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Fluorescein applications as fluorescent probes for the detection of analytes

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ABSTRACT

Fluorescein derivatives are important fluorescent probes which can be used for detection and optical imaging. Fluorescein derivatives are usually constructed by introducing aldehyde groups or esterified onto fluorescein xanthene ring and benzene moiety. Typically, the research direction of connecting amino groups with fluorescein monoaldehyde is in hot. Because of their high activity, these derivatives can be complexed with the analytes to produce changes of colors and the increase or decrease of fluorescence intensity. This article reviewed fluorescein probes in the past two years according to classification of different analytes including metal ions, anions, small molecules and biological macromolecules. The synthesis methods, optical properties, possible mechanisms and applications of fluorescein probes are summarized. This article provides a reference for the screening of fluorescein probes with high sensitivity and biological detection and can propel their further applications in sensing and detection of analytes.

1. Introduction

Fluorescein is widely employed as a platform for various fluorescent probes and fluorescent labels because of their high-intensity emission peaks, high molar absorption coefficients and quantum yields in aqueous media [1]. Since Bayer first synthesized fluorescein in 1871, it has received much attention and has shown great promise in a variety of applications, especially in the field of smart sensors and bioimaging [2].

Over the last decade, amazing progress has been made in the use of fluorescent probes for the development of probes due to their unique photoluminescence properties [3]. Compared with traditional techniques including titrimetry, chromatography, electrochemistry, chemiluminescence and flow injection analysis, these probes open up a new avenue for fast-responding, highly sensitive, non-sample destructing and on-site analysis of specific targets [4]. A great variety of fluorophores including organic molecules, metal nanoclusters, semiconductor quantum dots (QDS), etc. with improved properties are increasingly available. A benefit of synthetic organic molecules is the ability to employ chemical approaches to control the properties and direct the position of the fluorophore. Applying organic synthesis enables efficient tailoring of the structure to obtain fluorescent probes for specific sensitive experiments. Accordingly, the development of preparative strategy toward functional fluorescein structure is highly desired.

Fluorescein has been frequently utilized as the fluorescent core due to its characteristic spirolactam structure, which can 'close—open' with an 'turn-on' fluorescence response in specific environments or as a result of specific events, and made it an excellent dye for the design of probes. Recently, functional fluorescence probes based on the fluorescein structure have attracted growing interest [5]. Functional fluorescence probes based on the fluorescein structure are available for modification by organic synthesis at two moieties: the xanthene ring and the benzoic acid moiety (hereafter called the benzene moiety). Its unique characteristic in every moiety is worth summarizing.

Herein, the past two-years' advances of fluorescein probes about the synthesis methods, optical properties, possible mechanisms and applications of fluorescein probes are summarized [6,7]. Common fluorescein probes mechanisms include the 'off—on' structure, fluorescence resonance energy transfer (FRET), photo induced electron transfer (PET) and chelation enhanced fluorescence (CHEF) [8,9]. This article reviewed fluorescein probes according to classifications of different analytes, including metal ions,

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anions, small molecules and biological macromolecules. It could facilitate the researchers to design and develop proper fluorescent probes in future work.

2. Fluorescein probes design mechanisms

Functional fluorescence probes based on the fluorescein structure are available for modification by organic synthesis at two parts: the xanthene ring and the benzene moiety. The two moieties are orthogonal to each other. Its unique characteristic in every moiety is worth summarizing. Rational design strategies based on the five positions of the two parts have allowed us to rapidly develop a wide range of fluorescein probes (Fig. 1).

The rational design of a chromogenic/ratiometric fluorescein probe is based on the abovementioned reaction with benzene moiety. In carboxyl of benzene moiety (1 site), fluorescein probes are synthesized through fluorescein hydrazine based on Schiff base reaction connecting with aldehyde containing compounds. Fluorescein moiety is used as the signaling unit and aldehyde containing compounds are used as the binding unit which is based on non-covalent supramolecular interaction. Due to the high activity of the Schiff base, fluorescein probes can provide more binding sites, which can improve the sensitivity for detection.

In the xanthene ring (2 site), fluorescein aldehyde was obtained through a Reimer–Tiemann reaction, which can be further used to prepare probes. The amino compounds can connect with fluorescein aldehyde based on Schiff base reaction. The probes as a result of the amino group contain a lone pair of electrons which can occur PET process within the molecule induces the quenching of fluorescence. The ions with an empty orbit can hinder the PET process, which leads to fluorescence recovery.

The above two species fluorescein probes have Schiff base structure, it can be divided into two types: bonding-signaling type probes and reactive fluorescein probes. Bonding-signaling type fluorescein probes produce an increase or decrease of fluorescence intensity due to electron transfer from amino group containing compounds to the xanthene ring. The ions with an empty orbit can hinder the electron transfer process, leading to fluorescence recovery. Due to the activity of the Schiff base, reactive fluorescein probes can facilitate a ring-opening of the fluorescein spirolactam and increase the rigidity of the molecular assembly. The purpose of detection can be achieved via the inhibition of C=N isomerization and the promotion of CHEF.

In hydroxyl of xanthene ring (3 site), fluorescein probes are synthesized through two phenolic hydroxyl groups of fluorescein based on esterification connecting with small organic molecules. The initial state of the third specie fluorescein probes is colorless and non-fluorescent due to the break of the π-conjugation. When a specific ion is added via an irreversible chemical reaction, ester bond breaking and small organic molecules are removed. The π-conjugation recovering leads to notable color and fluorescence changes. The transformation of the two states can achieve the goal of detection of different analytes.

In the benzene moiety (4 site), fluorescein probes are synthesized through fluorescein connecting with organic fluorophores or gold nanoparticles (AuNPs), there is an overlap between two fluorescence emissions. Fluorescein connecting with other fluorophores can be used as ratiometric fluorescein probes to detect analytes based on FRET process between fluorescein and other fluorophores. Ratiometric fluorescein probes allow a ratio of the fluorescence intensities at two wavelengths. After the recognition, the absorption in UV–vis spectra should be different, thus resulting in the color change. Fluorescence emission spectra are turned out to be an accurate and efficient method for detection of analytes. Fluorescein connecting with AuNPs based on FRET process induces the quenching of fluorescence. The fluorescence emission intensity of fluorescein is significantly quenched due to overlapping of its emission spectra and the absorption spectra of AuNPs at plasmon resonance wavelength. Some analytes can hinder the FRET process due to the reporter group's stronger binding capacity for the analyte than recognition group, which lead to fluorescence recovery.

In ortho position of hydroxyl group (5 site), fluorescein probes are synthesized through halogen substituted fluorescein based on substitution reaction connecting with small organic molecules. This can increase its complexation ability and selectivity to metal cations. Most of the probes constructed in this way are bonding-signaling type probes.

3. Detection application

3.1. Fluorescein probes detection of metal ions

Probes for metal ions based on the fluorescein structure are modified by the five positions. When the probes combined with metal ions, it can lead to the changes of colors and the increase or decrease of fluorescence intensity. Based on this, the purpose of detection can be achieved [10]. In aqueous solution and living cell, the fluorescein probes can be used to detect such as copper ions, zinc ions, mercury ions, gold ions, silver ions, palladium ions, iron ions, magnesium ions, cadmium ions and lead ions [11,12]. Detection of these ions has great significance for human health and environmental protection [13].

3.1.1. Fluorescein probes for copper ions

The safe concentration of Cu^{2+} in drinking water is limited to 31.48 μM [14], excessive Cu^{2+} have biologically toxic [15]. Therefore, there is a great demand for the development of fluorescein probes that can provide the selective analysis of Cu^{2+} with high sensitivity, low detection limit and quick response.

Imidazole is a π-electron-rich heteroaromatic molecule, its derivatives show excellent coordination properties toward metal ions. Helal et al. synthesized a bonding-signaling type fluorescein probe (F2) based on Schiff base reaction with fluorescein hydrazine and N-methylimidazole in 1 site. Owing to the electron donating nature of the methyl group on the imidazole ring, the electron-donating capability of imidazole increases, which makes F2 to produce a green fluorescence emission at 525 nm and specific selection for Cu^{2+}. Cu^{2+} being a paramagnetic cationic species, with open shell d-orbitals, quenches the fluorescence of F2 upon binding, due to the ET from imidazole to the Cu^{2+} that inevitably inhibits the ET

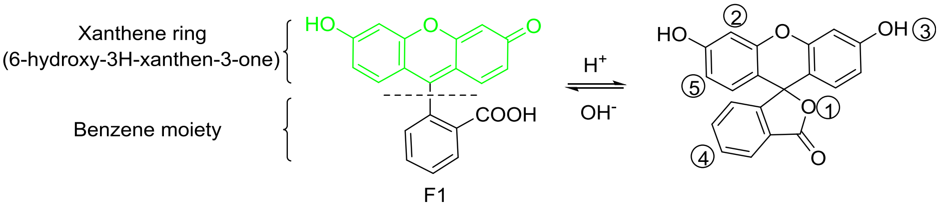

Fig. 1. Fluorescein fluorescence changes with pH and sites for modification.