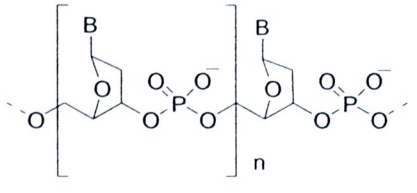
CHAPTER I

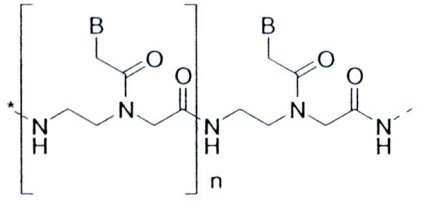
INTRODUCTION

Rationale for the study

The original Peptide Nucleic Acid (PNA) by Nielsen in 1991 [1] is DNA mimics in which the ribose-phosphate backbone is replaced by a *N*-2-aminoethylglycine unit backbone with the nucleobases attached through a methylenecarbonyl group at the glycine nitrogen so called *aeg*PNA (Figure 1). PNAs are chemically and biologically stable molecular that can mimic the most important feature of DNA. It can show the behavior of DNA and the ability to hybridize with DNA target through Watson-Crick base pairing rule. Furthermore, PNA are not substrates for common enzymes such as nucleases and proteases. The neutral backbone of PNA results in stronger binding and greater specificity than would normally achieved with DNA. The stronger binding properties and biological stability of PNA can be used to develop in a number of biotechnological and medical applications including device for genetic sequence determination [2].



DNA



PNA

Figure 1 Chemical structure of a DNA molecule and an *aeg*PNA molecule

PNA can be adapted to design in therapeutics particularly as antisense and antigene drugs. PNA can be designed to recognize and hybridize to the complementary sequences in a particular gene of interest whereby they should interfere with the transcription of the gene (antigene strategy). Alternatively, PNA can also be designed to recognize and hybridize to complementary sequences in mRNA and thereby inhibit its translation (antisense strategy) as show in Figure 2 [3]. The basic mechanism of antisense and antigene perform by oligonucleotide is rapidly degraded by nucleases.

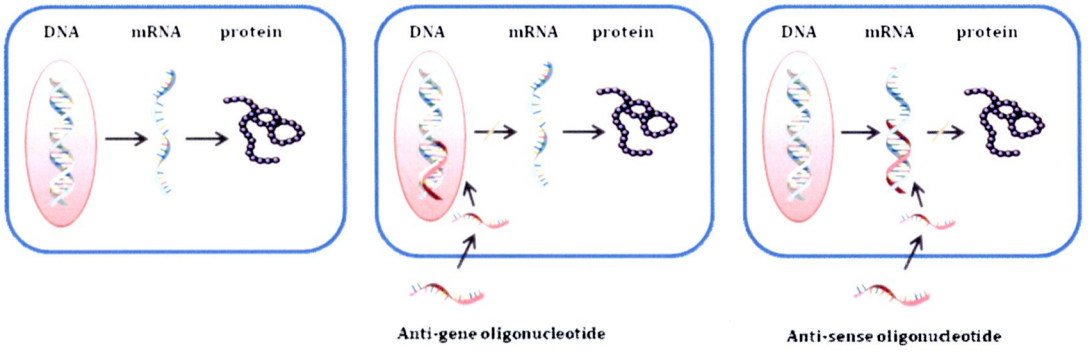
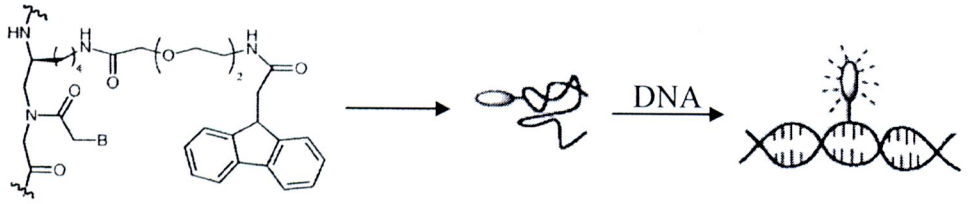


Figure 2 Schematic representation of the antisense inhibition and antigene inhibition. An antigene oligomer (e.g. PNA) could bind to complementary sequence in the DNA and inhibit transcription of the gene. On the other hand, cells can also be treated with an antisense oligonucleotide, and hybridization to a specific mRNA sequence can inhibit the expression of a protein at the level of translation [3].

Early interest in PNA came from the field of antisense research. Although this course has experienced significant progress, the bulk of the interest in PNA in the last few years has come from the use of PNA as a molecular hybridization probe. PNAs attach with a labeled probe is the practical way to detect a complementary target sequence in a complex nucleic acid mixture. PNAs may be used in many of the same applications as traditional synthetic DNA or DNA analogs, but with the added benefits of tighter binding that especially at low ionic strength and greater specificity. A large number of PNA research as a probe to detect DNA base sequence has been

demonstrated use of Nielsen's PNA in conjunction with different characterizing techniques such as optical, electrochemical or mass-sensitive transducer.

Recently, Appella and co-worker [4] reported the synthesis of the single-fluorophore-labelled PNA to be the probes for nucleic acid sequence detection. The probe exhibited a fluorescent signal only in the presence of the target oligonucleotide due to the separation of the fluorophore and the nucleobase which acted as quencher and to cross a restricted to fluorophore attachment to the ends of the PNA. PNA oligomers wherein fluorophores can be attached to the PNA backbone from novel γ-lysine PNA monomers. Oligomers incorporating the modified PNA showed comparable thermal stability to the corresponding *aeg*PNA oligomer with DNA. When the modified PNA oligomer was annealed with complementary DNA, the fluorescence intensity increased 4-fold over the unbound PNA.

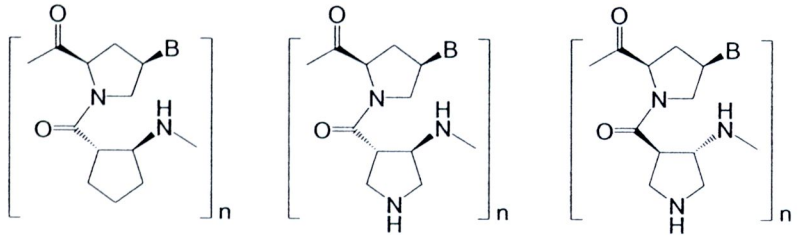


γ-Lysine PNA/Fluorene Fluorescent Probe

Figure 3 Structure and fluorescence of single-fluorophore-labelled PNA is observed upon duplex formation [4]

Any other interesting PNA system beside *aeg*PNA is the novel pyrrolidinyl PNA carrying β-amino acid spacers that developed by Vilaivan and coworker in 2005 (Figure 3) [5]. The conformationally rigid of PNA base on a pyrrolidine backbone bearing a (1*S*,2*S*)-2-aminocyclopentane carboxylic acid so called *acpc*PNA. This system showed very excellent binding properties to DNA compare to *aeg*PNA with strong affinity and high sequence specificity with its complementary DNA target and only form 1:1 hybrid. The melting tempareture (T_m) value of hybrid between the PNA and its complementary DNA is far more stable than natural DNA·DNA and other PNA·DNA hybrids [6]. This property is useful for application of detecting DNA sequence or gene therapies.

In this research, we were designed and synthesized a pyrrolidinyl PNA bearing a 3-aminopyrrolidine-4-carboxylic acid (APC) spacer so called *apc*PNA that have two configuration as *trans*-(3*R*,4*S*)-isomer and (3*S*,4*R*)-isomer. These spacers were designed based on *acpc*PNA system bearing (1*S*,2*S*)-2-aminocyclopentanecarboxylic acid spacer, with additional nitrogen atom in the cyclopentane ring of the *β*-amino acid. It is expected that the new spacer will improve the solubility of the PNA and provide a handle for internal modification by attach a fluorophore to addition nitrogen atom *via* amide linkage according to require positive.



(1*S*,2*S*)-*acpc*PNA (3*R*,4*S*)-*apc*PNA (3*S*,4*R*)-*apc*PNA

Figure 4 Chemical structures of pyrrolidinyl PNA molecules

Objectives of the study

1. To design and synthesis of novel pyrrolidine peptide nucleic acid carrying 3-aminopyrrolidine-4-carboxylic acid spacer.

2. To study nucleic acid binding properties of novel peptide nucleic acid *apc*PNA with oligonucleotide.

Scope of this Research

1. Synthesis of *trans*-(3*R*,4*S*) and *trans*-(3*S*,4*R*)-3-aminopyrrolidine-4-carboxylic acid.

2. Synthesis of pyrrolidine PNA carrying 3-aminopyrrolidine-4-carboxylic acid by solid phase peptide synthesis as *apc*PNA carrying the whole 3-aminopyrrolidine-4-carboxylic acid spacer and *apc*PNA carrying a single APC unit in the middle of the strand of the *acpc*PNA.

3. Study the nucleic acid binding properties of synthesized pyrrolidine peptide nucleic acid carrying 3-aminopyrrolidine-4-carboxylic acid by T_m measurement, UV titration and circular dichroism spectroscopy.