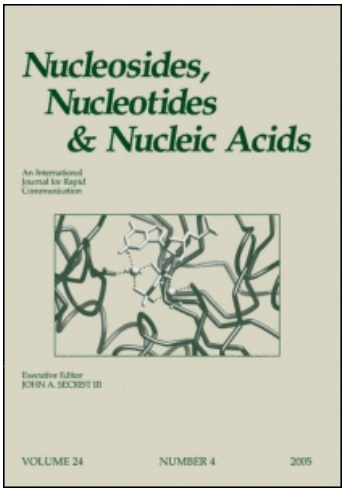

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Structure of Pna-Nucleic Acid Complexes

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STRUCTURE OF PNA-NUCLEIC ACID COMPLEXES

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ABSTRACT: The solution structure of the PNA-DNA hybrid H-GCTATGTC-NH_2·d(GACATAGC), determined by NMR methods, shows a new conformation which has elements of both A- and B-form DNA. Comparison with other PNA-nucleic acid complexes points to common structural features, but also demonstrates the ability of PNA to conform to nucleic acid partners of different conformations.

Peptide nucleic acid (PNA) is a DNA analogue that combines sequence specific binding to genetic targets with biostability and synthetic versatility.[1,2,3] In the PNA molecule the regular nucleobases (A, C, G, T and U) are attached to a backbone composed of pseudopeptide [N-(2-aminoehtyl)-glycine] units, rendering an achiral and uncharged molecule (Figure 1; the N-terminus normally carries an positively charged amino group, which together with any charged group attached to the C-terminus, commonly a lysinyl, gives the molecule a positive net charge). PNA hybridizes to DNA or RNA strands of complementary base sequence, and can also form complexes with matching PNA strands. Such complexes are characterized by high thermal stability and sensitivity to mismatches (for a review cf. ref. 4). Complexes with both antiparallel (DNA 5' end facing PNA C-terminus) and parallel strand orientation can form, but the former is generally more stable. Aside from potentially extensive future use in diagnostics, PNA is a promising lead compound for antisense and antigene therapeutic agents. However, prior to therapeutic use certain properties need to be optimized, e.g. bioavailability and base recognition code. Knowledge of molecular structure of PNA complexes may be important for guidance as to what modifications may convey any wanted properties.

To date three high resolution structures of PNA-nucleic acid complexes have been reported, the PNA-DNA[5] and PNA-RNA[6] duplexes and a PNA·DNA-PNA triplex[7]. This paper will focus on the PNA-DNA duplex structure, but will also include the others in a more general discussion of PNA structure.

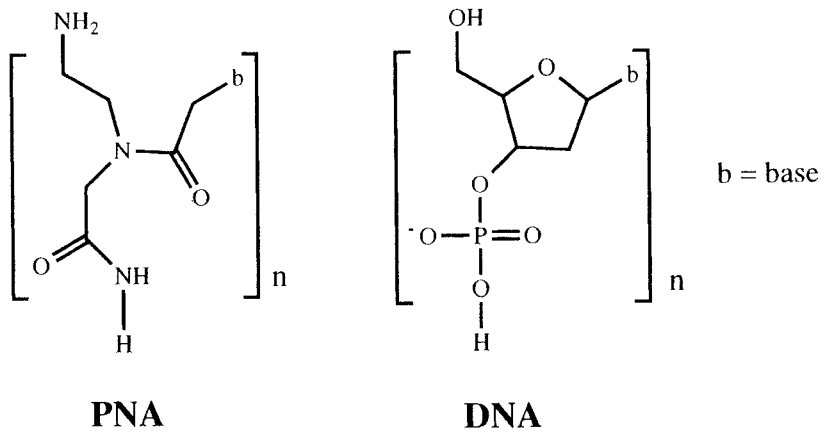

Figure 1. PNA and DNA monomers

PNA-DNA duplex structure

The solution structure of the PNA-DNA eightmer duplex H-GCTATGTC-NH_2-d(GACATAGC) has been determined by NMR-methods.[5] The ^{1}H-NMR spectrum of the complex is relatively well resolved, showing that the complex is structurally well defined. The spectrum was assigned from two dimensional NOESY and COSY data recorded in D_2O and H_2O solution. One interesting spectral feature is the several interstrand NOE crosspeaks observed (Figure 2) which, besides being crucial in the assignment procedure, demonstrate the right handed helicity of the duplex. The NOE crosspeak pattern in the DNA sugar-base region show typical characteristics of local B-conformation, such as *anti* glycosidic bonds and near C2'-*endo* sugar conformations[8]. The three dimensional structure was derived from interproton distances, derived from NOESY data, used as restraints in molecular dynamics calculations. The structure is at first sight reminiscent of B-form DNA, with a wide major groove and a narrower minor groove and the base pair planes approximately perpendicular to the helix axis. At closer inspection, however, significant differences become apparent, such as a displacement of the base pairs towards the minor groove, which is thus rather shallow, while the major groove reaches into the center of the helix. The helical dimensions also deviate from the B-conformation, with a diameter of approximately 23 Å, helical rise of 42 Å, and about 13 base pairs per turn. The primary amide bonds in the PNA backbone are all in the *trans* conformation with the carbonyl groups directed outward into solution. The flexible ethylene portions vary along the sequence, but the backbone-base linkers are consistently oriented with the carbonyl group pointing along the backbone in the C-terminal direction.

Comparison with other PNA hybrid structures

The structure of the PNA-RNA sixmer duplex, determined by NMR methods shows overall A-like features, apparently deriving from the RNA-strand, which is close to the standard

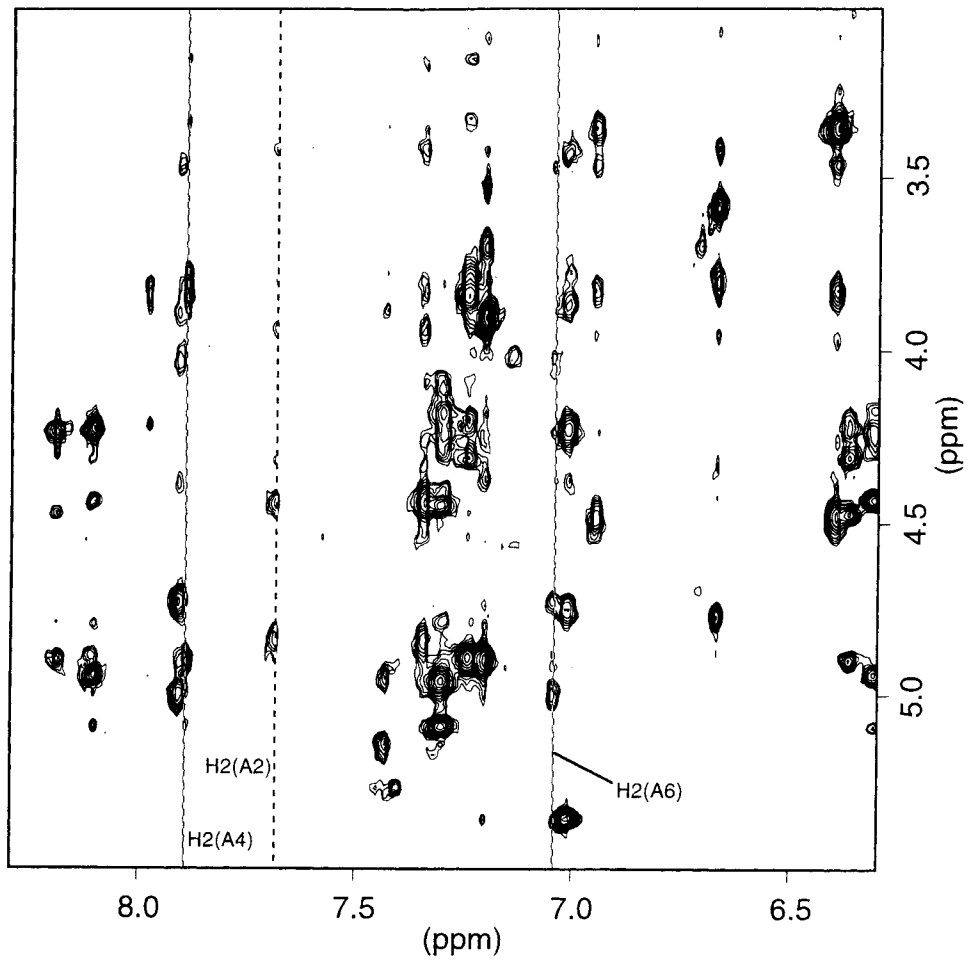

Figure 2. Aromatic-methylene proton region of NOESY of PNA-DNA duplex (200 ms mixing time at 21°C). Interstrand crosspeaks are marked.

A-conformation with C3'-*endo* sugars (Figure 3; ref. 6). In the PNA backbone the carbonyl groups on the backbone-base linkers are directed towards the C-terminus, similar to the PNA-DNA hybrid, and the primary amides are in the *trans* conformation.

The crystal structure of the PNA·DNA-PNA triple helix (Figure 3; ref. 7) differs from previously known nucleic acid triplexes[9]. It is composed of one all-purine DNA strand that makes Watson-Crick and Hoogsteen hydrogen bonds to two matching pyrimidine strands

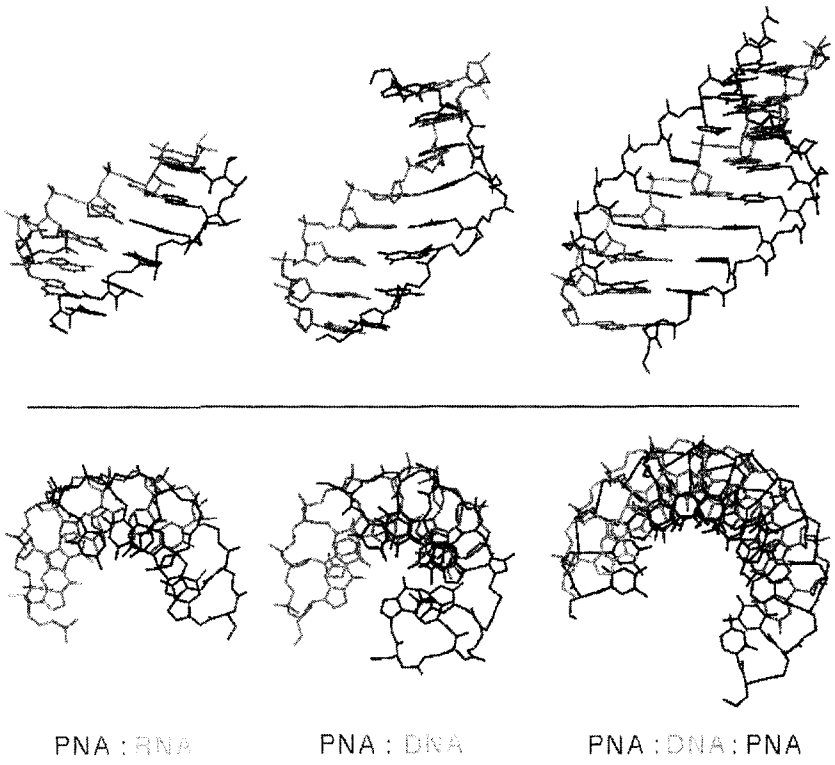

Figure 3

forming regular T·A-T and C^+·G-C triplets. This triple helix is wide (~26 Å in diameter), unwound (~16 base pairs per turn), has a helical rise of about 46 Å, and shows very close interactions, involving hyrdogen bonds, between the Hoogsteen PNA strand and the DNA strand. While the DNA backbone is A-like, the PNA strands are nearly identical and show resemblances with the PNA strand in the hybrid duplexes.

From the three structures described here a set of common features appear. First, the base pair displacement towards the minor groove is seen in all three structures. Second, the primary amide bonds of the PNA backbone are consistently found in the *trans* conformation. Third, the backbone-base linkers adopt similar conformations with the carbonyls pointing in the C-terminal direction. Fourth, many of the PNA backbone torsion angles are similar in the three structures, but large variations are seen for the ethylene regions, allowing the overall conformations to differ. It also appears that base pair stacking in an excentric manner is preferred. This notion is also supported by CD spectra of PNA-DNA and PNA-RNA spectra, which have similar shapes[10].

Structures studies of PNA-nucleic acid complex add new perspectives to our understanding of DNA and RNA structure. They show that lack of charge and chirality in one strand (two strands in the triplex case) has no major effects on conformation. The structures also suggest that base pair stacking is a major stabilizer of helicity, which is also supported by previous studies indicating that PNA-PNA duplexes adopt helical structures.[11,12]

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