Detection of A/G Single Nucleotide Alteration in RNA Using Base-discriminating Fluorescent Oligodeoxynucleotides

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We report the fluorescence properties of oligodeoxynucleotides (ODNs) containing a base-discriminating fluorescent nucleoside, benzopyridopyrimidine (BPP), in hybridizing with RNAs possessing different bases opposite BPP. BPP-containing ODN selectively exhibited a strong fluorescence when the RNA base opposite BPP was A, whereas the fluorescence of BPP-containing ODN hybridized with RNA possessing G opposite BPP was very weak. BPP acts as a good base-discriminating fluorescent nucleoside for A/G single base alteration of RNA.

Single nucleotide polymorphisms (SNPs) are single base-pair substitutions that occur within and outside genes.[1–4] Genes containing one or more SNPs can give rise to two or more allelic forms of mRNAs. mRNAs containing different bases at SNP sites may vary in their interactions with cellular components involved in mRNA synthesis, maturation, transport, translation, or degradation. It has been documented that a number of single base-pair substitutions alter or create essential sequence elements for splicing, processing, and translation of human mRNA.[1] These SNPs are associated with altered length and/or steady-state level of cytoplasmic mRNA.

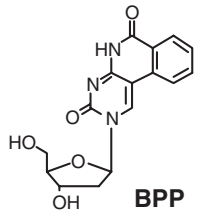

Figure 1. Base-discriminating fluorescent (BDF) nucleoside, benzopyridopyrimidine (BPP).

We have previously reported a fluorescent nucleoside, benzopyridopyrimidine (BPP),[5] which clearly distinguishes purine bases on complementary DNA using the change in fluorescence (Figure 1). Oligodeoxynucleotides (ODNs) containing BPP can be used as a powerful tool for the detection of A/G single nucleotide alteration in target DNAs. This observation has prompted us to apply BPP-containing ODNs to the detection of the single nucleotide alteration in RNA using the fluorescence emission from BPP. Here, we report the fluorescence properties of a BPP-containing ODN hybridized with RNAs possessing different bases opposite BPP. BPP-containing ODN selectively exhibited a strong fluorescence when the RNA base opposite BPP was A. In contrast, the fluorescence of BPP-containing ODN hybridized with RNA possessing G opposite BPP was very weak. BPP acts as a good base-discriminating fluorescent (BDF) nucleoside for RNA.

BPP-containing ODN **ODN(BPP)**, 5′-d(CGCAAT[BPP]-TAACGC)-3′, was prepared very efficiently, according to the protocol described in our previous report.[4,6] **ODN(BPP)** formed a thermodynamically stable duplex with the complementary RNA containing G opposite BPP (5′-r(GCGUUA-**G**AUUGCG)-3′, **RNA(G)**), because BPP is believed to be a C analog (Table 1). In addition, a large enhancement of the melting temperature (T_m) was observed in **ODN(BPP)/RNA(A)** duplex as compared with the T_m for the duplex containing a C/A mismatched base pair (+11.4 °C). The results of the T_m measurement suggest that BPP has a base-pairing degeneracy that can form hydrogen bonds with both G and A in complementary RNA.

Table 1. Melting temperatures (T_m) of duplexes formed by **ODN(X)** and the complementary RNA strands containing different bases opposite **X** **RNA(Y)**, 5′-d(CGCAAT**X**TAACGC)-3′/5′-r(GCGUUA**Y**AUUGCG)-3′ (**X** denotes BPP or C, and **Y** denotes C, G, U or A)[a]

Y	T_m/°C		ΔT_m
	X = BPP	X = C	
C	29.3	26.0	3.3
G	51.2	48.8	2.4
U	32.7	25.0	7.7
A	41.6	30.2	11.4

[a]All T_ms of the duplexes (2.5 μM, duplex concentration) were taken in a buffer containing 50 mM sodium phosphate and 100 mM sodium chloride, pH = 7.0. Absorbance *vs* temperature profiles were measured at 260 nm using a JASCO TPU-550 UV–vis spectrometer connected to a JASCO TPU-436 temperature controller. The absorbance of the samples was monitored at 260 nm from 2 °C to 80 °C with a heating rate of 1 °C/min. First derivatives were calculated from these profiles to determine T_m values.

The UV spectra observed for the duplexes containing BPP showed a characteristic absorption at 320–380 nm originating from BPP, the absorption maximum being observed at 349 nm, regardless of the type of base opposite BPP (Figure 2a). The CD spectra of the duplexes containing BPP exhibited the characteristic pattern of the A-type duplex with a large positive ellipticity at 270 nm and two negative peaks at 245 and 210 nm (Figure 2b).[7]

Next, the fluorescence spectra of the duplexes containing different bases opposite BPP were measured with excitation at 345 nm. In the spectrum of the "full-matched" duplex formed by **ODN(BPP)** and **RNA(G)**, a small fluorescence peak at 388 nm was observed ($\Phi = 0.009$), as shown in Figure 3. In contrast, the fluorescence spectrum for the "mismatched" du-

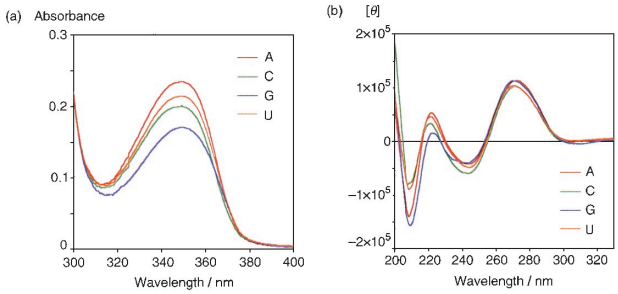

Figure 2. (a) Absorption spectra of 25 μM duplexes formed by **ODN(BPP)** and the complementary RNA strands containing different bases opposite BPP **RNA(Y)**, 5′-d(CGCAAT[BPP]TAACGC)-3′/5′-r(GCGUUA**Y**AUUGCG)-3′ (**Y** denotes C, G, U or A) in 50 mM sodium phosphate and 0.1 M sodium chloride (pH = 7.0) at 20 °C. (b) CD spectra of 2.5 μM duplexes formed by **ODN(BPP)** and **RNA(Y)** in 50 mM sodium pbosphate and 0.1 M sodium chloride (pH = 7.0) at 20 °C.

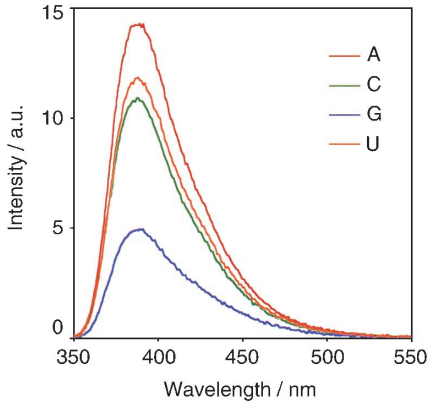

Figure 3. Fluorescence spectra of 25 μM duplexes formed by **ODN(BPP)** and the complementary RNA strands containing different bases opposite BPP **RNA(Y)**, 5′-d(CGCAAT[BPP]TAACGC)-3′/5′-r(GCGUUA**Y**AUUGCG)-3′ (**Y** denotes C, G, U or A) in 50 mM sodium phosphate and 0.1 M sodium chloride (pH = 7.0) at 20 °C. Excitation wavelength was 345 nm.

plex formed by **ODN(BPP)** and **RNA(A)** showed the strongest fluorescence ($\Phi = 0.020$). The fluorescence intensity of **ODN(BPP)/RNA(A)** at 388 nm, the peak of fluorescence spectra, was 2.9 times higher than that observed for the matched duplex, **ODN(BPP)/RNA(G)**. For other mismatched duplexes **ODN(BPP)/RNA(C)** and **ODN(BPP)/RNA(U)**, the fluorescence intensities were moderate ($\Phi = 0.017$ and 0.018, respectively). The fluorescence of **ODN(BPP)** was strongly influenced by the type of base opposite BPP, and particularly when G was the base opposite BPP, the fluorescence of **ODN(BPP)** was greatly weakened. Thus, the change in the fluorescence behavior of **ODN(BPP)** depending on the base opposite BPP facilitates identification of the type of bases, especially purine bases, located at a specific site on RNA.

The fluorescence peak of **ODN(BPP)** was broadened to nearly 500 nm, and thus, the fluorescence emission from a solution containing **ODN(BPP)** was visible to the human eye. As shown in Figure 4, **ODN(BPP)/RNA(A)** showed a pale blue fluorescence with excitation at 312 nm, and was clearly distinguishable from a very weak fluorescence observed for duplexes containing **ODN(BPP)/RNA(G)**. Therefore, the hybridization of **ODN(BPP)** with a target RNA facilitates the clear distinction of purine bases located at a specific site of RNA with the naked eye.

Figure 4. A/G discrimination using the change of BPP fluorescence. The fluorescence image from a solution containing 25 μM of **ODN(BPP)/RNA(A)** (left) or **ODN(BPP)/RNA(G)** (right) in 50 mM sodium phosphate and 0.1 M sodium chloride (pH = 7.0) was taken using a transilluminator at 312 nm.

In summary, we found that the fluorescence intensities of a fluorescent nucleoside, BPP, sharply changed depending on the RNA bases opposite BPP. The hybridization of a BPP-containing BDF probe with a target RNA facilitates detection of bases located at a specific site of the target RNA with the naked eye. The BPP-containing BDF probe will be useful for the detection of single nucleotide alterations in RNA strands as well as in DNA.

References and Notes

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