Crystal Structure of a B-form DNA Duplex Containing (L)-α-Threofuranosyl (3′-2′) Nucleosides (TNA): A Simple Four-carbon Sugar Is Easily Accommodated into the Backbone of DNA

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Introduction

TNA [(L)-α-threofuranosyl-(3′ → 2′)-oligonucleotides] containing vicinally connected phosphodiester linkages undergoes informational base-pairing in an antiparallel strand orientation and is capable of cross-pairing with RNA and DNA. TNA is derived from a sugar that contains only four carbon atoms and is one of the simplest, potentially natural, nucleic acid alternatives investigated thus far in the context of a chemical etiology of nucleic acid structure. Compared to DNA and RNA containing six covalent bonds per repeating nucleotide unit, TNA contains only five. We have determined the atomic-resolution crystal structure of the B-form DNA duplex $[d(CGCGAA)T^*d(TCGCG)]_2$ containing a single (L)-α-threofuranosyl thymine (T*) per strand. In the modified duplex base, stacking interactions are practically unchanged relative to the reference DNA structure. The orientations of the backbone at the TNA incorporation sites are slightly altered in order to accommodate fewer atoms and covalent bonds. The conformation of the threose is C4'-*exo* with the 2′- and 3′-substituents assuming quasi-diaxial orientation.

Methods and Materials

Oligonucleotide Synthesis

The native and TNA-modified oligonucleotides were synthesized on an Applied Biosystems 381A DNA synthesizer following slight modifications to published procedures. Monomer coupling times were 90 seconds for 3′-CE phosphoramidites and 10 minutes for the TNA amidite. Deprotection and cleavage of the oligonucleotides from the solid support were achieved by using 28% NH_4OH at 55°C for 8 hours. Reverse-phase (RP) high-pressure liquid chromatography (HPLC) analyses and purifications were carried out on an Applied Biosystems chromatograph with a Hewlett-Packard Hypersil ODS-5 column (4.6 × 200) and a 1% gradient of acetonitrile in 0.03 M triethylammonium acetate buffer (pH 7.0) with a flow rate of 1.0 mL/min. The crude oligonucleotides were isolated as "trityl-on" derivatives by RP HPLC and detritylated in 80% acetic acid for 30 minutes. The oligonucleotides were purified by RP HPLC a second time.

Crystallization and Data Collection

Crystals were grown at room temperature by the sitting-drop vapor-diffusion method. Droplets (20 μL) containing 1 mM oligonucleotide, 20 mM sodium cacodylate (pH 7), 10 mM magnesium acetate, and 3 mM spermine tetrahydrochloride were equilibrated against a reservoir of 25 mL of 40% 2-methyl-2,4-pentanediol (MPD). Large hexagonal rods appeared after a week. For data collection, a crystal (0.3 × 0.2 × 0.2 mm) was picked up from a droplet with a nylon loop and transferred into a cold N_2 stream (120K). High- and low-resolution data sets were collected on DND-CAT beamline 5-ID (λ = 0.978 Å) at the APS by using a MarCCD detector. Data were integrated and merged with DENZO/SCALEPACK.

Structure Determination and Refinement

The structure was solved by molecular replacement by using a DNA dodecamer as the search model and refined with the programs CNS and SHELX-97. After monitoring the R-free by using 10% of the reflections, all reflections were included in the final rounds of isotropic refinement. Hydrogen atoms were added in SHELX-97, and all atoms, including the solvent waters, were treated anisotropically.

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