Acridone-labeled Base-discriminating Fluorescence (BDF) Nucleoside: Synthesis and Their Photophysical Properties

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Acridone-labeled BDF bases ^{Ac}**A** and ^{Ac}**U** were incorporated into oligonucleotides (ODNs) and their photophysical properties were evaluated. The BDF probe containing ^{Ac}**A** is extremely powerful in recognizing opposite base **T** via enhancement of the fluorescence intensity. Furthermore, they emit strong fluorescence at a longer wavelength than previously reported pyrene-labeled BDF probes and thus can be used for the detection of SNPs.

The completion of the draft of the human genome prompted an unprecedented flurry of activity for screening genetic mutations to identify disease related genes on a genome-wide scale. Among many genetic variations, single nucleotide polymorphisms (SNPs) are the most common DNA variation.[1] Because of their medicinal and pharmacological importance, there has been current interest in SNP discovery and detection. While a large number of fluorescence-based detection techniques are available, almost all of the previous techniques rely upon the hybridization efficiency.[2]

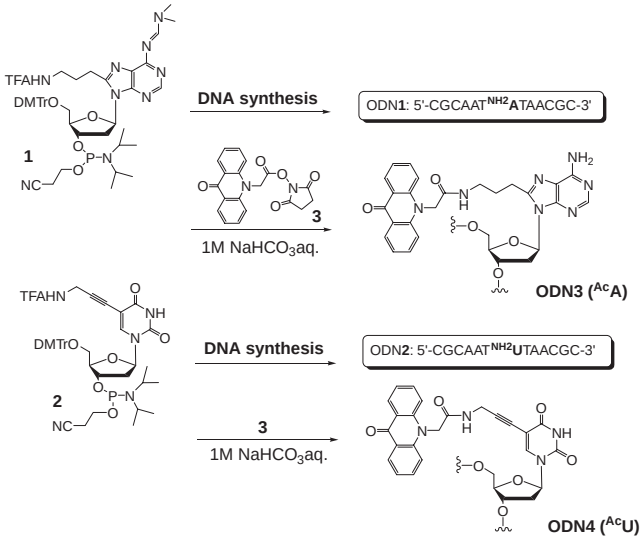
As an alternative to the presently available methods, we are involving in designing base-discriminating fluorescent (BDF) nucleobases usable in homogeneous detection of SNP. Our concept is based on the labelling of nucleobases by solvofluorochromic molecules that is capable of interacting specifically with DNA duplex, such as those of intercalators and DNA groove binders.[3] This concept of BDF probe design relies upon the fluorescence change of the BDF base itself in response to the opposite bases on a complementary strand, not on whether the probe is hybridized or not. Most of our research efforts exploited the fluorescent properties of pyrene-[3b] and anthracene-[3d] based BDF nucleosides. However, these fluorophores emit at a shorter wavelength region and the intensity of the emission is relatively low. Thus, these fluorophores are not always suitable to be used in DNA chip. Acridone derivatives are well-known antitumor drugs and their planar structure suggest the ability to bind DNA by intercalation.[4] Besides its bright fluorescence, acridone turns out to be photochemically quite stable. Also, acridone fluorophore has fluorescence lifetimes which are unaffected by pH in the range of 5 to 9. Due to the interesting fluorescence properties, acridone and its derivatives are widely used for labelling of biomolecules to probe biomolecular structure, function and dynamics.[4] Thus, we thought it will be worthwhile to use acridone as a fluorescence label for nucleobases and use them for the detection of SNP using our previously reported homogeneous detection protocol.[3]

The fluorescent property of acridone is strongly affected by the polarity of the solvent. For example, in hexane, the least polar solvent tested, the quantum yield of acridone fluorescence is 0.0015, and in methanol, the highest polar solvent, is 0.97, with emission wavelength at around 435 nm.[5] Thus, looking after the interesting fluorescent properties of acridon and to get a better signal to noise ratio along with a longer wavelength emission, we incorporated acridone in 2′-deoxyuridine (^{Ac}**U**) via a rigid acetylene linker and also in 2′-deoxyadenosine (^{Ac}**A**) via flexible methylene linker by post modification. We studied the photophysical and thermal melting properties in the presence of four complementary strands varying only by a single base opposite to the labeled BDF base. Our objective was to compare the present investigation with the previously reported result using pyrene as a fluorophore.[3b,6]

Thus, phosphoramidite derivatives **1** and **2** were synthesized according to our previously reported synthetic protocol.[6] Phosphoramidite **1** was incorporated into 13mer oligonucleotides [ODN**1**: 5′-CGCAAT^{NH2}**A**TAACGC-3′] by using automated DNA synthesizer. Similarly, the phosphoramidite **2** was incorporated into 13mer oligonucleotides [ODN**2**: 5′-CGCAAT^{NH2}**U**TAACGC-3′] (Scheme 1).

Finally, acridone fluorophore was introduced into both oligomers via post-modification according to previously reported synthetic protocol[6] to give desired oligonucleotides, ODN **3** and ODN **4** which were characterized by MALDI-TOF mass spectrometry. The newly designed ODNs are shown in Table 1.

After synthesizing the acridone-labeled oligonucleotides



Scheme 1. Synthesis of acridon-labeled ODNs by post modification.

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Table 1. Oligonucleotides used in this study

(ODNs)	Sequences
3	5′-d(CGC AAT ^{Ac}**A** TAA CGC)-3′
4	5′-d(CGC AAT ^{Ac}**U** TAA CGC)-3′
5	5′-d(GCG TTA **A** ATT GCG)-3′
6	5′-d(GCG TTA **G** ATT GCG)-3′
7	5′-d(GCG TTA **C** ATT GCG)-3′
8	5′-d(GCG TTA **T** ATT GCG)-3′

Table 2. UV visible spectroscopic data

(ODNs) for ^{Ac}**A**	λ_{max} /nm	ε_{max} /M^{-1} cm^{-1}	(ODNs) for ^{Ac}**U**	λ_{max} /nm	ε_{max} /M^{-1} cm^{-1}
3	392	6400	**4**	404	9200
3/**5**	391	5200	**4**/**5**	390	9600
3/**6**	391	4800	**4**/**6**	402	7200
3/**7**	390	5200	**4**/**7**	404	7600
3/**8**	390	6400	**4**/**8**	402	7200

ODN **3** and ODN **4**, we studied their fluorescence properties in the presence of complementary ODNs differing by a single base opposite to the BDF base ^{Ac}**A** (for ODN **3**) and ^{Ac}**U** (for ODN **4**). The mismatched duplexes [ODN **3**/ODN **5**, **6**, **7**] and the single-stranded oligonucleotide, ODN **3**, showed very weak fluorescence emission ($\Phi = 0.052$, 0.044, 0.133, 0.144, respectively), while for completely matched duplex [ODN **3**/**8**] almost 2 times enhancement of fluorescence was observed ($\Phi = 0.274$) at around 435 nm (Figure 1). Melting temperature (T_m) measurements showed that the fully matched duplex [ODN **3**/**8**, T_m = 53.4 °C] is more stable than natural A/T base pair containing duplex (T_m = 51.2 °C). Thus, novel acridone-labeled nucleoside ^{Ac}**A** is capable of sensing opposite base **T** selectively at longer wavelength than pyrene-labeled BDF probe ^{Py}**A** does. In the case of ODN **4**, for completely matched duplex (ODN **4**/**5**), an enhanced fluorescence ($\Phi = 0.237$) was observed but with low signal to noise ratio. Although ^{Ac}**A**-containing ODN showed a clear **T** selective fluorescence emission, a little selectivity was observed for ^{Ac}**U** containing BDF probe, possibly because of the flexibility of the longer linker attached to acridone moiety.

In the case of fully matched duplex (ODN **3**/**8**), the polarity-sensitive acridone fluorophore is extruded to the outside of the groove, a highly polar aqueous phase, hence a strong fluorescence emission is observed. In contrast, the mismatched base pair containing ^{Ac}**A** shows a weak fluorescence due to the lack of stable base pairing. Thus, the acridon fluorophore relocates via intercalative interaction inside the duplex, thus facing more hydrophobic microenvironment that weakened the intensity of fluorescence emission. For mismatched duplexes, slight red shift and hypochromicity of the acridone absorbance in the UV visible spectra also support our explanation (Table 2). Therefore, it is clear that ^{Ac}**A**-containing BDF probe is capable of sensing thymine on a target sequence by enhancement of fluorescence intensity and it can be used in homogeneous SNP typing. However, it should be noted that there are some sequence limitations for the use of ^{Ac}**A**-containing BDF probe in SNP typing.

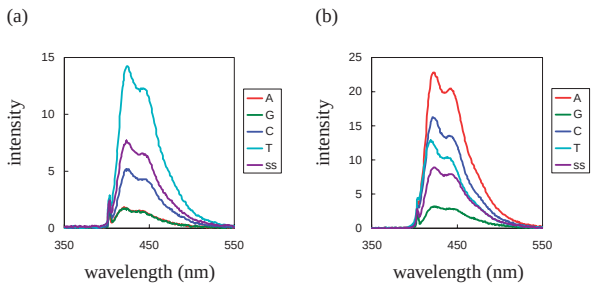

Figure 1. (a) Fluorescence spectra of **ODN 3** hybridized with 2.5 μM **ODN 5** (A), **ODN 6** (G), **ODN 7** (C), or **ODN 8** (T, full match) and single-stranded **ODN 3**. Excitation wavelength was at 402 nm. (b) Fluorescence spectra of **ODN 4** hybridized with 2.5 μM **ODN 5** (A, full match), **ODN 6** (G), **ODN 7** (C), or **ODN 8** (T) and single-stranded **ODN 4** (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). Excitation wavelength was at 404 nm.

In conclusion, we have developed novel types of acridone-labeled BDF probes. Among them ^{Ac}**A** is able to distinguish thymine base clearly on the complementary strand via enhancement of fluorescence intensity. Thus, the acridone-based BDF probes serve as a powerful alternative to current SNP-typing methods because of their simplicity in use, requires no stringent hybridization condition, involve no washing step and of low cost.

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