles and significance of these intracellular players. To that end, fluorescence imaging is a promising candidate to visualize living cells in their native environment, because it offers spatial and temporal resolution, high selectivity and sensitivity as well as real-time, fast, easy, and inexpensive imaging techniques thanks to a large variety of available probe (fluorophore) scaffolds and recent developments in fluorescence and confocal microscopy instrumentation.

2. Reaction-based fluorescent probes

Fluorescent molecular probe development has evolved into an attractive field of study particularly after Tsien's pioneering study on fluorescent Ca^{2+} detection in 1980 [2], followed by a large number of reports emerging at a steady pace with worldwide participation [3–8]. The common strategy, especially in the case of earlier examples is to use reversible and non-covalent supramolecular interactions in the design of fluorescent probes [9–15]. Accordingly, most of the synthetic fluorescent probes contain a binding site and a signaling core, which are linked or integrated to each other with a rapid communication in-between. The selective interaction of a probe with a target analyte through a binding site yields measurable optical changes in the signaling core (in the form of emission intensity or emission wavelength), which can be detected with various simple spectroscopic techniques.

The major requirement for fluorescent probe design is to ensure the high selectivity and affinity toward the analyte of interest in a complex intracellular medium, where many different types of reactions are taking place. In order to improve the selectivity of molecular probes in such a dynamic environment, it is highly rational to exploit different reactivities of target analytes. To that end, "reaction-based probes", also known as "chemodosimeters" have been employed extensively in bio-imaging studies during the last decade. In a reaction-based approach, the observable signal results from an analyte-specific bio-orthogonal reaction that is mostly irreversible. A typical chemodosimeter consists of a fluorophore core as a signaling unit that is modified with a functional group, which serves as a specific reaction site for the analyte. As in the case of a conventional supramolecular approach, the fluorescence response can either be modulated by OFF-ON/ON-OFF manner, or ratiometrically [16]. In the former case, the probe is either virtually non-fluorescent unless an analyte-specific reaction takes place and reveals its fluorescence, or it is initially emissive and a reaction quenches the fluorescence. On the other hand, ratiometric design results in an emission wavelength shift following the reaction between species of interest and the probes. An efficient reaction-based probe should have: (i) a high selectivity in the presence of competing species that may have similar reactivities, (ii) a less tendency to interfere with endogenous processes taking place in the cellular environment, (iii) enough product stability to yield an optical signal change [1].

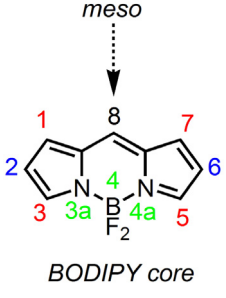

Fig. 1. Molecular structure of a BODIPY core.

3. BODIPY-based chemodosimeters

The choice of a signaling unit while designing a reaction-based probe is highly critical to harvest the best optical performance from the probe. Among potential fluorophore scaffolds BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) (Fig. 1) dye has attracted great attention as a fluorescent module and has been widely applied in bio-imaging applications because of its high absorption coefficient, high fluorescence quantum yield, relatively sharp absorption and emission spectra, photostability, easy functionalization, and neutral net charge [16,17]. Current advances in BODIPY chemistry also allow the synthesis of red-shifted BODIPYs [18]. Far-red and near-IR probes have advantages in the development of small molecule fluorescent probes for biological applications since absorption and emission in long-wavelength region generate low autofluorescence, minimal phototoxicity, and negligible background from biomolecules [18]. Furthermore, red-shifted probes can also be suitable for *in vivo* imaging, which is highly useful for practical applications due to deeper tissue penetration of the incoming and outgoing light. The major drawback of BODIPY derivatives is their high hydrophobicity leading to low water solubility. However, this problem can be simply addressed by decorating the core structure with hydrophilic moieties through well-established examples of BODIPY chemistry. There are several excellent reviews on literature regarding the chemistry and spectroscopic properties of BODIPYs as well as some others highlighting BODIPY-based fluorescent probes [16–20]. This review, however, focuses on only the reaction-based BODIPY probes, which have been used to detect biological thiols, reactive oxygen/nitrogen species, and gaseous molecules in living cells. For further reading about reaction-based probes, readers may refer to previously published reviews on the literature [1,16,21,22].

3.1. Selective detection of bio-thiols

Biological thiols, namely cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) are vital molecules for cells due to their important roles in maintaining redox homeostasis in biological systems [23,24]. These low molecular weight thiols are also known to be significant biomarkers for several acute and chronic diseases [24]. High Cys concentration, for instance, is clearly associated with myocardial and cerebral infractions, whereas Cys deficiency can induce liver damage, muscle and fat loss, skin lesions, growth problems in children, and cancer [25]. Moreover, Cys plays crucial roles in oxidation/reduction reactions of mitochondria related electron transport, as it is the main thiol source for iron-sulfur clusters. On the other hand, elevated level of Hcy has been linked to several vascular and renal disorders as well as Alzheimer's diseases [26]. Moreover, change in total plasma concentration of Hcy can be the risk factor for birth abnormalities and cognitive impairment in elder people [23]. Intracellular concentrations of Cys and Hcy are at micromolar levels; however, plasma concentration can reach up to a millimolar level (0.25–0.38 mM) [23]. GSH is the most abundant intracellular bio-thiol, which is the tri-peptide of cysteine, glycine,

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