Nucleic Acids

Why Does TNA Cross-Pair More Strongly with RNA Than with DNA? An Answer From X-ray Analysis**

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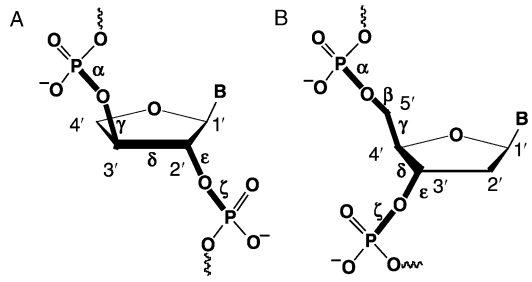
Research directed toward a chemical etiology of nucleic acid structure has recently established that L-α-threofuranosyl (3′→2′) nucleic acid (TNA) is an efficient Watson–Crick base-pairing system capable of informational cross-pairing with both RNA and DNA.[1–3] TNA is constitutionally the simplest oligonucleotide-type nucleic acid system found so far that possesses this capability. Its cross-pairing with RNA is stronger than with DNA.[1,2] The backbone of TNA is shorter than that of DNA and RNA since the sugar moiety in TNA contains only four carbon atoms and the phosphodiester groups are attached to the 2′- and 3′-positions of the threofuranose ring, as opposed to the 3′- and 5′-positions as in DNA and RNA (Scheme 1).

TNA and RNA, and to a lesser extent DNA sequences can act as templates for the ligation of TNA fragments. Such ligations proceed with high fidelity and are greatly accelerated when adenine is replaced by 2,6-diaminopurine in the TNA ligands.[4] Nitrogenous analogues of TNA in which either O3′ or O2′ is substituted by an NH group are equally efficient Watson–Crick base-pairing systems; they also cross-pair with TNA, RNA, and DNA.[5] It was recently shown that certain DNA polymerases are able to copy a TNA template sequence, albeit of limited length and at a slower rate than with a DNA template.[6] Moreover, a DNA polymerase has been identified that is capable of significant TNA synthesis.[7] The combination of a DNA-dependent TNA polymerase and a TNA-directed DNA polymerase may enable in vitro evolution experiments to be carried out with TNA molecules in the future.[7]



Scheme 1. Configuration, atom numbering, and backbone torsion angle definition in a) TNA and b) DNA.

The remarkable properties of TNA warrant a detailed investigation of its three-dimensional structure and potential conformational plasticity. Specifically, structures of TNA may provide insight into the origins of the more stable pairing between TNA and RNA relative to that between TNA and DNA. We initially analyzed the conformational properties of TNA nucleotides in a B-form DNA dodecamer.[8] The crystal structure of the oligonucleotide with sequence CGCGAAT*TCGCG (T* = TNA T; residues of strands 1 and 2 are numbered 1–12 and 13–24, respectively) was determined at 1.20-Å resolution and revealed a C4′-*exo* conformation of the threose sugar, coupled with a quasi *trans*-diaxial orientation of the 3′ and 2′ substituents (Figure 1A). The TNA backbone conformation can be characterized by five torsion angles (instead of the six needed for DNA; Scheme 1). The values of these angles in the single TNA thymidine residue present per strand in the B-form DNA duplex fall into the sc^-, *ap, ap,* ac^-, ac^- ranges (*α, γ, δ, ε,* ζ; Table 1, Figure 1A).

The structure of the B-form dodecamer with a single TNA thymidine residue per strand also shows a virtually seamless fit of TNA within a B-DNA context.[8] However, the intra-nucleotide P···P distance in the two TNA residues (5.79 Å in residue T*7; Figure 1A) is significantly shorter than that between the 5′ and 3′ phosphorus atoms in 2′-deoxyribonucleotides in B-form DNA (for example, 6.77 Å in residue T20; Figure 1B).[9,10]

To assess the potential of the TNA nucleotide unit to conformationally adapt to different overall helix geometries, we analyzed the crystal structure of an A-form DNA duplex containing a single TNA adenosine residue (A*) per strand. The structure of the decamer with the sequence GCGTAT ^{M}A*CGC (T^{M}, 2′-*O*-methyl-thymidine) was refined to an R factor of 13.3% at 1.16-Å resolution. The T^{M} residue was

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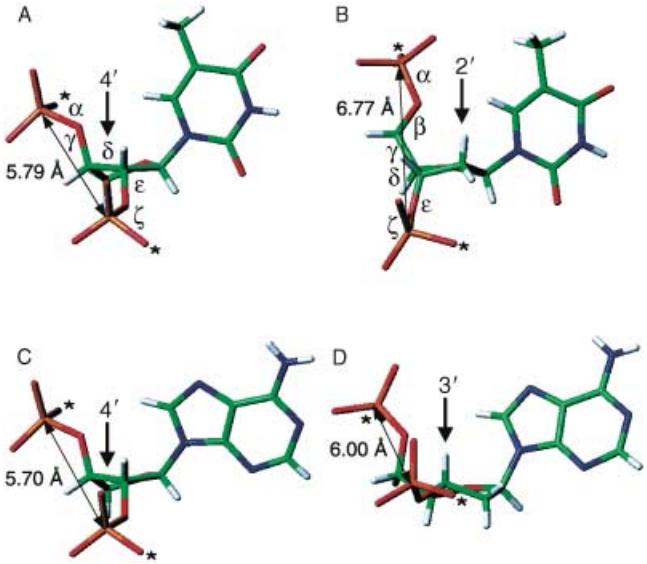

Figure 1. Conformations adopted by TNA and DNA nucleotides in B- and A-form duplexes: a) B-TNA (with residue T*7); b) B-DNA (T20); c) A-TNA (A*7); d) A-DNA (A15). Asterisks in the figure designate the directions of the backbone chain, torsions angles in TNA and DNA are labeled, and carbon atoms of the L-α-threofuranose and D-β-deoxyribose sugars positioned either *exo* (TNA) or *endo* (DNA) to the best plane (defined by the other sugar ring carbon and oxygen atoms) are indicated by arrows.

Table 1: TNA backbone torsion angles in degrees.

Residue	Helix	α	γ	δ	ε	ζ
T*7	B-form	−84	−173	157	−100	−136
T*19	B-form	−86	−170	160	−130	−98
A*7	A-form	−88	−172	152	−97	−132
A*17	A-form	−87	−166	153	−108	−115

incorporated to lock the duplex in the A-form geometry. Residues in strands 1 and 2 of the decamer are numbered 1 to 10 and 11 to 20, respectively. A summary of the crystal data, data statistics, and refinement parameters is provided in Table 2.

Table 2: Selected crystal data and refinement parameters.

space group	orthorhombic $P2_12_12_1$
unit cell constants [Å]	$a=24.43$, $b=44.52$, $c=47.33$
resolution [Å]	1.16
no. of unique reflections	18289
completeness (1.20–1.16 Å) [%]	98.8 (94.5)
R-merge (1.20–1.16 Å) [%]	6.2 (29.7)
R-work (1.20–1.16 Å) [%, no σ cutoff]	13.3 (21.1)
R-free [%]	18.8
no. of refinement parameters	4921
data-to-parameter ratio	3.53
no. of restraints	10197
no. of DNA atoms	404
no. of water molecules	125
r.m.s[a] deviation bonds [Å]	0.017
r.m.s.[a] deviation angles [1···3 dist., Å]	0.026

[a] r.m.s. = root mean square.

The conformations of the two adenine TNA nucleotides in the A-form environment are remarkably similar to those of the two thymidine TNA residues in the B-form duplex (Figure 1C, A). In both environments, the conformation of the two threose sugar units is C4′-*exo* and the intranucleotide P···P distances of the TNA residues in the B- and A-DNA duplexes differ only by 0.1 Å. The five backbone torsion angles in the TNA residues in the A-form duplex also fall into the *sc*$^-$, *ap*, *ap*, *ac*$^-$, *ac*$^-$ ranges (α, γ, δ, ε, ζ; Table 1, Figure 1C). In both the A- and B-form duplexes with incorporated TNA nucleotides, the sugar puckers of residues surrounding the TNA nucleotide are altered minimally relative to those in the respective unmodified duplexes, and local helical parameters (i.e. rise and twist) are only slightly changed (data not shown).[8]

The combined structural data demonstrate that TNA behaves in a conformationally rigid fashion in these duplexes. Minor deviations between the geometries of L-α-threofuranosyl nucleotides in A- and B-form duplexes are limited to the ε and ζ backbone torsion angles (Table 1; the glycosidic torsion angles of DNA and TNA nucleotides in the A- and B-form environments differ to a similar extent). The preferred sugar pucker is C4′-*exo*, independent of whether TNA is in an RNA or DNA environment, or indeed exists as an isolated nucleotide (Figure 1).[1] This observation is also in accordance with the conformation of the TNA sugar in the recently determined NMR solution structure of an all-TNA duplex with sequence CGAATTCG.[11] The quasi *trans*-diaxial orientation preferred by the 3′ and 2′ phosphate groups leads to an intranucleotide P···P distance with an upper limit of around 5.8 Å. This distance is similar to that between adjacent phosphate groups in A-form DNA double helices (up to about 6 Å; see Figure 1D).[9,10]

The structural results lead us to conclude that cross-pairing between TNA and DNA is possible because the latter can conformationally adjust to the structural requirements of TNA and not vice versa. To achieve this adjustment, DNA sugars have to assume a conformation in which the inter-phosphate P···P distance is reduced from approximately 7 Å (B-form; Figure 1B) to around 6 Å (A-form; Figure 1D), a conformational adaptation that is similar to that required for DNA when it pairs with RNA. Double-helical RNA cannot assume an extended backbone variant with the sugars in a C2′-*endo* conformation because the quasi-equatorial orientation of the 2′-hydroxy group associated with this pucker would result in a steric clash with the 5′-phosphate oxygen atom of the following ribose unit.[12] Contrary to the apparent conformational uniformity of threoses in TNA–DNA and TNA–RNA hybrid duplexes, the 2′-deoxyribose sugars in DNA–RNA hybrids assume a variety of conformations, including the C3′-*endo* and O4′-*endo* puckers.[13–15] A more important fact with regard to the relative pairing stabilities of TNA with RNA and DNA is that the thermodynamic stability of DNA–RNA hybrids is commonly lower than that of RNA duplexes with identical sequence.[16]

We hypothesize that TNA is a poor substitute for DNA in chemical reactions and molecular recognition events that require a canonical B-form helix geometry. Conversely, TNA appears to be an excellent mimic of A-form DNA and

therefore also of A-form RNA as far as geometry and conformation of the nucleotide building blocks are concerned. Thus, our structural observations provide a convincing qualitative rationalization of the experimental observations that 1) TNA hybridizes more strongly with RNA than with DNA,[1,2] and 2) RNA constitutes a better template for ligating TNA fragments than DNA.[4]

The close and specific resemblance between TNA and RNA with respect to the intranucleotide P···P distance, one of the structural parameters that determine the capabilities of oligonucleotide systems for cross-pairing and for serving as cross-templates, is of interest in the context of the notion of a possible etiological relationship between RNA and TNA.

Experimental Section

Materials: The synthesis of the 3′-*O*-dimethoxytrityl-L-α-*threo*-furanosyladenine-2′-*O*-(2-cyanoethyl)-*N,N*′-diisopropyl phosphoramidite monomer has been described previously.[1,2] 2′-Deoxynucleotide phosphoramidites and 3′-terminal nucleoside controlled-pore glass support were purchased from Glen Research (Sterling, VA). All other chemicals for solid-phase oligonucleotide synthesis were also purchased from Glen Research (Sterling, VA).

Oligonucleotide synthesis: The TNA-modified oligonucleotide was synthesized on an Applied Biosystems 381A DNA synthesizer with slight modifications to published procedures.[17] Monomer coupling times were 90 sec for 3′-CE phosphoramidites and 10 min for the TNA amidite, with the oligomer synthesized with the 5′-terminal trityl group "off". Deprotection and cleavage from the solid support were achieved by treatment with 28–30% NH_4OH at 55 °C for 8 h. The sequence was analyzed and purified by strong anion exchange chromatography on a Pharmacia AKTA Purifier HPLC instrument with a DIONEX DNAPAC PA-100 analytical column (4 × 25 mm) purchased from Dionex Corp (Sunnyvale, CA). For preparative runs, 25 OD units were purified on the analytical column at a time with a gradient of 25 mM tris(hydroxymethyl)aminomethane-HCl (pH 7.8) to 1 M NaCl over 30 min and a flow rate of 1.0 mL min^{-1}. The purified oligomer was desalted on a SEP PAK cartridge (Waters Inc., Milford, MA). The cartridge was pre-equilibrated with acetonitrile followed by water. The oligonucleotide solution was applied to the cartridge, washed with water, and a solution of 75% methanol in water was then used to elute the desalted oligomer with a final yield of 56%. Molecular weights of the products were determined by MALDI-TOF mass spectrometry.

Crystallization and data collection: Crystals were grown at 18 °C by the hanging drop vapor diffusion method. Droplets (4 μL) containing oligonucleotide (0.5 mM), sodium cacodylate (20 mM, pH 6.0), sodium chloride (40 mM), barium chloride (10 mM), spermine tetrahydrochloride (6 mM), and 2-methyl-2,4-pentanediol (MPD; 5% (v/v)) were equilibrated against a reservoir of MPD (1 mL, 35%). Hexagonal rods of reasonable size appeared after two weeks. For data collection, a crystal (0.3 × 0.1 × 0.05 mm) was picked up from a droplet with a nylon loop and transferred into a cold N_2 stream (120 K). High- and low-resolution data sets were collected on the 5-ID beam line ($\lambda = 1.0$ Å) of the DND-CAT at the Advanced Photon Source (Argonne, IL) by using a MARCCD detector. Data were integrated and merged with the DENZO and SCALEPACK programs, respectively.[18] Selected crystal data and data quality and completeness are summarized in Table 2.

Structure determination and refinement: The structure was determined by the molecular replacement technique by using the program CNS,[19] with an A-form DNA decamer duplex as the search model. Initially, the decamer duplex was refined as an all-DNA molecule. At a later stage, atoms of TNA A*7 and A*17 were renumbered and the former 2′-deoxyribose C5′ atoms in these residues were deleted. Isotropic and anisotropic refinements of the model were carried out with the program SHELX-97,[20] and 5% of the reflections were set aside at random for calculating the R-free value.[21] Hydrogen atoms were added to the SHELX-97 calculations and all DNA and TNA atoms, as well as selected solvent water molecules, were treated anisotropically. Final refinement parameters and r.m.s. deviations from ideal bond lengths and angles are listed in Table 2.

Data deposition: Final coordinates and structure factors have been deposited in the Protein Data Bank[22]: PDB entry code 1PWF.

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