ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 5 (5): 257-262, 2012
ISSN 1996-3351 / DOI: 10.3923/ajbs.2012.257.262

Sequence Discriminating Ability of OligoDNA Bearing Silylated Pyrene Derivatives through Excimer Formation

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ABSTRACT

To develop an efficient and simple method for detecting Single Nucleotide Polymorphisms (SNPs) is important because nucleobase mutations may cause genetic abnormalities. Excimer formation by modified pyrene has been utilized by many research groups for designing unique classes of DNA probes but the intensity of excimer fluorescence may be influenced by terminal overhangs in the complementary sequences. The aim of the study was to justify the effect of chain length of the complementary strands on the excimer fluorescence intensity. In the present study, a simple method has been developed for SNP detection using a novel 12-mer DNA probe bearing two consecutive silylated pyrene units that could efficiently detect perfectly matched and single base mismatched complementary sequences. The oligoDNA probe has been synthesized by automated DNA synthesizer using phosphoramidite chemistry. The probe gives marked excimer fluorescent signal only upon binding to the fully matched complementary DNA strand. However, the excimer signal was significantly decreased in the single stranded form as well as in the duplexes with single base mismatched complements. And virtually there was no effect of the length of the complementary DNA strands on the intensity of excimer fluorescence.

Key words: DNA probe, silylated pyrene, excimer intensity, chain length, excimer fluorescence

INTRODUCTION

Common genetic variations in the human genome are Single Nucleotide Polymorphisms (SNPs), which are important for identifying disease-causing genes and for pharmacogenetic studies (Suman and Jamil, 2006; Abbasi *et al*., 2009; Javed and Mukesh, 2010; Lajin and Alachkar, 2011; Furukawa *et al*., 2012; Uddin and Azam, 2012). Therefore, sequence-selective detection of nucleic acids has become an important research area in bioorganic chemistry, medicine and molecular biology. Accordingly, fluorescent oligoDNA probes have now widely been introduced for the detection and study of nucleic acids. Of the commonly used fluorophores, functionalized pyrenes have become popular in recent years as fluorescent labels because of pyrene's photophysical properties, such as the long lifetime of its excited state, sensitivity towards microenvironmental changes and propensity to pie-stacking (Astakhova *et al*., 2008; Nakamura *et al*., 2005; Winnik, 1993).

Several modified oligonucleotide probes utilizing pyrene to exhibit excimer fluorescence upon hybridization to their complementary oligonucleotides have been reported (Lewis *et al*., 1997;

Kostenko *et al*., 2001; Yamana *et al*., 2002; Hrdlicka *et al*., 2005). Besides, silylated pyrene bearing a modifiable functional group is a recently developed new derivative of pyrene capable of introduction into biological molecules such as nucleic acids and lipids (Sekiguchi *et al*., 2007; Moriguchi *et al*., 2009).

Recently we have reported a molecular beacon type hybridization probe bearing silylated pyrene as a nucleobase that could efficiently detect fully matched and point mutated complementary DNA sequences through excimer formation (Uddin *et al*., 2012). In addition, very recently another molecular beacon type novel oligoDNA probe carrying non-nucleosidic silylated pyrene that exhibited excimer fluorescence has been reported (Uddin and Azam, 2012).

The synthesis and fluorescence properties of this modified oligoDNA probe (DN-1) have already been reported by our group where the complementary target sequences were equal in chain length (12-mer) to the modified probe (Mogi *et al*., 2010). In this case, relatively longer complementary oligoDNAs (20~21-mer) have been used with a view to reporting the effect of chain length of the complementary sequences on the excimer fluorescence intensity.

MATERIALS AND METHODS

Reagents: Pyrene, N-bromosuccinimide, *n*-butyl lithium, chloromethyl dimethyl chlrorosilane, phthalimide potassium, hydrazine monohydrate, 2, 2 bis-(hydroxymethyl) propionic acid, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl) 4-methylmorpholinium chloride (DMT-MM), DMTrCl, N,N-Diisopropylethylamine (DIPEA), 2-cyanoethyl diisopropylchlorophosphoramidite.

Instruments: Automated DNA synthesizer (Applied Biosystems 392), UV spectrophotometer (Shimadzu, Japan), Fluoroscence spectrophotometer (Hitachi F-4010).

OligoDNA synthesis: The synthesis of modified oligoDNA (DN-1) was carried out by an automated DNA synthesizer (Applied Biosystems 392) using standard protocol on 1.0 μmol scale starting from CPG-bound β-deoxyguanosine. After the assembly, the support-bound oligoDNA was treated with concentrated ammonium hydroxide (60°C, 12 h) followed by reversed-phase HPLC, ethanol precipitation and gel-filtration (Sephadex G-25). All other oligoDNAs (Fig. 1) were also synthesized and purified in the same manner.

High-performance liquid chromatography (HPLC): The oligomer was purified by reverse-phase HPLC using quaternary solvent systems (Shimadzu, Japan).

Nuclear magnetic resonance (NMR) data: ^{1}H NMR data were recorded on JEOL 300 MHz and ^{31}P NMR was done on JEOL 500 MHz NMR spectrophotometer using $CDCl_3$ by using standard protocol.

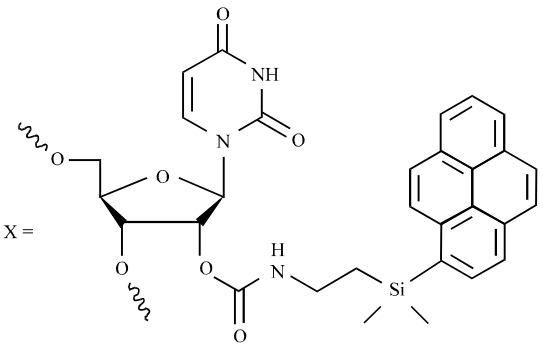
Fluorescence spectroscopy: Fluorescence spectra were taken on Hitachi F-4010 fluorescence spectrophotometer by using standard protocol on 1.5 mM scale (Uddin *et al*., 2012).

Electrospray ionization mass spectrometry (ESI-MS): Mass of the oligomer was determined by JEOL LC-MS spectrophotometer by using standard protocol.

RESULTS

The synthesis and fluorescent behavior of the modified oligomer (DN-1) bearing silylated pyrene has already been reported by our group (Mogi *et al*., 2010). Sequences of the oligomer used in this study have been displayed in Fig. 1. Fluorescent properties of DN-1 (1.5 μM) were examined in both a single-stranded form and a double-stranded form in buffer solution (10 mM sodium phosphate containing 100 mM NaCl) using excitation wavelength of 314 nm. Interestingly, fluorescence of silylated pyrene molecules in DN-1 is effectively quenched while the DNA stays as a single-stranded form.

A vigorous change was observed in the fluorescent signal containing the mixtures of DN-1 (12nt) and its fully matched equal length (12nt, DN-2S) as well as longer complementary sequence DN-2L (21nt) at room temperature. The excimer signal was significantly increased in both the cases after hybridization (Fig. 2).



DN-1: 3' - GCC TCX XTC GCT - 5'
DN -2S: 5' - CGG AGA AAG CGA - 3'
DN-2L: 5' - AAG AAG CGG AGA AAG CGA CGA - 3'
DN -3: 5' - AAG AAG CGG AGA TAG CGA CGA - 3'
DN-4: 5' - AGA AG CGA AGA CAG CGA CGA - 3'
DN-5: 5' - AAg AAG CGG AGA GAG CGA CGA - 3'

Fig. 1: Sequences of the oligoDNAs used in this study

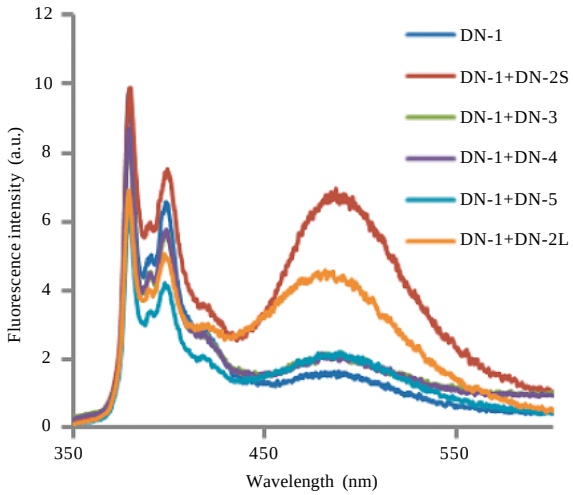


Fig. 2: Fluorescence spectra of the oligoDNAs

In the first case where the probe was hybridized with short sequenced (12nt) complementary strand, the intensity of excimer fluorescence was little bit higher than that of the second case (21nt). The actual reason behind this is not yet been elucidated.

Next we examined the effect of the single-base substitution in the complementary strand having longer chain length (20~21nt) at the opposite position of X in DN-1 to the fluorescent signal. The fluorescent spectrum of the mixture containing DN-1 and DN-3, in which an A residue at the middle region of the sequence of DN-2 was substituted with T-residue (Fig. 2). As it is clear from the spectrum, the increment of neither the monomer state signal nor the excimer-state signal was observed in the mixture. The same results were obtained in mixtures containing other mismatched oligomers (DN-4 and DN-5).

DISCUSSION

Substantial attempts have been made to develop oligonucleotide probes utilizing both monomer as well as excimer emission of pyrene for the last few years. Pyrene excimer fluorescence is less subject to quenching by donors and acceptors than monomer fluorescence. Therefore, contrary to pyrene monomer emission, an excimer forming probe would be a more efficient and selective probe for detecting certain fragments of nucleic acids (Lewis *et al*., 1997).

Modified oligonucleotide probes utilizing pyrene to exhibit excimer fluorescence upon hybridization to their complementary oligonucleotides have been reported by several research groups (Paris *et al*., 1998; Yamana *et al*., 2002; Langenegger and Haner, 2004). In all those cases, pyrene either in unmodified or modified form has been incorporated in DNA probe that exhibited excimer fluorescence in addition to monomer emission. Pyrene excimer formation has also been utilized for designing fluorescent probes for RNA structure investigation (Kostenko *et al*., 2001). In contrast to these instances, we have already reported several excimer forming novel oligoDNAs bearing more emissive silylated pyrene moieties having capability to effectively distinguish between a full-match and a mismatch DNA target (Mogi *et al*., 2010; Uddin *et al*., 2012; Uddin and Azam, 2012).

In the present study, the silylated pyrene derivatives have been introduced at 2'-position of uridine. Fluorescence spectra of the modified oligoDNA (DN-1) and its duplexes with full match (DN-2S and DN-2L) and point mutated complementary sequences (DN-3, DN-4 and DN-5) have been displayed in Fig. 2. The figure shows that the excimer fluorescence of silylated pyrene molecules in DN-1 is effectively quenched while the probe stays in single stranded form. It is worthy to mention here that excimer formation results from aromatic pie-stacking between two adjacent pyrene molecules.

It is presumed that before hybridization to the complementary nucleotide, the consecutive pyrenes cannot attain proper position for sufficient pie-stacking. As a result, no prominent excimer is formed in this case. Conversely, once the probe binds to the fully matched target, by dint of appropriate positioning of nearby pyrenes, characteristic excimer fluorescence signals were observed at around 485 nm in both the cases (DN-2S and DN-2L) despite the intensity was little bit decreased in the second case (DN-2L). In addition to the excimer signal, there was monomer fluorescence signal of pyrene at approximately 370 nm in all the cases (Fig. 2).

It is interesting that whatever is the intensity difference, excimer signal is distinctive in both the cases. So, the effect of chain length of the complementary strands on the intensity of excimer fluorescence is not so significant.

CONCLUSION

The modified oligoDNA can effectively discriminate a single-nucleotide substitution in the complementary DNA strand through its excimer fluorescent signal. It would be a practically feasible probe to detect certain gene fragments and the effect of chain length of the complementary sequences on the excimer fluorescence intensity is insignificant.

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