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Dual hairpin-like molecular beacon based on coralyne-adenosine interaction for sensing melamine in dairy products

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ABSTRACT

This study presents a novel dual hairpin-like molecular beacon (MB) for the selective and sensitive detection of melamine (MA) based on the conjugation of MA and thymine. In this protocol, the coordination between coralyne and adenosine (A) leaded a dual hairpin-like MB and the fluorophore–quencher pair is close proximity resulting in the fluorescence quenching. With the addition of MA, it conjugated with thymine in the loop part of dual hairpin-like MB by triple H-bonds, triggering the dissociation of the dual hairpin-like MB. The resulting spatial separation of the fluorophore from quencher induced the enhancement in fluorescence emission. Under the optimized conditions, the sensor exhibited a wide linear range of 8×10^{-9}–1.6×10^{-5} M (R^2=0.9969) towards MA, with a low detection limit of 5 nM, approximately 4000 times lower than the Drug Administration and the US Food estimated MA safety limit. The real milk samples were also investigated with a satisfying result.

1. Introduction

Since Tyagi and Kramer first described the molecular beacons (MBs) which can produce fluorescence without having to separate probe-target hybrids from excess probes in hybridization assays [1], it has received remarkable attention for wide applications in areas such as genetic screening [2–4], the monitoring of living systems [5–7], the investigation of enzymatic processes [8–10], the development of biosensors [11,12], the study of protein–DNA interactions [13,14], and the construction of biochips [15,16]. Generally, typical MBs are single-stranded oligonucleotides that contained a stem-loop structure with a fluorophore–quencher pair at 5′- and 3′-ends. The stem can self-hybridize that brings the fluorophore–quencher pair into close proximity, whereby fluorescence is quenched effectively. Hybridization to a complementary target disrupts the self-hybridization of the stem resulting spatial separation of the fluorophore from quencher and inducing the enhancement in fluorescence emission [1,11,16]. Although MB-based detection system is one of the most successful separation-free probes [17,18], typical MBs do not perform well at room temperature without significant and empirical optimization of their thermodynamics [19]. To solve these problems, T (thymine)–Hg^{2+}–T and C (cytosine)–Ag^{+}–C based MBs have been studied due to that the stable T–Hg^{2+}–T or C–Ag^{+}–C structures make the sensors possess relatively high stability, selectivity and short analysis time [20,21]. Nevertheless, as we know, either Hg^{2+} or Ag^{+} is harmful to aquatic ecosystems and human health [22–24]. Hence, it is required to explore other lowly toxic molecules for MBs.

Coralyne (5,6,7,8,13,13a-hexadehydro-8-methyl-2,3,-10,11-tetramethoxy berbinium chloride) (see Scheme 1A), a small crescent-shaped planar heterocyclic molecule, possesses pronounced antitumor activity among the protoberberine alkaloids [25,26]. It is found that its fused planar cationic aromatic ring system provides the capability of interaction with polyadenosine [27]. In the neutral pH solution coralyne promotes an excellently stable antiparallel duplex of polyadenosine, with an association constant of 1.8×10^{6} M^{-1} and a stoichiometry of one coralyne to four adenine bases (A_2-coralyne-A_2) [27,28–30]. What attracts the researchers most is the activity coupled with relatively low toxicity and high stability of A_2-coralyne-A_2 cooperation [31]. Thus, it is hopeful to build a MB based on the interaction between coralyne and polyadenosine.

Melamine (MA, 2,4,6-triamino-1,3,5-triazine), a chemical compound broadly used in the synthesis of melamine resins for manufacturing laminates, coatings, adhesives, dishware, plastics and so on [32,33]. Nevertheless, because of its high nitrogen level (about 66% by mass) and low cost, it has been illegally added to the protein-rich food such as infant formula and pet food abused to increase the apparent protein level and cannot be detected by

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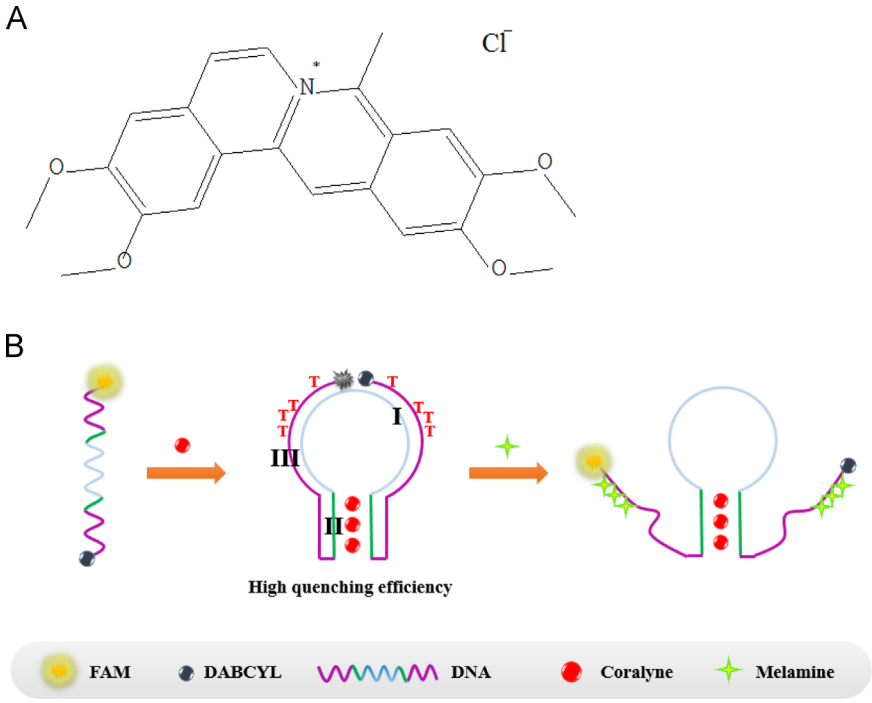

Scheme 1. (A) The structure of coralyne; (B) Schematic illustration of dual hairpin-like MBs based on A_2-coralyne-A_2 complexes and the formation of triple H-bonds for MA Assay .

kjeldah method [34]. However, MA cannot be absorbed by animals metabolically, leading to subsequent tissue injury and even death above the safety regulation level. Therefore, the detection of MA are of particular significance in people's daily living and production. Recently, various methods for the detection of melamine are developed including chromatography (GC) [35–38], capillary electrophoresis [39,40], surface-enhanced Raman spectroscopy (SERS) [41–47], electrochemiluminescence (ECL) [48], and fluorescence [49,50], et al. However, some of them are not readily adaptable to routine analysis due to their time-consuming and complicated processes. Thus, developing rapid, simple and sensitive methods to detect MA becomes especially urgent.

In this work, we designed a coralyne-adenosine based dual hairpin-like MB probe for the detection of MA. We demonstrated that the presence of coralyne can efficiently form a dual hairpin-like MB. Because there is a stable triple H-bond between thymine and MA (Fig. 1) [51], the conjugation of thymine with MA triggered the separation of fluorophore–quencher pair, resulting in the restoration of fluorescence. This made the dual hairpin-like MB can detect MA at neutral pH and room temperature with high sensitivity and selectivity, even for the real sample detection.

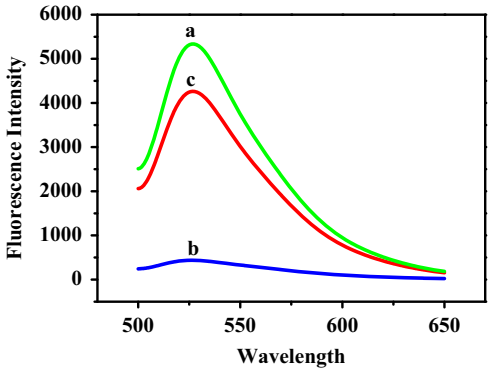

Fig. 1. Fluorescence spectra of solutions containing (a) 20 nM S1; (b) 20 nM S1 after 40 min of self-assembly; (c) 20 nM S1 and 4 μM coralyne; (d) 20 nM S1 and 4 μM coralyne in the presence of 2000 μg/L MA.

2. Experimental

2.1. Reagents and instrument

Oligonucleotide (S1: 5′-FAM-TCC TTT GGC GCG C6 A6 GGA GCC CCC GGA AGG CCC CCG AGG A6 C6 GCG CGG TTT CCT-DABCYL-3′) was synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and purified by HPLC. It was dissolved in Tris (hydroxymethyl) aminomethane (Tris)–HCl buffer (pH 7.4) as stock solution and the concentration was identified according to UV absorption at 260 nm. FAM: 5-carboxyfluorescein (at the 5′-end); DABCYL: 4-([4-(dimethylamino) phenoyl] azo)-benzoic acid (at the 3′-end), coralyne chloride hydrate and Tris were purchased from Sigma-Aldrich (St. Louis, MO, USA) used without further purification. MA was purchased from Sinopharm Chemical Reagent Co., Ltd. All the stocks and buffer solutions were prepared by using ultrapure water (PSDK2-10-C, Beijing, China). Fluorescence spectra were recorded using F-4600 fluorescence spectrophotometer (Hitachi, Japan).

2.2. Preparation of dual hairpin-like MB

DNA probe was prepared in a solution containing 20 mM Tris–HCl buffer (pH 7.4). The mixture was heated at 88 °C for 10 min, and gradually cooled to the room temperature. After heat treatment, DNA probe solution (20 nM) was mixed with coralyne solution at 1:1 volume ratio and incubated for 50 min at room temperature to form dual hairpin-like MB. The fluorescence emission spectrum was used to characterize the formation of MB at an excitation wavelength of 490 nm.