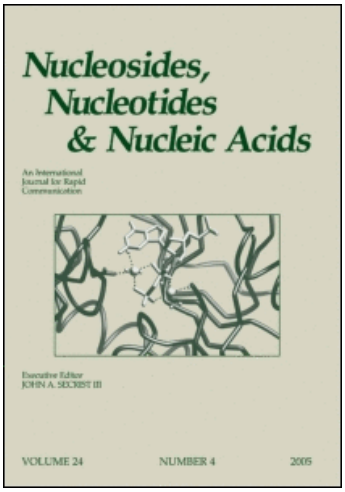


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Recognition of Double-Stranded Dna by Peptide Nucleic Acid

Pernilla Wittung[a]; Peter Nielsen[b]; Bengt Nordén[b]
[a] Center for Biomolecular Recognition, Department of Biochemistry B, The Panum Institute, University of Copenhagen, Copenhagen N, Denmark. [b] Department of Physical Chemistry, Chalmers University of Technology, Gothenburg, Sweden

To cite this Article Wittung, Pernilla , Nielsen, Peter and Nordén, Bengt(1997) 'Recognition of Double-Stranded Dna by Peptide Nucleic Acid', Nucleosides, Nucleotides and Nucleic Acids, 16: 5, 599 — 602
To link to this Article: DOI: 10.1080/07328319708002922
URL: http://dx.doi.org/10.1080/07328319708002922

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 599-602 (1997)

RECOGNITION OF DOUBLE-STRANDED DNA BY PEPTIDE NUCLEIC ACID

Pernilla Wittung[a]*, Peter Nielsen[b] and Bengt Nordén[a]

[a] *Department of Physical Chemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden*
[b] *Center for Biomolecular Recognition, Department of Biochemistry B, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen N, Denmark.*

ABSTACT: Peptide nucleic acid (PNA) is an oligonucleotide mimic in which the backbone of DNA has been replaced by a pseudopeptide. We here show that there are distinct variations as to how PNA oligomers interact with double-stranded DNA depending on choice of nucleobases. Thymine-rich homopyrimidine PNA oligomers recognise double-stranded polynucleotides by forming PNA_2-DNA triplexes with the DNA purine strand. By contrast, cytosine-rich homopyrimidine PNAs add to double-stranded polynucleotides as Hoogsteen strands, forming PNA-DNA_2 triplexes, while homopurine, or alternating thymine-guanine, PNA oligomers invade DNA to form PNA-DNA duplexes.

Reagents that can bind sequence-selectively to double-stranded DNA can be useful for artificial gene regulation as well as for mapping and isolating fragments of genomic DNA. Single-stranded oligonucleotides and their close analogs, which bind to double-stranded DNA by triplex-formation, are the main candidates for such reagents[1,2]. So far only purine stretches of dsDNA may be targeted this way by oligonucleotides[3]. Oligonucleotides with cytosines and thymines bind to DNA by Hoogsteen base-pairing forming T-AxT and C-GxC$^+$ triplets, whereas purine oligonucleotides can form reverse Hoogsteen triplets, T-AxA and C-GxG. Finally, oligonucleotides containing guanines and thymines may form T-AxT and C-GxG triplets with DNA.

PNA is an oligonucleotide mimic in which the entire deoxyribose phosphate backbone of DNA has been replaced by a peptide-like backbone[4]. Thymine-rich homopyrimidine PNA oligomers have been found to recognize double-stranded DNA targets by a mechanism in which two PNAs bind to the complementary DNA strand to form a PNA_2-DNA triplex, the non-complementary DNA strand being displaced into a loop[5]. This type of PNA binding is inhibited by salt, thereby limiting the use of PNA as a DNA-directed drug in living cells.

Therefore, for future medicinal applications it is essential to understand the mechanism of such PNA-DNA interactions, and factors that control them. It is also interesting to address the possibility of targeting other DNA sequences with PNA, to extend the recognition code of double-stranded DNA.

By using circular dichroism spectroscopy, which reflects helical base stacking, we have followed the kinetics of the invasion of homopyrimidine PNA into double-stranded DNA[6]. As a model system we used double-stranded polynucleotide poly(dA):poly(dT) and thymine-PNA decamers. From the temperature dependence of the PNA binding rate we could estimate an activation energy of about 60 kJ/mole. This value is similar to activation energies found for opening of a few base-pairs in DNA[7], suggesting DNA opening to be the rate-limiting step of the reaction. Introducing a mismatch in the PNA drastically reduced the PNA binding rate and also increased the activation energy[6]. Investigating the PNA binding rates for various PNA concentrations showed an approximately quadratic dependence on the PNA concentration. This result indicates that the two PNA molecules are both involved in the activation step. One may speculate about a precursory complex with the PNA strands associated loosely to the intact DNA before opening of the DNA. We found groove-binding DNA ligands, prebound to poly(dA):poly(dT), to decrease the rate of PNA invasion, whereas intercalating ligands enhanced the rate of PNA binding[6]. Covalently attached intercalators might thus be a way to enable PNA to perform invasion also at higher salt concentrations.

To elucidate the sequence dependence for PNA binding to dsDNA we studied the interactions of different PNA oligomers (see Table 1 for sequences), with their respective double-stranded target polynucleotides[8]. In addition to circular dichroism to monitor the formation of PNA-DNA complexes, we also used linear dichroism which reflects the orientation of the nucleobases.

Both CD and LD titrations of the PNAs with their matching double-stranded DNAs showed that 1:1 (PNA bases to DNA base-pairs) complexes were formed (Table 1, column 3). The CD spectra for the complexes formed between the homo-pyrimidinic PNA-$(TC)_5$ and PNA-C_{10} and their respective target dsDNA molecules were similar to that of poly(dC)-poly(dG)-poly(dC^+) triplexes. The CD spectrum of the resulting complex between the PNA-A_{10} oligomer and poly(dA):poly(dT) instead resembles that of a PNA-DNA duplex[9]. The CD kinetics of PNA binding to these polynucleotides, upon varying the PNA concentration, showed the reaction rates to depend approximately linearly on the PNA concentrations in all cases.

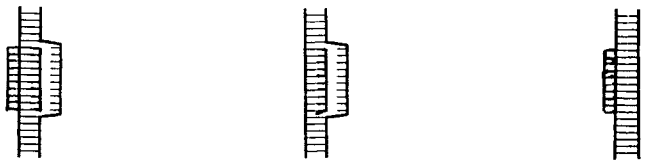
Table 1. Experimental findings for binding of various PNAs to corresponding double-stranded DNA based on circular dichroism and (last column) linear dichroism measurements.

Reaction:	E_a (kJ/mole)	Binding stoichiometry	NaCl dependence	Change in negative LD
poly(dG):poly(dC) + PNA-C_{10}	not detectable	1	intermediate	increase
poly(dAdG):poly(dTdC) + PNA-$(TC)_5$	13 (50mM NaCl)	1	intermediate	increase
poly(dA):poly(dT) + PNA-A_{10}	90 (25mM NaCl)	1	strong	decrease
poly(dAdG):poly(dTdC) + PNA-$(AG)_5$	84 (50mM NaCl)	1	strong	decrease
poly(dAdC):poly(dTdG) + PNA-$(TG)_5$	74 (25mM NaCl)	1	strong	decrease
poly(dA):poly(dT) + PNA-T_{10}	60 (50mM NaCl)	2	intermediate	increase

The activation energies for the PNA binding reactions were found to be large for the homopurine PNAs, PNA-A_{10} and PNA-$(AG)_5$, and also for the alternating PNA-$(TG)_5$ (Table 1, column 2), and similar in size to the activation energy determined for binding of PNA-T_{10} to poly(dA):poly(dT)[6] suggesting base-pair opening to be involved also here. By contrast, we observed almost no temperature-dependence for binding of the cytosine-rich homopyrimidine oligomers PNA-C_{10} and PNA-$(TC)_5$ (Table 1, column 2), indicating a binding mode for these PNAs that does not involve base-pair opening.

Furthermore, the binding rates of PNA-C_{10} and PNA-$(TC)_5$ are only slightly reduced upon increasing the concentration of NaCl. By contrast, very strong ionic-strength dependencies are observed for binding of the purine oligomers (PNA-A_{10} and PNA-$(AG)_5$), as well as PNA-$(TG)_5$, and at moderate NaCl concentrations the reactions are more or less inhibited (Table 1, column 4).

The negative LD signal around 260nm increased approximately linearly with amount of added PNA upon titrating the respective DNAs with PNA-C_{10} or PNA-$(TC)_5$, in agreement with a direct association of the PNA to the intact DNA duplex, resulting in an increased stiffness of the complex. By contrast, a gradual decrease of the negative LD signal of DNA was observed upon addition of increasing amounts of homopurinic PNAs, as well as the alternating thymine-guanine PNA, to their matching DNAs. This may be explained by a resulting PNA-DNA duplex, containing a polymeric DNA strand covered with numerous short PNA oligomers, which would be more flexible than the original DNA duplex.



Invasion triplex *Invasion duplex* *Hoogsteen triplex*

Figure 1. Observed PNA-binding modes to double-stranded polynucleotides.

From these experiments we can conclude that cytosine-rich PNA oligomers add directly to double-stranded target polynucleotides as Hoogsteen strands, forming PNA-DNA_2 triplexes. On the other hand, purine-rich PNAs, homopurine as well as thymine-guanine PNA sequences, invade their corrsponding Watson-Crick complementary DNA targets, by displacing the identical DNA strands, to form new PNA-DNA duplexes. Our experiments thus demonstrate an extended repertoire of PNA recognition of double-stranded DNA, in addition to the invasion reaction observed by thymine-rich PNAs, which could be of importance for future design of gene-specific reagents.

REFERENCES

1. Dervan, P.B. *Nature* **1992**, 359, 87-88.
2. Hélène, C. *Curr. Opin. Biotechnol.* **1993**, 4, 29-36.
3. Sun, J. and Hélène, C. *Curr. Opin. Struct. Biol.* **1993**, 3, 345-356.
4. Nielsen, P.E., Egholm, M., Berg, R.H. and Buchardt, O. *Science* **1991,** *254*, 1497- 1500.
5. Demidov, V.V., Yanilovich, M.V., Belotserkovskii, B.P., Frank-Kamenetskii, M.D. and Nielsen, P.E. *Proc. Nat. Acad. Sci. USA* **1995,** *92*, 2637-2641.
6. Wittung, P., Nielsen, P.E., Nordén, B. *J. Am. Chem Soc.* **1996**, *31*, 7049-7054.
7. Braunlin, W.H. and Bloomfield, V.A. *Biochemistry* **1988**, 27, 1184-1191.
8. Wittung, P., Nielsen, P.E., Nordén, B. **1996**, submitted.
9. Egholm, M., Buchardt, O., Christensen, L., Bejrens, C., Freier, S.M., Driver, D., Berg, R.H., Kim, S.K., Nordén, B. and Nielsen, P.E. *Nature* **1993,** *365*, 566-568.