Peptide Nucleic Acid (PNA)

Eurogentec is a member of the Licensed Providers of PNA.

Over the last few years, the use of Peptide Nucleic Acids as synthetic oligomers has proven their powerful usefulness in Molecular Biology Procedures, Diagnostic assays and Antisense therapeutics.

STRUCTURE OF PNAS

The backbone is made from repeating N-(2-aminoethyl)-glycine units linked by peptide bonds. The different bases (purines and pyrimidines) are linked to the backbone by methylene carbonyl linkages. Unlike DNA or other DNA analogs, PNAs do not contain any pentose sugar moieties or phosphate groups.

PNAs are depicted like peptides with the N-terminus at the first (left) position and the C-terminus at the right.

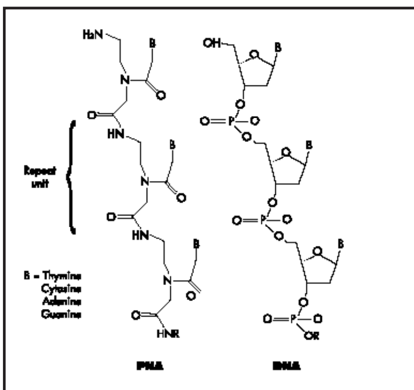

Figure 1: Comparison of PNA and DNA structures

BINDING PROPERTIES OF THE PNA STRANDS

The PNA backbone is not charged, this confers to this polymers a much more stronger binding between PNA/DNA strands than between PNA strands and DNA strands. This is due to the lack of charge repulsion between PNA and DNA strand.

Early experiments with homopyrimidine strands have shown that the Tm of a 6-mer PNA T/DNA dA was determined to be 31°C in comparison to a DNA dT/DNA dA 6-mer duplex that denatures at a temperature less than 10°C.

The formation of triple-stranded complex also confers a tighter binding. Homopyrimidine PNAs typically binds with a 2PNA-1DNA stoichiometry. The complex has been shown to be comprised of a PNA/DNA double helix (formed by Watson-Crick hydrogen bonds) with a second PNA strand lying in the major groove of the duplex (formed by Hoogsteen hydrogen bonds). The stability of these triple helix structures was found to be so high that strand invasion of DNA/DNA was possible. In general, a 10-mer homopyrimidine PNA binds to its target with Tm upper than 70°C.

The consequence is that 2 PNA molecules can displace a duplex DNA strand to form a triplex with the complementary fragment.

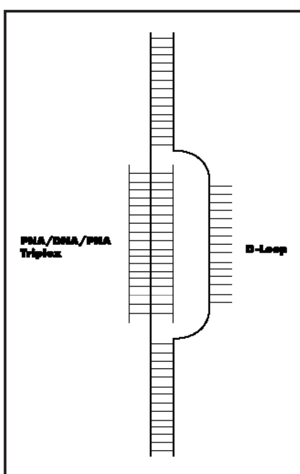

Figure 2: Strand displacement

The conditions that favor triplex formation are : many pyrimidines and low pH (4.5 to 6.5).

A triplex can also be formed even if there is a couple of purines in the sequence.

Experiments done with PNA sequences containing all four bases also showed that the Tm are higher for PNA hybrids than for either DNA/DNA or DNA/RNA, in general, there is an increase of the Tm of about 1°C per base pair in 100 mM NaCl (Figure 3).

In the case of mixed base sequences, the tighter binding of PNA/DNA is due to the neutrality of the PNA, only, and not to the formation of triplex structures. Thus, mixed base PNA are true mimics of DNA molecules in terms of base-pair recognition.

Due to this higher binding property, it is not necessary to design long PNA-oligomers, in comparison to the 20-25-mer which are the typical length of oligonucleotide probes.

The main concern of the length of the PNA-oligomers is to guarantee the specificity. A 10-mer PNA typically has a Tm of about 50°C and a 15-mer of about 70°C.

In addition to their higher binding properties, PNAs also show greater specificity in binding to complementary DNAs. A PNA/DNA mismatch is more destabilizing than a mismatch in a DNA/DNA duplex. A single mismatch in mixed PNA/DNA 15-mer decreases the Tm by 15°C, on average. In the corresponding DNA/DNA interactions, a single mismatch decreases the Tm by only 11°C, on average.

HYBRID	**Tm**
6-mer PNA T/DNA A	*31 °C*
6-mer DNA T/DNA A	*<10 °C*
10-mer PNA T/DNA A	*73 °C*
10-mer DNA T/DNA A	*23 °C*
15-mer mixed PNA/DNA	*69 °C*
15-mer mixed PNA/DNA	*54 °C*
15-mer mixed PNA/RNA	*72 °C*
15-mer mixed PNA/RNA	*50 °C*

Figure 3: The higher stability of PNA/DNA or PNA/RNA complexes is reflected in the higher Tm over the corresponding DNA/RNA or DNA/RNA duplexes.

The greater stability of PNA/DNA or PNA/RNA complexes is reflected in the higher Tm over the corresponding DNA/RNA or DNA/RNA duplexes.

The greater specificity is reflected in the larger decreases in Tm caused by a single mismatch in the base sequences.

RESISTANCE TO NUCLEASES AND PROTEASES

PNAs with their peptide backbone bearing purine and pyrimidine bases are not amolecular species easily recognized by nucleases or proteases. They are thus resistant to the enzyme degradation.

PNAs are also stable over a wide pH range.

These features confer to the PNA a good position among the candidates for Antisense Research.

Since they are not easily degraded by enzymes, the lifetime of these polymers is extended both in vitro and in vivo.

In addition to that, the fact that they are not charged should facilitate their crossing through the cell membranes and their stronger binding properties should decrease the amount of antisense needed for the inhibition of gene expression.

APPLICATIONS

PNAs can be used in the same applications than traditional synthetic DNA or RNA, but with the added benefits of tighter binding and greater specificity.

For instance, PNAs labeled with biotin, digoxigenin, fluorescent dyes or reporter enzymes are powerful probes in hybridization experiments (DNA array, Northern or Southern blot, FISH, detection of single point mutations, DNA mapping).

HOW TO USE YOUR PNA OLIGOMERE?

To dissolve it

Resuspend your PNA in 200-1000 µl of 0.1 % aqueous trifluoroacetic acid (TFA) and fractionnate the solution into aliquots. Stock your aliquots frozen.

Some particular sequences are hard to dissolve, in this case, add 10-20 % of acetonitrile to the TFA solution and heat the sample to 50°C.

It has been reported that PNAs have an affinity for glass surfaces, so, if you work with sub-micro molar concentrations of PNA, the majority of the PNA may be bound to the glass.

So, if possible, use polypropylene or polyethylene materials during handling and storage of PNA.

Ionic Strength and Buffers

Due to the fact that PNAs are not charged, no salt is necessary to favor and to stabilize the formation of duplex PNA/DNA or PNA/RNA, in comparison of DNA/DNA or DNA/RNA duplex. This means that the Tm of PNA/DNA duplex is almost independent of ionic strength.

It has been reported that the Tm of a 15-mer duplex decreased by only 5°C as the NaCl concentration was raised from 10 mM to 1 M.

The consequence is that a PNA, at low ionic strength (10 mM phosphate buffer, without NaCl), will effectively bind to its target in the presence of competing DNA strand because the stability of the DNA/DNA duplex is small in these conditions.

It is important to keep in mind that the hybridization of PNA must always be done in the presence of a buffer, because PNA/DNA duplexes precipitate at low pH or in absence of counterions.

When using RNA, the low ionic strength permits to destabilize intramolecular hybridization favoring the hybridization with the PNA.

It is strongly recommended to avoid self-complementary, hairpin and palindromic sequences in the design of PNAs since PNA/PNA interactions are stronger than PNA/DNA interactions.

Strand displacement

For experiment of strand displacement, the PNA-oligomer should be parallel to the targeted segment and the pH should be low (5 to 5.5). The ionic strength must be as low as possible due to the buffer, only, without any divalent metal ions. The low salt concentration is only necessary to permit the formation of the complex by favoring the strand displacement, once it is formed, the salt concentration can be increased to carry out enzymatic reactions, for instance.

However, it is necessary to use a buffer to perform the experiment of strand displacement since PNA/DNA complexes precipitate at low pH or when no counter ions are present.

Aminic acid residue	*OL-PNAA-A*
Biotin labelling	*OL-PNAB-IOT*
DNA inside chimmeric PNA/DNA	*OL-PNAD-NA*
Fluorescein labelling	*OL-PNAF-LUO*
FPLC purification	*OL-PNAF-PLC*
HPLC for PNA	*OL-PNAH-PLC*
C6 carboxylate linker synthesis	*OL-PNAL-INKC6*
MMTC6 aminolink synthesis	*OL-PNAM-MTC6*
Base	*OL-PNAP-NA*
Spacer between PNA and Amino Acid	*OL-PNAS-PAC*