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Peptide nucleic acid (PNA) From DNA recognition to antisense and DNA structure

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Abstract

The biophysical and biological properties of PNA (peptide nucleic acid) is briefly reviewed with special emphasis on recent three dimensional structures of PNA-nucleic acid complexes and on structure-activity relations in terms of nucleic acid hybridization properties. © 1997 Elsevier Science B.V.

Keywords: PNA; Peptide nucleic acid; Antisense; Nucleic acid hybridization

1. Introduction

Peptide nucleic acid (PNA) was originally synthesized as a DNA mimic designed to bind sequence specifically in the major groove of double stranded DNA in a manner similar to triplex forming oligonucleotides. We wished to retain the nucleobases thymine and cytosine as recognition units for *adenine*–thymine and *guanine*–cytosine base pairs, and connect these via a charge neutral and preferably achiral peptide based backbone. Computer modelling suggested that ornithin could, in principle, fill that bill (except for the chirality), but for reasons of synthetic availability, synthetic flexibility and to avoid chiral centres, we settled on another homomorphous backbone composed of *N*-(2-aminoethyl)glycine units with the nucleobase connected to the glycine nitrogen via a methylene carbonyl linker [1–3] (Fig. 1). (This was a fortunate choice since the ornithin type backbone which was subsequently prepared by us [4] and others [5] has less desirable properties in terms of a DNA mimic). We reasoned that choosing the from a synthetic point of view quite easily accessible aminoethyl glycin backbone would allow for subsequent optimization and also for testing and designing other (novel) 'nucleobases' for recognizing *A*–T and *G*–C, but also *T*–A and *C*–G base pairs.

Experiments soon revealed that the PNAs are very powerful DNA mimics indeed for recognizing both double stranded DNA as well as single stranded DNA or RNA. However, whereas binding to single stranded nucleic acids takes place via conventional Watson–Crick base pairing, binding to (homopurine sequences) in double stranded DNA revealed a novel binding mechanism, P-loop formation via strand displacement (Fig. 2) [1,6,7].

The DNA and RNA binding properties of PNA as well as its chemical and biological stability has made PNA a very attractive molecule for the development

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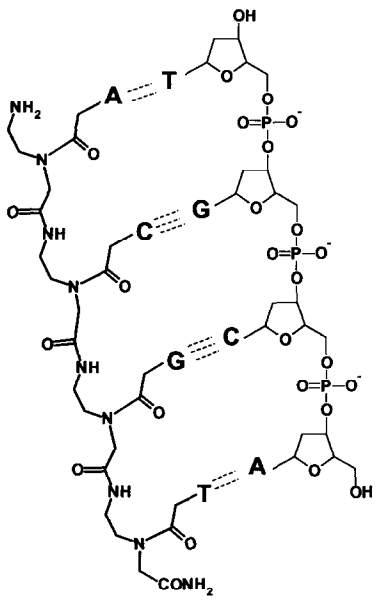


Fig. 1. Chemical structures of PNA and DNA.

of antisense and antigene therapeutic agents as well as biomolecular tools in diagnostics and molecular biology. For instance, it has been shown that protein synthesis (translation) can be sequence selectively and efficiently blocked by PNA bound to the mRNA [8–10] (Fig. 3). Analogously, PNA bound to the promoter [8,11] or within the coding region of a gene can inhibit transcription by blocking the DNA access or the progression of the RNA polymerase (Fig. 4). Finally, PNA targeted to telomerase RNA is a very potent inhibitor of this cancer related enzyme [12]. Thus, PNA is considered a promising gene therapeutic drug lead [13,14].

Likewise, several molecular biology methods based on PNA technology have been developed. These include sequence specific cleavage of plasmid DNA by PNA targeting of nuclease S_1 [15], a rare cleavage strategy for genome mapping using the achilles heel restriction enzyme/methylase principle [16], modulation of the PCR technique to detect

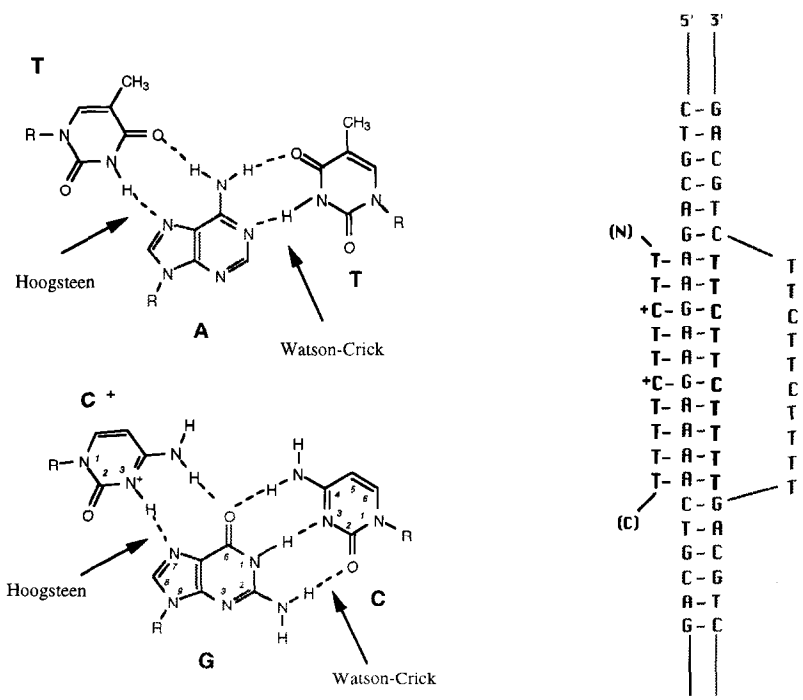


Fig. 2. Schematic representation of a P-loop in which a very stable, internal PNA_2–DNA triplex is formed with the complementary homopurine DNA strand via conventional Watson–Crick and Hoogsteen base pairing.

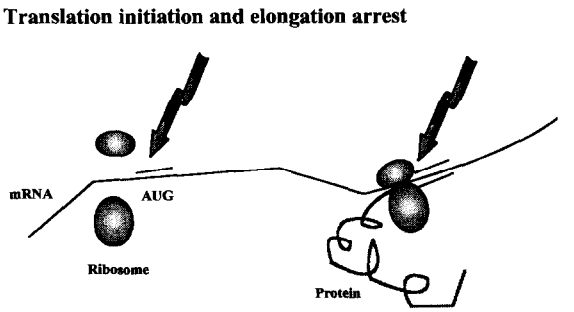


Fig. 3. Translation initiation can be blocked by either a duplex- or a triplex-forming PNA, whereas a triplex-forming PNA is required for translation elongation arrest of the ribosome [9].

single base mutations [17] as well as a PNA-based nucleic acid capture technique [18].

Finally, the fact that a peptide–(protein)-like structure is able to efficiently mimic DNA and, in principle, be the carrier of genetic information has prompted speculations and experiments using PNA as a model for a primordial genetic material with implications for the origin of life on earth [19,20].

These properties of PNA have spurred an interest in exploring further the structure of PNA complexes, and a desire to understand the chemical structure-constraints responsible for these properties.

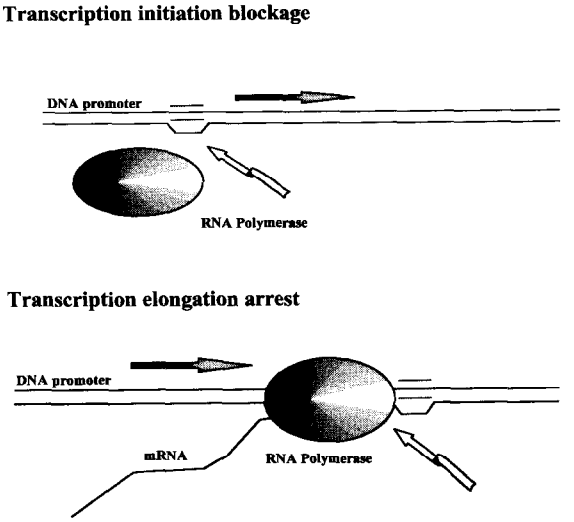


Fig. 4. Transcription inhibition can be accomplished by homopyrimidine PNAs that form P-loops either at the promoter thereby interfering with the DNA binding of RNA polymerase or required transcription factors [8,11] or by PNAs targeted to the coding region of the gene since bound to the template DNA strand can arrest elongating RNA polymerases [8–11].

2. Structure of PNA complexes

Three-dimensional molecular structures are now available for the basic PNA complexes. The structures of a hexamer PNA–RNA [21] and an octamer PNA–DNA [22] duplex were determined by NMR, while the structures of a nonamer PNA_2–DNA triplex [23] and a hexamer PNA–PNA duplex [24] were determined by X-ray crystallography. Several general conclusions can be drawn from these structures. From the heteroduplexes, it is clear that binding of PNA allows the backbone of the nucleic acid partner to retain close to its 'natural' conformation: The RNA in the PNA–RNA duplex is close to an A-form with $C_{3'}$-endo sugar puckering; whereas the DNA in the PNA–DNA duplex is closer to B-form with $C_{2'}$-endo sugar puckering. It is striking, however, that the base pair positions in both duplexes are more A-like in terms of helix-displacement, but are, in contrast to a canonical A-form helix, more perpendicular to the helix axis. The two duplexes also show a tendency towards a larger pitch than the usual 10–11 base pairs seen in nucleic acid duplexes, although this parameter is not that accurately deter-

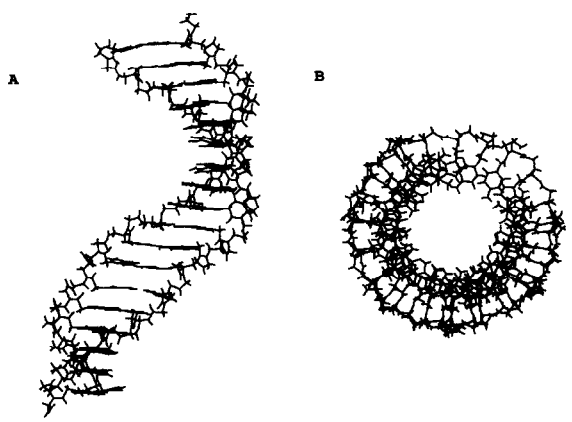


Fig. 5. Structure of an 18-mer PNA duplex based on the crystal structure of a self-complementary PNA hexamer [24]. The side and end views are shown.

mined in NMR structures. However, both the PNA_2–DNA triplex and especially the PNA–PNA duplex both show a very wide (26 and 28 Å, respectively) and a very large pitched helix (16 and 18 bases for the triplex and duplex, respectively) strongly suggesting that this latter type of helix, termed the P-form [23,24] (Fig. 5), is the natural conformation for helices with a PNA backbone. It is further notable that the base pairs in the PNA–PNA duplex exhibit close to A-form interbase–pair stacking overlaps (Fig. 6). Thus, it is clear that PNA helices have a structure of their own, which both resemble but is also distinctly different from known nucleic acid helices. By incorporating alternative nucleobases–aromatic moieties into PNAs, the effects on helical parameters of changes in hydrogen bonding patterns and stacking interactions may be studied, and a correlation between nucleobase interactions and helix structure may emerge.

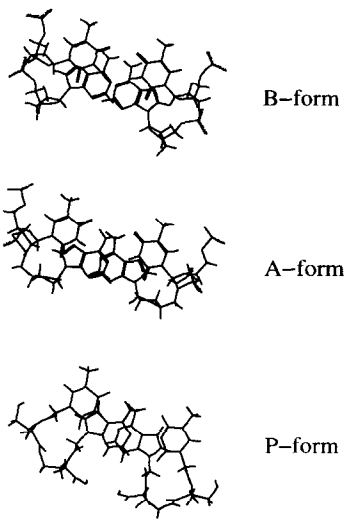
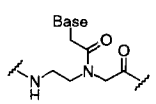
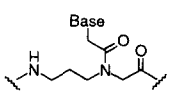
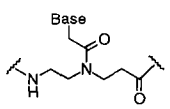
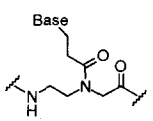
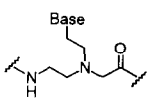
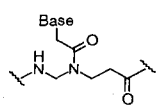


Fig. 6. Stacking structures of two A–T base pairs in canonical B- or A-form DNA and in the P-form PNA duplex.

Table 1
Effects on thermal stability (ΔT_m, °C) for structurally modifed PNA T-monomers when incorporated into the oligomer sequence H-GTA GA*T* CAC T-NH_2 [27]

Entry	Structure	Backbone/linker	ΔT_m DNA	ΔT_m RNA	reference
1		ethylglycine	0	0	
2		propylglycine	-8.0	-6.5	25
3		ethyl-β-alanine	-10	-7.5	25
4		propionyl linker	-20	-16	25
5		ethyl linker	-22	-18	26
6		retro-inverso	-6.5	nd	27

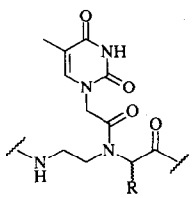
3. Structure–activity studies

A large number of backbone-modified PNAs have been made in order to define the structural space within which efficient DNA mimics exist and also with the aim of developing even better antisense compounds (Tables 1 and 2). Overall, it can be concluded from these results that deviations from the original aminoethylglycine backbone can only be minor. Extensions in any of the three possible directions (compounds **2–4**) [25] are not 'allowed', and the restricted flexibility imposed by the secondary amido group also seems necessary since reduction of this to a tertiary amine (compound **5**) is deleterious to the hybridization potency [26]. Also, an isomerization of the backbone to the 'retro-inverso' structure (compound **6**) which essentially moves a methylene group from the 'ethyl' to the 'glycine' moiety results in PNAs of low hybridization efficiency.

All available structures of PNA–DNA complexes suggest that substitutions at the α-position of the glycine moiety of the backbone is feasible without steric interference. Concordingly, the results presented in Table 2 [28] clearly demonstrate that PNA backbones based on other natural aminoacids than glycine result in fair to good DNA mimicking properties with some variations amongst the different amino acids.

By using such PNA monomers, chemical functionality can be introduced into the backbone and the physical properties of the PNAs, such as hydrophilicity, hydrophobicity, ionic character, etc. can be finely tuned and controlled. This should greatly facilitate the optimisation of, e.g., the pharmacokinetic behaviour of PNA. Especially it was shown that PNAs containing a few lysine-monomers (compounds **14** and **15**) have significantly increased aqueous solubility.

Table 2
Effects on thermal stability (ΔT_m, °C) for the PNA sequence H-G*T*A GA*T* CAC *T*-NH_2^a incorporating three chiral monomers as compared to an unmodified PNA [28]



Entry	R	chirality	ΔT_m DNA	ΔT_m RNA
7	CH_3	L	-1.8	nd
8	CH_3	D	-0.7	nd
9	*sec*-Bu	L	-2.6	-3.0
10	CH_2OH	L	-1.0	-1.0
11	CH_2OH	D	-0.6	-1.0
12	CH_2CO_2H	L	-3.3	nd
13	$CH_2CH_2CO_2H$	D	-2.3	nd
14	$(CH_2)_4NH_2$	L	-1.0	-1.3
15	$(CH_2)_4NH_2$	D	+1.0	0

Several reports have described the synthesis of PNA–DNA chimera of both DNA–3′-N-PNA and DNA–5′-C-PNA type junctions as exemplified in Fig. 7, [30,31]. Such chimerae hybridize less efficiently than pure PNAs to complementary oligonucleotides, but could confer RNaseH activating capacity. It has also reported that PNA–DNA chimera are taken up by mammalian cells as efficiently as normal oligonucleotides [32], but since the latter are

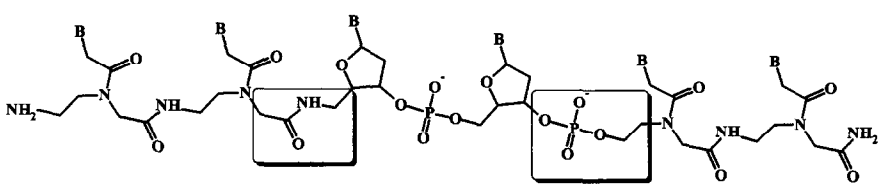


Fig. 7. Chemical structure of a PNA–DNA–PNA chimera.

taken up very poorly anyway, this does not appear a significant feature of the chimera.

3.1. Closing remarks

In conclusion the development of PNA has shed new light on many areas of DNA chemistry, biology and technology, giving new insight into both structural and functional aspects of nucleic acids. Judging from the expanding literature in this field, this development seems to be continuing, and I have no doubts that we can soon look forward to new interesting aspects of 'PNA technology'.

Acknowledgements

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