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A novel fluorescent sensor for triplex DNA

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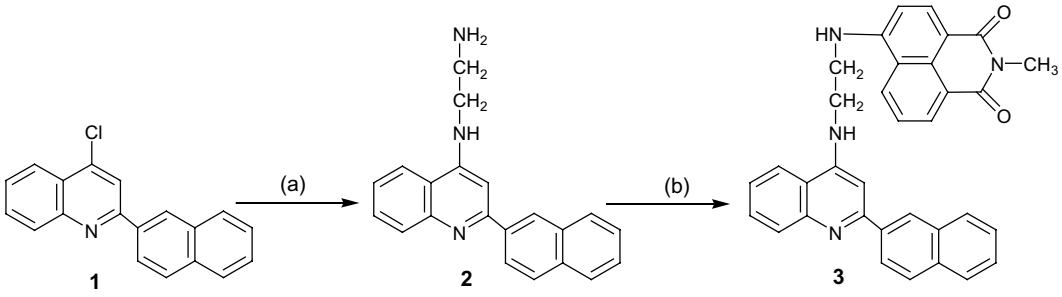
Abstract—A triplex DNA fluorescent sensor based on PET is described. The sensor takes 4-aminonaphthalimide as a reporting group and a triplex-select intercalator as a recognizing group. The results show that it is a selective sensor for T·AT triplex DNA in compared with duplex and ssDNA by fluorescence enhancement in PIPES 20 buffer.

At present, many advances are being made in the understanding of the formation and the recognition of DNA triple helices which should help the development of the great potential in a variety of analytical and therapeutic applications of triple helix forming oligonucleotides (TFOs).[1–6] Recognition and detection of triple helix are of special importance for useful therapeutic agents as modulators of gene expression in the 'antigene' therapeutic strategy.[7–10] Therefore, there is a need to develop new selective and sensitive methods for triple helix. Of all detected signals, fluorescence is the favored signaling technology for molecular diagnoses on account of its simplicity and high sensitivity. Fluorescence assay such as molecular beacon that is covalently linked in triplex has been used to study the structure and thermodynamic characterization of the triplex DNA.[11,12] On the other hand, there is few fluorescence sensor which is selective for triplex DNA in comparison with other structural forms of DNA, such as single and double stranded DNA. These observations prompted us to develop a new fluorescent biosensor for triplex structure DNA.

In this paper, we synthesized a new triplex-selective fluorescent sensor **3** (Scheme 1). The 4-aminonaphthalimide was chosen as fluorescent-monitoring unit which has been used in many studies such as sensors, special interactions with some biomolecules (DNA, enzymes, etc.) because of its outstanding spectroscopic properties.[13–18] Ethylene group as a spacer linked with 4-aminonaphthalimide could be used to build highly sensitive photoinduced electron transfer (PET) sensors.[19–21] 2-(2-Naphthyl)quinoline moiety, whose derivatives are proved highly selective triplex intercalators



Scheme 1. Reaction conditions: (a) $SnCl_4$, ethylenediamine, 135 °C, 4 h, 85%; (b) C_6H_5N, *N*-methyl-4-bromo-1,8-naphthalimide, reflux 10 h, then chromatography separation (CH_2Cl_2:MeOH: 20:1), 70%.

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for T·AT triplex,[22–24] was used as triplex receptor. The results showed that **3** displayed a highly selective response of fluorescence enhancement in the presence of T·AT triplex in PIPES 20 (pH 7.0) buffer.

Compound **3** was obtained in two steps. Compound **1**, prepared according to literature,[25] was used as a starting material. A nucleophilic displacement of chloride from **1** by the reaction with ethylenediamine in the presence of a catalytic amount of $SnCl_4$ furnished **2**. *N*-Methyl-4-bromo-1,8-naphthalimide was readily prepared from 4-bromo-1,8-naphthalimide and 33% aq methylamine by stirring at room temperature for 2.5 h. Then it was refluxed with **2** in pyridine solution to afford **3**, which was characterized by ^{1}H NMR spectroscopy, and high resolution mass spectrometry.[26] The $\lambda_{max(ab)}$ and $\lambda_{max(em)}$ of **3** are 457 nm and 538 nm, respectively, in PIPES 20 buffer (10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 1 mM EDTA, and 200 mM NaCl adjusted to pH 7.0). The fluorescent emission spectrum was recorded on a spectrometer with excitation at 457 nm.

2-(2-Naphthyl)quinoline derivatives are highly selective triplex intercalators for T·AT triplex which is widely used in studies of ligand–triplex interactions, and is one of the best characterized triple helix structures.[24] Thus, T·AT was chosen as our target triplex structure. Calf thymus, purchased from Sigma, was chosen as a mixed sequence duplex at hand for comparison purpose. Nucleic acid concentration was expressed in terms of the monomeric unit for each polymer, that is, nucleotides for single strands, base pairs for duplex forms and triplets for the triplex.

The nucleic acids oligomers, dA_{19} and dT_{19}, were purchased from Takara, and dissolved in PIPES 20 buffer. The concentration of dA_{19} and dT_{19} were determined spectrophotometrically according to the reported extinction coefficients (L/mol of base)/cm, at 260 nm and 25 °C) for dA_{19} (9000) and dT_{19} (8200), respectively.[27] T·AT was prepared by mixing 1:2 molar ratio of dA_{19} and dT_{19}, and heating the mixture in a water bath to 90 °C then slowly cooling to 5 °C to form the final sample before use according to literatures.[22,27] AT was prepared by mixing 1:1 molar ratio of dA_{19} and dT_{19} with the same method. All of the fluorescence experiments were carried out with the samples in PIPES 20 buffer.

To ensure that the T·AT remains in its triplex structure, we investigated the fluorescent response of **3** to DNA triplex at 5 °C. As expected, **3** showed a very clear fluorescence enhancement by the addition of T·AT triplex. When the intercalation of 2-(2-naphthyl)quinoline moiety into the bases of T·AT triplex, hydrogen bond between the electron pair of the N atom of amino-quinoline and the triplex bases or π stacking between the naphthylquinoline and triplex bases or both might cause the decrease of PET to the fluorophore, which results in the fluorescence enhancement. There was about 6-fold fluorescence enhancement, when **1** was 1.0 μM and T·AT was 60 μM (Fig. 1).

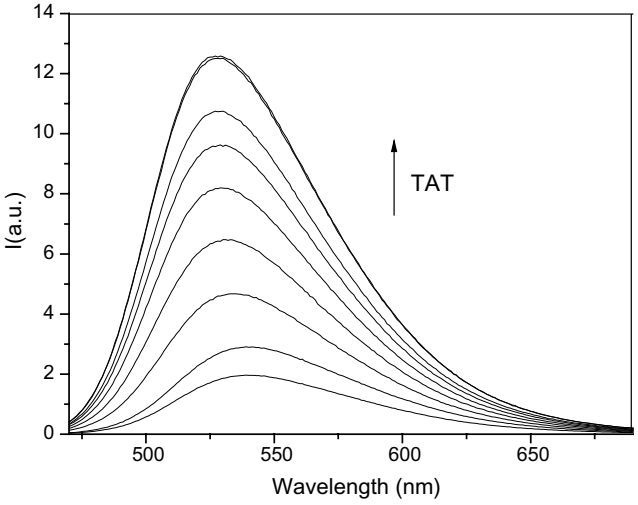

Figure 1. Emission spectra (excitation at 457 nm) of **3** (1 μM) in the presence of various concentrations of T·AT ranging from 0, 1, 5, 10, 20, 40, 60, 80, 100 μM. These spectra were measured in pH 7.0 PIPES 20 buffer.

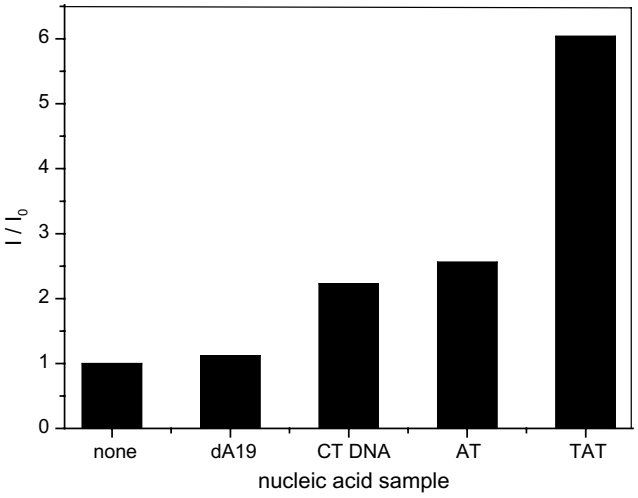

Figure 2. Fluorescence response of 1 μM **3** to nucleic acid samples (50 μM) in PIPES 20 buffer (pH 7.0). The temperature was 5 °C.

To clarify the selectivity of fluorescence enhancement of **3** toward T·AT triplex, several kinds of DNA with different structures were studied. As shown in Figure 2, the addition of ssDNA (dA_{19}, 50 μM) to the solution of **3** has little increase in fluorescence emission. Addition of AT (50 μM) or calf thymus (50 μM) duplex shows a modest increase in fluorescence emission. However, the significant fluorescence enhancement was observed upon adding 50 μM T·AT, suggesting that compound **3** is a triplex-selective DNA sensor.

Fluorescence recovery is often occurred when a PET sensor combines with a proton. As shown in Figure 3, the fluorescence intensity is high in the low pH, this is due to the protonation of N atom of amino-quinoline and quinoline. Therefore, the normal fluorescent quenching pathway by PET is blocked. The pK_a of **3**

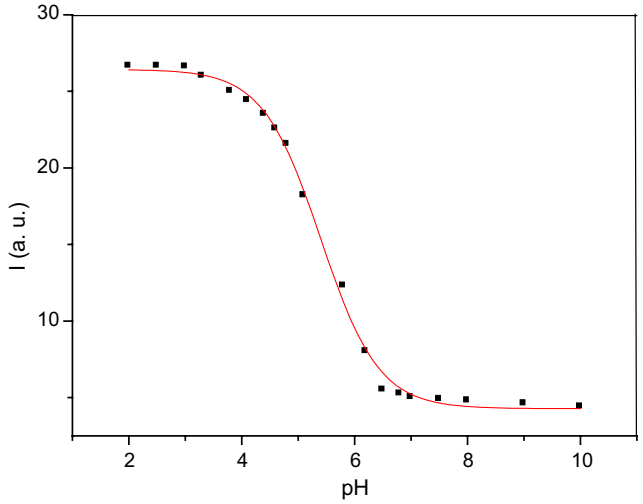

Figure 3. Effect of pH on the fluorescence intensity of 1 μM **3** (excitation at 457 nm, emission from 470 to 680 nm) at 25 °C.

is about 5.4 obtained from the fluorescence titration curve (Fig. 3). The fluorescence intensity of **3** decreases along with the increase of pH value, but when pH > 6.5, the fluorescence intensity changes very little in PIPES 20 buffer. This pH range might be helpful to avoid the interference of possible pH change induced by biological stimulation.[28]

In summary, a simple fluorescent biosensor **3** for recognition of T·AT was designed and synthesized, and it displays selectivity for T·AT triplex by fluorescence enhancement when pH > 6.5.

Acknowledgements

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