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“Molecular beacon”-hosted thioflavin T: Applications for label-free fluorescent detection of iodide and logic operations

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ABSTRACT

In this work, we presented a simple, label-free and rapid-responsive fluorescence assay for iodide (I^-) detection based on “molecular beacon (MB)”-hosted thioflavin T (ThT), achieving a limit of detection as low as 158 nM. The proposed method exhibited very good selectivity to I^- ions over other anions interference due to the strong binding force between I^- ions with Hg^{2+}. Upon the addition of I^- ions, it would capture Hg^{2+} from a T–Hg^{2+}–T complex belonging to the MB-like DNA hairpin structure, which eventually quenched the initial fluorescence as output. In addition, it was successfully applied for operation of an integrated DNA logic gate system and to the determination of I^- in real samples such as human urine.

1. Introduction

Over the past few years, substantial efforts have been devoted to the recognition and sensing of anions owing to their importance in many biological, chemical, industrial, and environmental processes [1]. Among these anions, iodide has attracted much attention due to its immense biomedical significance. Iodide is known to be one of the essential micronutrients in the human body, and it plays a crucial role in normal growth, neurological development and thyroid gland function. In fact, iodide deficiency or excess can lead to relevant thyroid disease and mental defects. The World Health Organization (WHO) has recommended that daily iodine intake for adults is 150 μg per day. Excess iodine intake by humans is excreted through urine in the form of iodide since the organs cannot store iodine. Hence, measuring the urinary iodide concentration does help to diagnose transient thyroid dysfunction and iodine-induced diseases [2,3]. Until now, various analytical methods, such as ion chromatography, surface-enhanced Raman scattering, electrochemical methods, fluorometry and colorimetry, have been proposed to detect iodide in different samples [1,3–6]. However, many of these methods are expensive, time-consuming, involved in multistep sample preparation and professional instrument operation. Therefore, the development of a cheap, simple and sensitive method toward detection of iodide is urgent needed.

Molecular beacons (MBs), first reported by Tyagi and Kramer in 1996, are hairpin-shaped nucleic acid probes. In the traditional format, MBs are dually labeled with a fluorophore and a quencher at either ends in close proximity, which can reduce fluorescence emission due to fluorescence resonance energy transfer. While binding to a complementary sequence or target, the hairpin structure will be destroyed and the distance between the fluorophore and the quencher increases resulting in fluorescence enhancement [7]. The double labeling MBs have presented great activity in a wide range of applications, but they need relatively long assay time and some complicated or expensive operations. Furthermore, the quencher may not completely quench fluorescence, which gives rise to a high background signal and reduces the sensitivity of detection [8]. To address this, many new types of MB-like probes solely labeled with fluorophores at one end have been developed, in which nanomaterials are used as quenchers. However, these type of MB-like probes are totally dependent on the particular structural and photophysical features of nanomaterials [9,10]. As a result, many research efforts have been devoted to develop label-free MB-like probes to overcome these drawbacks. Recently, our group have developed label-free MB-like probes for fluorescent detection of varied target molecules such as Hg^{2+}, biothiols, H_2O_2, glucose and adenosine, etc. [11–15]. These probes have more advantages than traditional MBs, because they are label-free, low-cost and easy to synthesis. Even so, it is still desirable to develop more novel label-free MB-likes probe with appealing performances and expand their applications in diverse fields.

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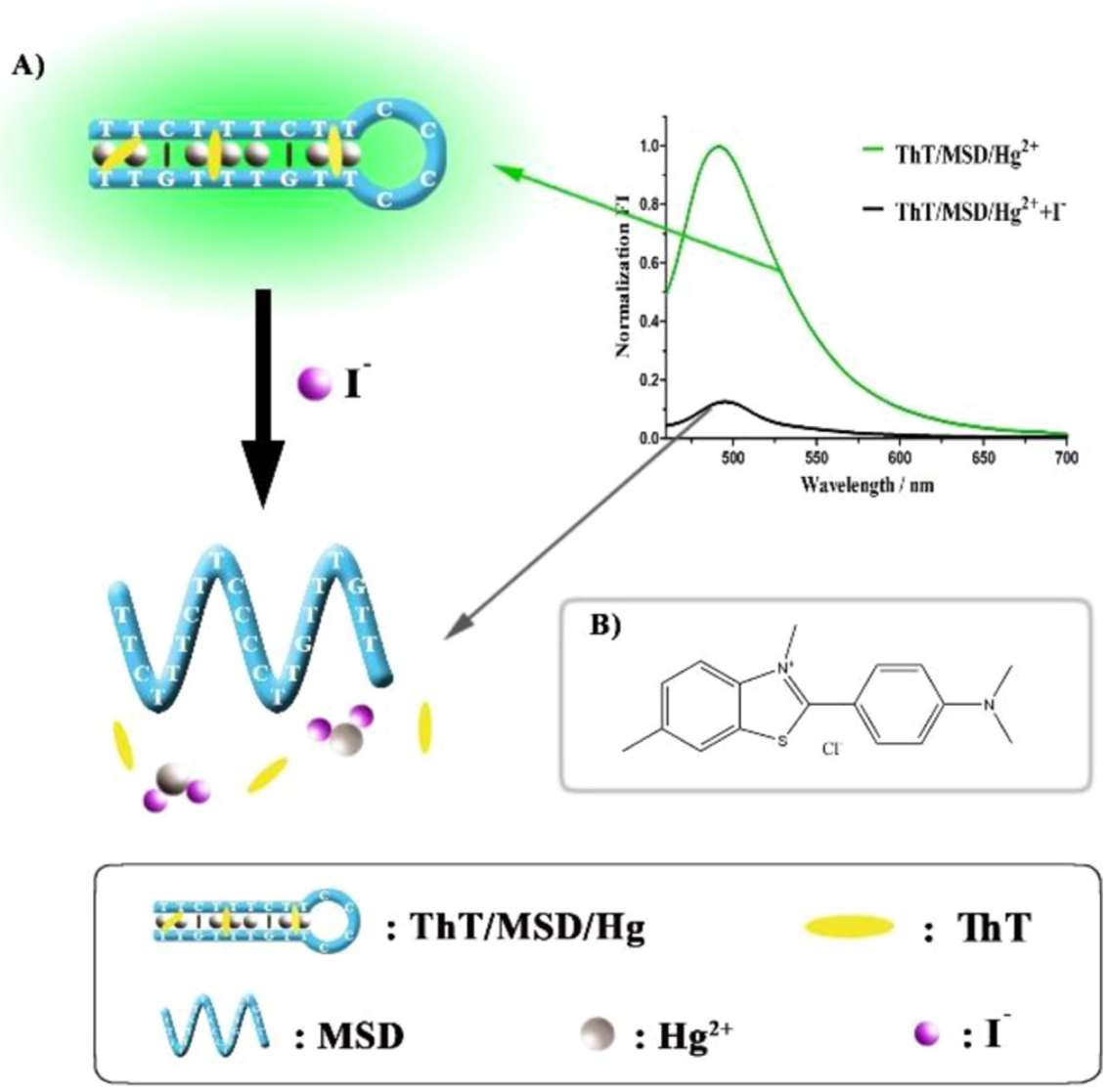

Scheme 1. (A) Schematic representation of the visual fluorescence sensing of iodide based on the "molecular beacon"-hosted thioflavin T; (B) Structure of thioflavin T.

Thioflavin T (ThT), a common commercial dye molecule, is easy to purchase, of low toxicity and cheap. ThT is composed of benzothiazole and dimethylaminobenzene rings unit linked by a C–C bond, which can be twisted around. Due to the efficient internal non-radiative twisting process, ThT has negligible fluorescence in aqueous solution. When the free rotation of the rings is inhibited, ThT shows remarkable fluorescence enhancement [16]. It has been reported that ThT can display greater fluorescence enhancement when it interacts with double-stranded DNA than with single-stranded DNA [17–19]. Enlightened by the above facts, we have developed a novel detection method for iodide utilizing ThT dyes, a label-free MB-like probe consisting of a mercury ions (Hg^{2+})-specific DNA (MSD: 5′-TTCTTTCTTCCCCTTGTTTGTT-3′) and Hg^{2+}. It has been previously demonstrated that Hg^{2+} can bond with the MSD sequence to form a hairpin structure because of the strong interaction between Hg^{2+} and thymine (T), and then ThT can be incorporated into the DNA grooves of stem region and exhibit intense emission [20]. As shown in Scheme 1, the as-prepared ThT/MSD/Hg^{2+} system exhibits green fluorescence emission. However, in the presence of iodide (I^-), Hg^{2+} has a higher affinity with I^- than thymine (T), so I^- can effectively grab Hg^{2+} from the T–Hg^{2+}–T complex through the strong interaction between Hg^{2+} and I^- [21]. Consequently, the hairpin structure will be destroyed and the MSD sequence changes into freed, causing a fluorescence decrease. In this case, a simple and facile "turn off" fluorescence assay for I^- can be constructed based on regulating the fluorescence of ThT hosted by the label-free MB-like probes, in which ThT/MSD/Hg^{2+} complexes act as a signal indicator and I^- as a target-responsive element. Biothiols, e.g. cysteine (Cys), have high affinity for Hg^{2+} through the strong thiol-Hg^{2+} [12]. In this respect, the fluorescence of ThT/MSD/Hg^{2+} system will be quenched upon addition of I^- or biothiols. To improve the detection selectivity, a common the thiol-based compound scavenger, N-ethylmaleimide (NEM) [22], was introduced to eliminate the interference of biothiols in the assays for I^- with high selectivity. With the introduction of NEM, which showed a corresponding recognized masking effect for Cys, the aforementioned ThT/MSD/Hg^{2+} system has also been constructed as an integrated DNA logic gate system by utilizing ThT/MSD as a signal transducer and Hg^{2+}, I^-, Cys, NEM as mechanical activators.