American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 3 (1): 175-181, 2013
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2013.175.181

A Novel Oligo-DNA Probe Carrying Non-nucleosidic Silylated Pyrene Derivatives: Synthesis and Excimer Forming Ability

[1,2]Md. Gias Uddin and [2]A.T.M. Zafrul Azam

[1]Department of Chemistry and Chemical Biology, Graduate School of Engineering, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515, Japan

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Corresponding Author: Dr. Md. Gias Uddin, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

ABSTRACT

Sequence-selective DNA detection is an important tool for monitoring many biological processes and for other biotechnological applications. In this way, molecular beacons (MBs) are highly selective in detecting oligonucleotides that have got widespread applications in nucleic acids chemistry. Excimer formation is a fundamental property of pyrene that can be used for designing unique class of DNA probe such as molecular beacons. To develop a new DNA detection probe that gives specific excimer fluorescent signal before hybridization to the target, a novel 29mer stem-loop structured (molecular beacon) DNA was synthesized. Then two non-nucleosidic silylated pyrene units have been incorporated into this novel oligo-DNA by standard phosphoramidite method using automated DNA synthesizer. Molecular beacon probe with silylated pyrene has already been reported by our group where pyrene was used as a part of nucleobase. In the present case, pyrenes are incorporated as non-nucleobase that have successfully displayed excimer fluorescence signal. Interestingly, the resulting modified DNA exhibited strong excimer fluorescent signal both before and after hybridization to the fully matched complementary target. Careful selection of the nucleobases in the stem segment could result in a more efficient sequence discriminating oligo-DNA probe.

Key words: Molecular beacon, non-nucleosidic silylated pyrene, oligo-DNA, excimer, fluorescence

INTRODUCTION

Single Nucleotide Polymorphisms (SNPs), common genetic variations in the human genome, 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). At present, there are about 13 thousand human diseases which are identified as genetic disorder and the number is increasing day by day. It is estimated that approximately one in 100 new born babies is likely to have a genetic problem (Afzal *et al*., 2008). With the introduction of relatively inexpensive fluorescent labeling agents, oligonucleotides tagged with fluorescent labels have now widely been introduced for the detection of nucleic acids. Since pyrene has some inherent chemical and photo-physical properties such as long life-time

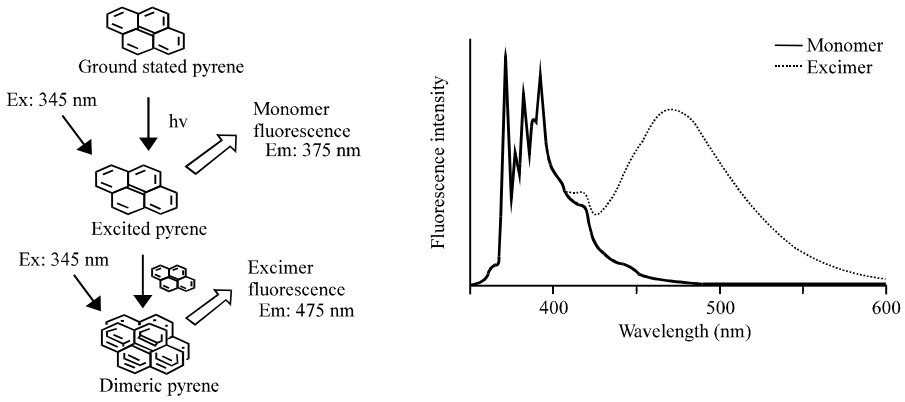


Fig. 1: Excimer formation by pyrene, Ex: Excitation, Em: Emission

of excited state, high fluorescence quantum yield, propensity to pie-stacking, sensitivity towards micro environmental changes, long wave emission (Winnik, 1993; Mahara *et al.*, 2002; Astakhova *et al.*, 2008). Therefore, pyrene became an attractive fluorophore for nucleic acid labeling for the last few decades. Excimer formation is a fundamental property of pyrene that can be used for designing a unique class of DNA probe such as molecular beacons. When two pyrene molecules exist in close proximity, they form excimer that fluoresces prominently at a longer wavelength compared to monomer emission. Excimer is a bimolecular complex where one molecule exists in an excited state and the other molecule is in a ground state. Monomer emission of pyrene occurs within the range of 370-390 nm whereas, the excimer emission is obtained within the wavelength limit of 465-500 nm. A typical monomer and excimer emission spectra of pyrene (Mahara *et al.*, 2002; Wilson and Kool, 2006) has been shown schematically in Fig. 1.

Several modified oligonucleotide probes utilizing pyrene to exhibit excimer fluorescence upon hybridization to their complementary oligonucleotides have been reported (Hrdlicka *et al.*, 2005; Astakhova *et al.*, 2008). On the other hand, silylated pyrene derivative, capable to incorporate into biological molecules such as nucleic acids and lipids, is recently developed (Sekiguchi *et al.*, 2007; Moriguchi *et al.*, 2009). The compound exhibits enhanced fluorescent quantum yield along with a bathochromic shift in absorption and emission, due to the specific sigma-pie interaction (Kyushin *et al.*, 1996; Maeda *et al.*, 2001). Thus, the compound is more advantageous as a fluorescent labeling agent compared to original pyrene. We have recently reported two novel oligo-DNAs bearing silylated pyrene that utilizes the excimer forming capability of pyrene to effectively distinguish between a full-match and a mismatch DNA target (Mogi *et al.*, 2010; Uddin *et al.*, 2012). Herein, the study has reported a new strategy for designing MBs by using the unique property of modified oligo-DNA possessing silylated pyrene as non-nucleosidic unit.

MATERIALS AND METHODS

Reagents: Pyrene, N-bromosuccinimide, *n*-butyl lithium, chloromethyl dimethyl chlorosilane, 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), Fluorescence spectrophotometer (Hitachi F-4010).

Oligo-DNA synthesis: The synthesis of modified oligo-DNA (GK2097, Scheme 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 oligo-DNA was treated with concentrated ammonium hydroxide (60°C, 12 h) followed by reversed-phase HPLC, ethanol precipitation and gel-filtration (Sephadex G-25) (Uddin *et al*., 2012).

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

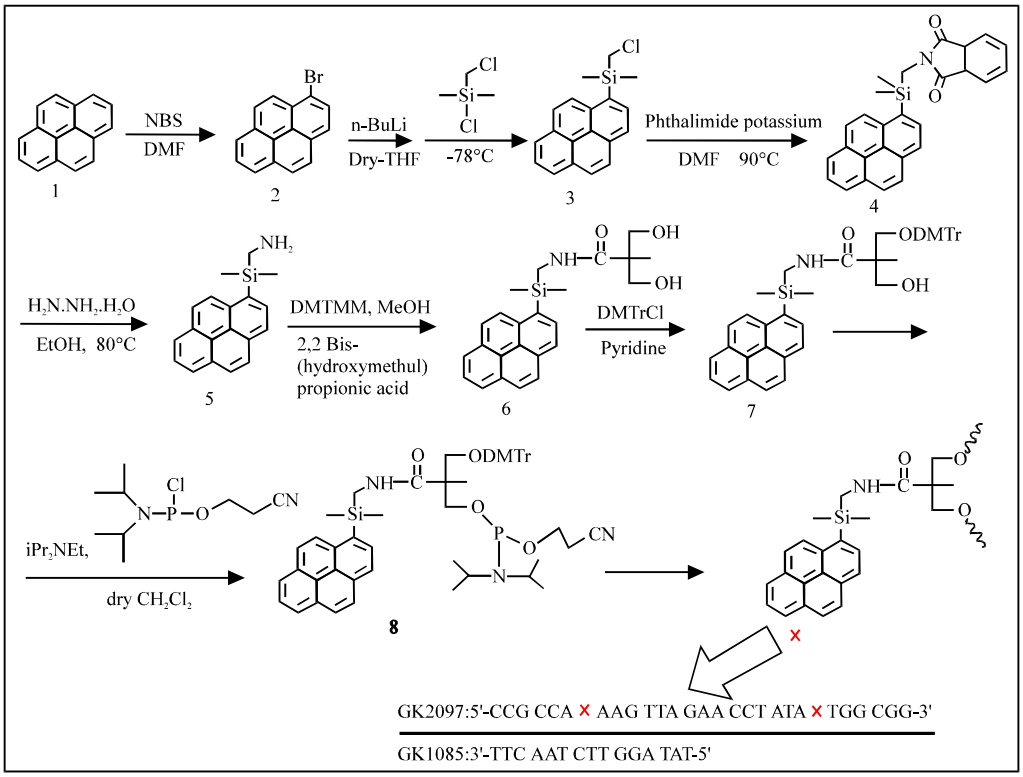
Nuclear magnetic resonance (NMR) data: ^{1}H NMR data were recorded on JEOL 300 MHz and ^{31}P NMR was done JEOL 500 MHz NMR spectrophotometer using $CDCl_3$ using standard protocol.

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

Synthesis of oligo-DNA bearing silylated pyrene is summarized in Scheme 1. In brief, the key starting material 1-bromopyrene (2) was prepared from pyrene by stirring it (1) with n-bromosuccinimide (NBS) in Dimethyl Formamide (DMF) at room temperature for 1 h. The resulting compound (2) was allowed to react with n-BuLi in dry tetrahydrofuran (THF) followed by the addition of chloromethyl dimethylsilyl chloride at -78°C for about 2 h. The compound thus obtained (3) was then converted to corresponding phthalimide derivative (4) by reacting with phthalimide potassium in DMF at temperature near about 90°C. The reaction of compound 4 with hydrazine hydrate in ethanol at 80°C afforded the compound aminomethyl dimethyl 1-silyl chloride (5) which was then condensed with 2,2 bis-(hydroxymethyl) propionic acid by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl) 4-methylmorpholinium chloride (DMT-MM) in MeOH to afford the amide linked propionic acid derivative of silylated pyrene (6). One hydroxyl function of the resulting compound was selectively protected by DMTrCl to obtain compound 7 which was then converted to corresponding phosphoramidite derivative (8) by standard phosphoramidite method. Its structure was confirmed by ^{1}H-NMR and ^{31}P-NMR (146 ppm in $CDCl_3$). The incorporation of compound 8 to the two consecutive positions in oligo-DNA molecule was accomplished using an automated DNA synthesizer (ABI 392). It would be worth to note that prolonged coupling period (360 sec) was required to achieve satisfactory coupling yield (ca. 90%). After the assembly, the support-bound fluorescent oligo-DNA(GK-2097) was treated with conc. ammonium hydroxide (60°C, 12 h) followed by reversed-phase HPLC, acid treatment to remove DMTr group and gel-filtration as usual. The isolated yield of the modified oligo-DNA (GK2097) was 13.6%. ESI-MS of the oligomer was found to be 9227.5 (calculated 9228.4).



Scheme 1: Synthesis of oligo-DNA (GK2097) bearing silylated pyrene

Fluorescent properties of the modified oligo-DNA GK2097 (1.5 μM) bearing non-nucleosidic silylated pyrene were examined in both before and after hybridization with fully matched complementary target sequence (GK1085) in buffer solution (10 mM sodium phosphate containing 100 mM NaCl, pH 7.2 at room temperature) using excitation wavelength of 314 nm. It was found that the modified oligomer (GK 2097) itself as stem-loop structure exhibited excimer fluorescence signal because of two pyrene units in the opposite stem (Fig. 2). But after hybridization to the fully matched complimentary target, the excimer signal was not disappeared completely (Fig. 2).

DISCUSSION

As it is mentioned earlier that we have already reported two novel oligo-DNAs bearing silylated pyrene that utilizes the excimer forming capability of pyrene to effectively distinguish between a full-match and a mismatch DNA target (Mogi *et al*., 2010; Uddin *et al*., 2012). In those cases, pyrene units were used as part of respective nucleobases. In the present case the modified oligomer (GK2097) is a stem-loop structure DNA bearing two silylated pyrene units as non-nucleobase at the opposite complementary stems. Before hybridization to the target strand which is complementary to the loop segment, the probe is presumed to give excimer emission of pyrene because of adjacent positions of pyrenes. Upon hybridization to the target strand, the stem-loop structure is expected to resolve into linear form. And accordingly the pyrene units would go apart from each other and give monomer emission. The working principle of the molecular beacon DNA has been displayed in Fig. 3 which has been proposed on the basis of the fundamental properties of molecular beacon probe (Tyagi and Kramer, 1996; Tyagi *et al*., 1998).

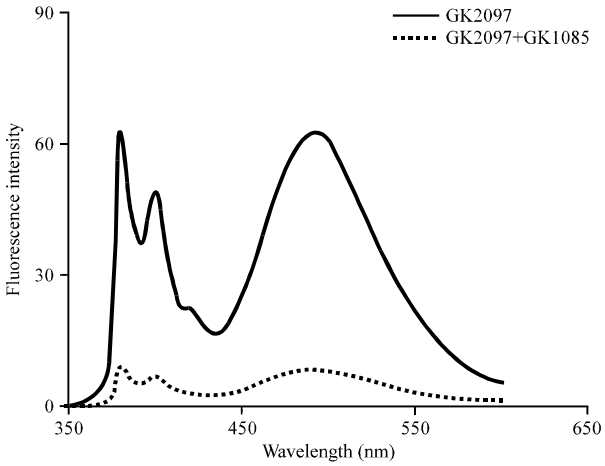


Fig. 2: Fluorescence spectra of modified oligomer GK2097 and its duplex with full match target (GK1085)

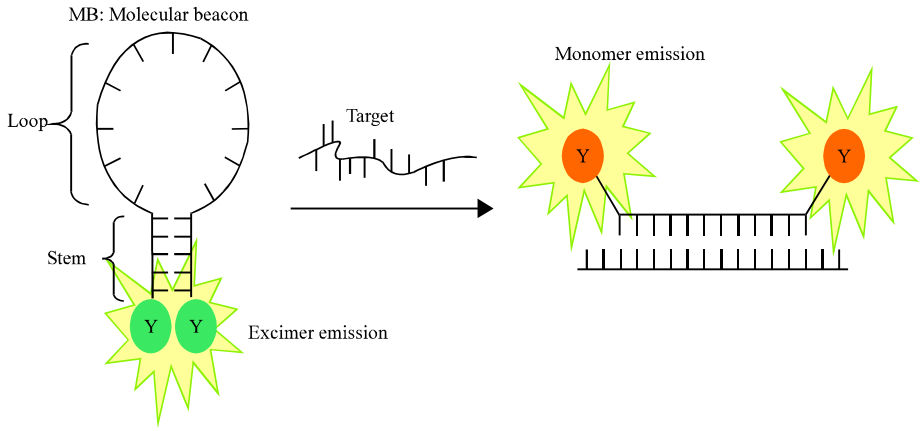


Fig. 3: Working principle of molecular beacon DNA

As it is shown in Fig. 2, the modified oligomer (GK2097) gave a characteristic excimer fluorescence signal at around 480 nm. The excimer signal is presumed to be attributed to the aromatic pie stacking between the Si-pyrene moieties in the opposite strands before hybridization. The probe (GK2097) also gave a monomer fluorescence signal of pyrene at 370 nm. It was anticipated that upon hybridization to the complementary target (GK1085), the native stem-loop structure would resolve completely into a linear form giving rise to a distinctive monomer signal.

However, in the presence of the full-matched complementary sequence (GK1085), the excimer signal was not disappeared completely and the monomer emission was also very faint. At this moment, the exact reason behind this has not been elucidated. It is speculated that this is probably due to the strongly hydrogen-bonded framework by G-C pairs in the stem portion.

CONCLUSION

Two non-nucleosidic silylated pyrene moieties were successfully incorporated into a stem-loop structured oligo-DNA probe. The oligomer showed strong excimer fluorescence signal both

before and after binding to complementary target. Proper selection of sequences in the stem portion could result in a more efficient excimer forming oligomer.

REFERENCES

Abbasi, S., P. Ismail., F. Othman., R. Rosli and C. Azimi, 2009. Estrogen receptor-α gene, codon 594 (G3242A) polymorphism among Iranian women with breast cancer: A case control study. Asian J. Sci. Res., 2: 51-60.

Afzal, M., Y.H. Siddique, T. Beg, G. Ara and J. Gupta, 2008. Genetics and public health in post-genomic era. Trends Med. Res., 3: 41-63.

Astakhova, I.V., V.A. Korshun and J. Wengel, 2008. Highly fluorescent conjugated pyrenes in nucleic acid probes: (Phenylethynyl) pyrenecarbonyl-functionalized locked nucleic acids. Chemistry, 14: 11010-11026.

Furukawa, K., M. Hattori, T. Ohki, Y. Kitamura, Y. Kitade and Y. Ueno, 2012. Nucleic acid probe containing fluorescent tricyclic base-linked acyclonucleoside for detection of single nucleotide polymorphisms. Bioorg. Med. Chem., 20: 16-24.

Hrdlicka, P.J., B.R. Babu, M.D. Sorensen, N. Harrit and J. Wengel, 2005. Multilabeled pyrene-functionalized 2;-amino-LNA probes for nucleic acid detection in homogenous fluorescence assays. J. Am. Chem. Soc., 127: 13293-13299.

Javed, R. and Mukesh, 2010. Current research status, databases and application of single nucleotide polymorphism. Pak. J. Biol. Sci., 13: 657-663.

Kyushin, S., M. Ikarugi, M. Goto, H. Hiratsuka and H. Matsumoto, 1996. Synthesis and electronic properties of 9,10-disilylanthracenes. Organometallics, 15: 1067-1070.

Lajin, B. and A. Alachkar, 2011. Detection of Arg72Pro polymorphism of the tumor suppressor gene (TP53) by a rapid one-step tetra-primer amplification refractory mutation system-PCR. Am. J. Biochem. Mol. Biol., 1: 231-236.

Maeda, H., Y. Inoue, H. Ishida and K. Mizuno, 2001. UV absorption and fluorescence properties of pyrene derivatives having trimethylsilyl, trimethylgermyl and trimethylstannyl groups. Chem. Lett., 12: 1224-1225.

Mahara A., R. Iwase, T. Sakamoto, K. Yamana, T. Yamaoka and A. Murakami, 2002. Bispyrene-conjugated 2-O-methyloligonucleotide as a highly specific RNA-recognition probe. Angewandte Chem., 114: 3800-3802.

Mogi, M., M.G. Uddin, M. Ichimura, T. Moriguchi and K. Shinozuka, 2010. Modified OligoDNA having two consecutive silylated-pyrene moieties in minor groove exhibiting an excimer fluorescent signal upon binding to fully complementary DNA strand. Chem. Lett., 39: 1254-1255.

Moriguchi, T., M. Hosaka, A. Yoshinari, A. Ozaki, T. Takeuchi and K. Shinozuka, 2009. Cholesterol analogs labeled with novel silylated fluorescent compounds. Chem. Lett., 38: 966-967.

Sekiguchi, T., Y. Ebara, T. Moriguchi and K. Shinozuka, 2007. Novel sequence-responding fluorescent oligo DNA probe bearing a silylated pyrene molecule. Bioorg. Med. Chem. Lett., 17: 6883-6886.

Suman, G. and K. Jamil, 2006. Novel *CYP3A4* gene polymorphisms in post chemo breast cancer patients. Int. J. Cancer Res., 2: 358-366.

Tyagi, S. and F.R. Kramer, 1996. Molecular beacons: Probes that fluoresce upon hybridization. Nat. Biotechnol., 14: 303-308.

Tyagi, S., D. Bratu and F.R. Kramer, 1998. Multicolor molecular beacons for allele discrimination. Nat. Biotechnol., 16: 49-53.

Uddin, M.G., T. Moriguchi, M. Ichimura and K. Shinozuka, 2012. Synthesis and properties of molecular beacon DNA probe bearing novel silylated pyrene derivative. Key Eng. Materials, 497: 47-50.

Wilson, J.N. and E.T. Kool, 2006. Fluorescent DNA base replacements: Reporters and sensors for biological systems. Org. Biomol. Chem., 4: 4265-4274.

Winnik, F.M., 1993. Photophysics of preassociated pyrenes in aqueous polymer solutions and in other organized media. Chem. Rev., 93: 587-614.