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Detection of hydrogen sulphide based on a novel G-quadruplex selective fluorescent probe

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ABSTRACT

It is of great importance to detect hydrogen sulfide (H_2S) simply and sensitively for its role in various physiological processes as well as its inherent toxicity. In this work, two 3-Hydroxychromone (3HC) derivatives were prepared from 3HC-N_3 based on HS^- mediated reduction of azides, which was found to recognize G-quadruplexes (G4) with strong fluorescence emission through strong π-π stacking interactions and hydrogen bonding. Moreover, the mixture of 3HC-N_3 and G4 was used as the H_2S sensor with a limit of detection (LOD) as low as 2.64 nM in aqueous solution, which was much lower than most of current approaches. Given the fine performances and striking properties, this G-quadruplex-selective fluorescent turn-on probe would achieve a promising application of H_2S detection in low fluorescence interfering environment samples.

1. Introduction

Hydrogen sulfide (H_2S) is a highly toxic and flammable gas with rotten egg smell, which is mainly produced in sewage plants, oil and natural gas industries, and coal mines. Besides, H_2S gas is also extensively used in many chemical industries, scientific research institutions and companies of heavy water production. H_2S is also a broad-spectrum poison, meaning that it can poison several different systems in the body through inhibition of cytochrome c oxidase, formation of reactive oxygen species (ROS) and direct toxic effects on the brain [1,2]. Amongst all pollutants in the environment, H_2S is the major threat to human health even at very low concentrations [3]. Therefore, it is imperatively necessary to develop highly selective and sensitive detection methods of H_2S.

Variety of methods have been applied for H_2S detection, such as colorimetry [4], inductively coupled plasma-atomic emission [5], electrochemical analysis [6], gas chromatography [7] and optical sensors [8]. Colorimetric assays of H_2S mainly involves the reaction of sulfide with *N, N*-dimethyl-p-phenylenediamine sulfate to form methylene blue [9]. However, methylene blue only obeys Beer's Law at very low concentrations and the accuracy of colorimetric is very low [10]. Besides, inductively coupled plasma-atomic emission, electrochemical analysis and gas chromatography usually require expensive and sophisticated instruments, complicated preprocessing and post-mortem processing. Based on polyaniline nanowires-gold nanoparticles, electrochemical analysis of H_2S can obtain extremely low detection limit (0.1ppb), but the preparation of this sensor are relatively complicated [11]. Besides, optical H_2S sensors are mainly prepared using metal organic framework (MOF) fluorescent materials, fluorescent organic molecules, and fluorescent nanoclusters, which can offer high sensitivity and selectivity [12–15]. Furthermore, based on surface plasmon resonance (SPR) and lossy mode resonance (LMR) techniques, Gupta and coworkers prepared various fiber optic H_2S with high sensitivity and long-term stability, but the experimental setup of these excellent performance sensors is relatively complex [16]. Complex synthetic steps or experimental setups [16,17], lower specificity [18], high working temperature [19], and dry testing conditions [19] are hindrances that need to be overcome in the development of H_2S sensors.

One famous DNA conformation widely employed in sensing is G-quadruplexes (G4), which are non-canonical four-stranded structures formed by at least four tracts of consecutive guanines [20]. The rich diversity in structural topologies of G4 has made them attractive and versatile signal-transducing elements for the development of label-free detection of important species in biology or environment [21,22]. Therefore, G4 has already been used to develop various sensors for

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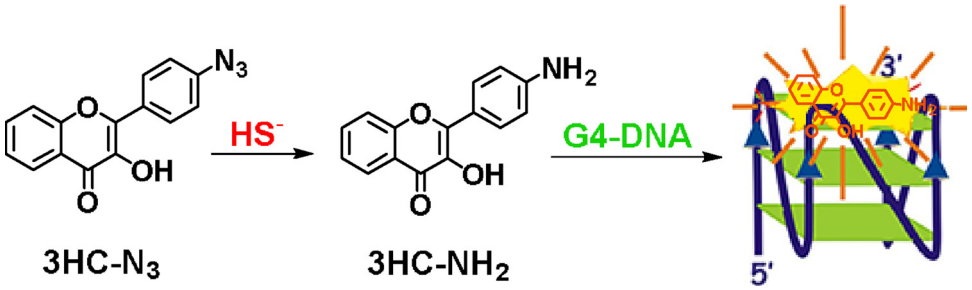

Scheme 1. Compound 3HC-N_3, 3HC-NH_2 and sensing of hydrogen sulfide based on G-quadruplex recognition.

protein [23], nucleic acids [24], metal ions [25] and small molecules [26].

3-hydroxychromone (3HC) derivatives with excellent sensitivity to the polarity of microenvironment [27], which are important excited-state intramolecular proton transfer (ESIPT) based molecules, have been used to investigate micelles [28], phosphor lipid vesicles [29], metals [30] and proteins [31]. According to our previous reports, hydrophobic microenvironment provided by low-polar solvent [32], high α-helical level proteins [32,33], and intracellular environment [33], could enhance the fluorescence intensity of ESIPT molecules. Recently, many works have also been done to use 3HC dyes in the studies of structures and interactions of nucleic acids [34]. For example, a 3HC fluorophore being conjugated to polycationic spermine was able to easily distinguish its binding to double-stranded DNA (dsDNA) from single-stranded DNA (ssDNA) by dramatic changes in the spectra [35]. Thus, application of environment-sensitive dyes undergoing ESIPT constitutes a new approach for H_2S probing.

In this work, we screened out a 3HC derivative 3HC-NH_2, which was able to recognize G4 with obvious. Then we modified the $-NH_2$ group of 3HC-NH_2 to $-N_3$ group and obtained 3HC-N_3, and further applied 3HC-N_3 to detect HS^- in the presence of G4, as the $-N_3$ group could be reduced to the $-NH_2$ group through HS^- [36]. The results displayed that the mixture of 3HC-N_3 and G4 was capable of acting as the HS^- sensor with a limit of detection (LOD) as low as 2.64 nM in aqueous solution, which was much lower than most of the previous methods. Through strong π-π stacking interactions and hydrogen bonding, G4 DNA could function as fluorescent activator for 3HC-NH_2. Therefore, the studies here would provide a new perspective to develop the sensing application of the ESIPT-based 3HC derivatives, and then be helpful for future potential biology imaging.

2. Material and methods

2.1. Materials and oligonucleotides

Unless otherwise noted, materials were purchased from commercial suppliers without further purification. All the solvents were treated according to standard methods. The oligonucleotides were purchased from Invitrogen Technology (Shanghai, China), purified over polyacrylamide gel electrophoresis (PAGE), and then dissolved in deionized water, which was finally stored at −20 °C. Concentrations of oligonucleotides were determined by ultraviolet (UV) spectrometry using extinction coefficients according to the manufacturer's instructions. All other reagents were purchased from commercial suppliers as analytical grade. Full synthesis details and characteristics of the novel compounds were shown in ESI.

2.2. General spectroscopic methods

Deionized water was used to prepare all aqueous solutions. The compounds were dissolved in dimethyl sulfoxide (DMSO) to make 10 mM stock solutions, which were diluted to the required concentrations for measurement. All spectroscopic measurements were working in 50 mM Tris-buffer at 37 °C, pH 7.4. Fluorescence spectra were obtained on a Cary Eclipse fluorescence spectrophotometer (Varian, USA) using a 1 cm quartz cuvette with a total volume of 400 μL, unless otherwise stated, 5 nm slit widths and a photomultiplier tube power of 700 V were used. The LOD was defined as 3 × standard deviation of the blank, which was calculated according to standard methods in linearity H_2S response range of 3HC-N_3/ILPR [37]. Absorption spectra were recorded on a SPECORD 210 Ultra-visible spectrophotometer (Analytic Jena AG) using 1 cm path length quartz cuvette with a total volume of 200 μL.

3. Results and discussion

3.1. Design and synthesis of 3HC-N_3 and 3HC-NH_2

ESIPT-based 3HC derivatives have excellent sensitivity to the polarity of microenvironment [27], and the hydrophobic microenvironment could enhance the fluorescence intensity of certain 3-hydroxychromone derivatives. Because of the hollow structure inside G4 [38], it might enhance the fluorescence intensity of 3HC derivatives by the hydrophobic interactions similar to bovine serum albumin. However, so far as we know, few examples of the interaction between 3HC derivatives and G4 DNA are available in the literature. In our study, we synthesized two novel 3HC derivatives, 3HC-NH_2 and 3HC-N_3, which were able to transform into each other via the diazotization reaction and reductive reaction, respectively (Scheme 1 and S1). The fluorescence experiments revealed that the two 3HC dyes had a single and weak emission band in Tris-buffer (50 mM Tris-HCl, 50 mM K^+, pH 7.4), however, when in the presence of most, but not all, G4-forming sequences, a dramatic increase can be observed in 3HC-NH_2 emission intensity, whereas the presence of control dsDNA and ssDNA showed only moderate effect on emission. As H_2S can reduce 3HC-N_3 and yield 3HC-NH_2 under mild conditions, herein, the mixture of 3HC-N_3 and G4 was used as a fluorescence sensor for H_2S.

The synthesis of 3HC-N_3 was achieved based on reported procedures for 3HC derivatives (Scheme S1) [39]. An oxidative cyclization between 2′-hydroxyacetophenone and 4-acetamidobenzaldehyde yield the flavonol unit (the intermediate ***1***), in which the amino group was protected. Afterward, 3HC-NH_2 was obtained via deprotection using HCl. Then, 3HC-N_3 was formed based on the diazotization reaction. The chemical structures of the two 3HC derivatives were fully characterized by NMR (^1H and ^{13}C) and HRMS. Besides, 3HC-NH_2 was additionally characterized by elemental analysis as well as X-ray single crystal diffraction analysis (ESI, detailed crystallographic data were summarized in Table S1).

3.2. Fluorescence properties of 3HC-NH_2 in the presence of G4 DNA

In order to demonstrate the selective fluorescent recognition of 3HC-NH_2 toward the G4-forming oligonucleotides of ILPR (sequence was shown in Table S2), having parallel and antiparallel G4 forms co-existed under physiological conditions [40], ILPR was used to evaluate the interactions through absorption, fluorescence spectroscopy and CD