Sensors and Actuators B 171–172 (2012) 666–671

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

DNA abasic site-based aptamer for selective fluorescence light-up detection of fisetin by excited-state intramolecular proton transfer

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ARTICLE INFO

Article history:
Received 21 February 2012
Received in revised form 13 May 2012
Accepted 15 May 2012
Available online 24 May 2012

Keywords:
Aptamer
Fluorescence detection
Abasic site
Fisetin
Flavonoids
Excited-state intramolecular proton transfer

ABSTRACT

Many efforts have been made for the developments of aptamers due to their wide applications for various targets. In this work, we tried to use an abasic site (AP site) that was embeded in a double-stranded DNA as a binding pocket for fisetin. With the AP site being wholly opposed and flanked by thymines (named T-T aptamer), the aptamer exhibits high binding selectivity and sensitivity for fisetin over the other flavonoids such as morin, rutin, apigenin, kaempferol, myricetin, quercetin, luteolin, baicalin, naringenin, genistein, chrysin, and galangin. Upon binding to the AP site, fisetin experiences a significant enhancement in fluorescence emisson by favoring the occurrence of its excited-state intramolecular proton transfer (ESIPT) reaction. The detection limit is about 50 nM at a signal-to-noise ratio of 3. Therefore, the realization for fisetin's selective analysis is beneficial from the novel aptamer design without any fluorophore modification. Thus, the almost negligible fluorescence background from the aptamer is achieved in our method.

1. Introduction

Aptamers, which are short, single-stranded oligonucleotides (including ss-DNA or ss-RNA) with high affinity to certain targets, have received much attention due to their wide applications such as molecule sensing, medicine discovering, drug delivering, and therapeutics developing [1–4]. As a recognition element for analytical application, aptamers can target many substrates including small molecules (such as ATP [5,6], adenosine [6–9], Hg^{2+} [10]), proteins (such as thrombin [11–14]), and even intact cells [1,2]. The key issues for the aptamer developments are their selection out from the oligonucleotide random library and transducing the target's recognition event to a readout signal. The well-known SELEX (systematic evolution of ligands by exponential enrichment) process has been normally used to resolve the former issue by starting from 10^{13} to 10^{16} ss-DNA or ss-RNA molecules having different sequences and lengths. Nevertheless, for the latter issue, labeled tags have been usually introduced into the aptamers or their targets to response to the target's recognition event. The selective recognition of an aptamer to its corresponding target brings its tag having, for example, electroactive or fluorescent moieties, approaching (or detaching away) to (or from) an electrode surface or a quencher [15]. During this process, the aptamers' conformation is switched. The obtained signal distinction is strongly dependent on the manner how the conformation is changed. However, these strategies frequently suffer from the high background signals happened to the tagged aptamers. Thus, label-free strategies [16–19] are desired to some analytes such as solvent-sensitive fluorescent substrates for achieving low background signals.

Herein, double-stranded DNAs (ds-DNA) containing an abasic site (AP site) were employed as potential aptamers to detect fisetin, one of the natural flavonoids. The DNA AP site is produced in vivo by enzyme cleavage of the damaged nucleobases and then a void is left and spatially constrained by an opposing base that is unpaired and two flanking bases (Scheme 1) [20]. The AP site can be served as the binding pocket for target molecules having a comparable size and substituent distribution. For example, the presence of the AP site in a DNA can be identified by some small molecules such as binder/insertor heterodimers [20–22], metalloinsertors [23,24], naphthalene derivative [25], nitroxide spin labels [26], and fluorescent DNA base analogs [27]. Moreover, fluorescent ligands possessing complementary hydrogen bonding moieties to the unpaired base opposite the AP site have been employed as probes to detect DNA mutations [28]. Recently, the AP site has been developed as aptamer for fluorescence detections of riboflavin [29] and adenosine [30] but suffer from low selectivity and high background signal in their cases, respectively. However, binding-induced fluorescence light-up recognition for certain small molecules based on the AP-site aptamer still remains in progress. Previously, we found that fisetin binding at the AP site exhibited a sequence-dependent

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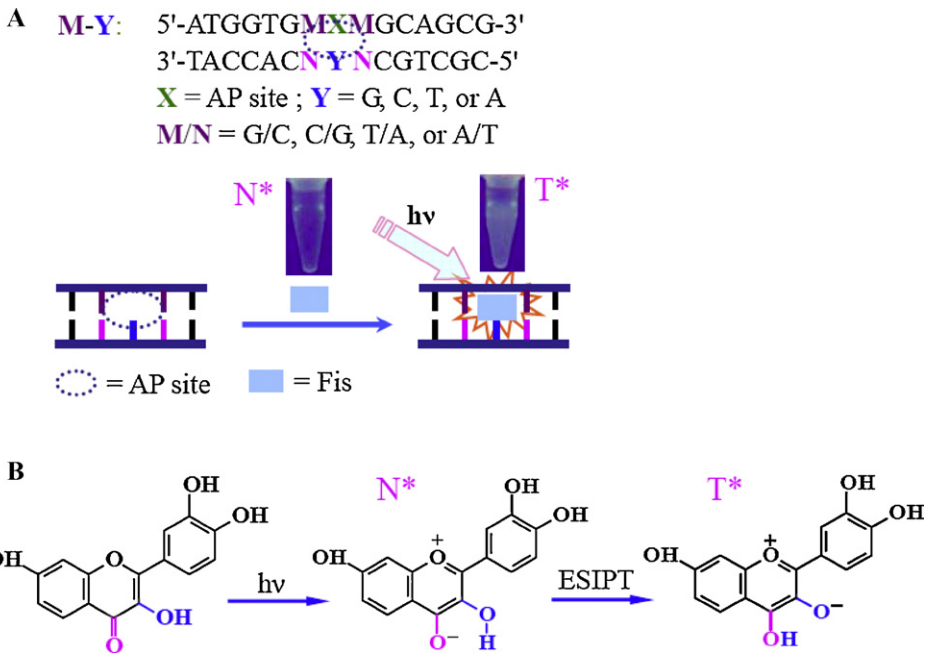
http://dx.doi.org/10.1016/j.snb.2012.05.051



Scheme 1. (A) The used DNA sequences (**M-Y**, named by the flanking and opposing bases) and schematic representations of the AP site-directed association of fisetin. (B) The structure transitions of fisetin from the species responsible for the normal emission (N*) to that responsible for the tautomer emission (T*) by the ESIPT process.

fluorescence behavior [31]. Fisetin, which is abundant in common plants possessing a broad range of biological activities, belongs to 3-hydroxyflavone in the group of flavonoids. Up to now, there is no reliable method to directly detect fisetin without a separation procedure because of similarity in the molecule structures for the related natural flavonoids [32,33]. Excited-state intramolecular proton transfer (ESIPT) between the 3-OH and 4-carbonyl (as shown in Scheme 1) moieties of such 3-hydroxyflavone can occur in a non-polar environment, which results in appearance of a new long-wavelength tautomer emission band (T*) besides the normal emission band (N*) [34–39]. Due to the very low quantum yield of the N* emission band, fisetin is weakly fluorescent in aqueous solution. However, the T* emission band can be enhanced upon binding to the AP site [31]. In this work, among all the investigated flavonoids, fisetin shows a selective affinity to the AP site that is opposed and flanked by thymines. Thus, this AP site-containing aptamer for fisetin detection is unnecessarily labeled with any foreign fluorophore, which results in no any interference from the background emission.

2. Experimental

DNA strands were synthesized by TaKaRa Biotechnology Co., Ltd. (Dalian, China). All DNA samples were purified by HPLC. All commercially available flavonoids including morin (Mor), rutin (Rut), apigenin (Api), kaempferol (Kae), myricetin (Myr), quercetin (Que), fisetin (Fis), luteolin (Lut), baicalin (Bai), naringenin (Nar), genistein (Gen), chrysin (Chr), and galangin (Gal) were purchased from Aladdin Reagent Co., (Shanghai, China) and used as received without further purification. Other reagents were of analytical grade and used without purification. Tetrahydrofuran residue was used as the chemically stable abasic site (AP site) for replacement of the naturally tautomeric deoxyribose structure. Prior to hybridization, the DNA concentrations in single-stranded format were measured at 260 nm in pure water on the basis of extinction coefficients calculated by the nearest neighbor analysis [40].

To prepare DNA duplex solutions, the two single-strand DNAs were mixed in equimolar amounts and annealed in a thermocycler (first at 92 °C, then cooled down to room temperature slowly) in 20 mM phosphate buffer (pH 6.5) containing 0.1 M NaCl and 1 mM EDTA. Flavonoid was added to the duplex DNA solution to an appropriate molar ratio at 20 mM phosphate buffer (pH 6.5) containing 0.1 M NaCl and 1 mM EDTA. After mixing, the solution was incubated for 15 min with gentle stirring. The resulting solutions were examined at room temperature within 2 h. Nanopure water (18.2 mΩ; Millipore Co., USA) was used in all experiments. Fluorescence spectra were acquired with a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., UK) at 18 ± 1 °C, equipped with a temperature-controlled circulator (Julabo, Germany). The DNA melting temperatures (T_m) were determined with a UV2550 spectrophotometer (Shimadzu Corp., Japan), equipped with an accessory of TMSPC-8 T_m analysis system which can simultaneously control the chamber temperature and detect up to 8 samples by a micro multi-cell with a low deviation in temperature between samples.

3. Results and discussion

Due to the weak binding of flavonoids with the fully matched DNAs (FM) [41], FM is impossible to be served as an aptamer for selective analysis of one of flavonoids. On the basis of our previous report related to fisetin (Fis) [31], we tried to make an effort to use the AP site as the binding pocket for the development of an aptamer to Fis. The used sequences (**M-Ys**) are shown in Scheme 1 with the AP site located at the very central position and named with its flanking (M) and opposing (Y) bases. The binding of Fis at the AP site exhibits a clear dependence on the Ys. The Fis' tautomer emission band (T*, Scheme 1B) is favored with Ys being pyrimidines by comparison to purines [31]. In order to get a high sensitivity, we checked the M-T sequences to investigate the effect of the flanking Ms on the T* emission band of Fis by employing thymine (that is, Y = T) as the opposing base. As shown in Fig. 1, the DNA duplexes with thymine (T), adenine (A), and guanine (G) flanking the AP site induce stronger T* emission bands of Fis by comparison to the case with cytosine (C), whereas the normal (N*) emission bands seem to be a little varied, supporting the point that the binding of Fis at