Crystal structure of tricyclo-DNA: an unusual compensatory change of two adjacent backbone torsion angles†

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Received (in Cambridge, UK) 24th October 2007, Accepted 29th November 2007
First published as an Advance Article on the web 21st December 2007
DOI: 10.1039/b716390h

The crystal structure of a DNA duplex with tricyclo-DNA (tc-DNA) residues explains the increased RNA affinity of tc-DNA relative to DNA and tc-DNA’s superior resistance to nucleases.

Conformationally restricted oligonucleotide analogs have been widely investigated in antisense applications and as diagnostic tools1,2 as well as in etiological studies of nucleic acid structure.3,4 Among the chemical modifications of DNA and RNA that lead to structural reorganization are those that target either the sugar moiety alone3,5–7 or both the sugar and adjacent phosphate backbone region.8,9 Tricyclo-DNA (tc-DNA; Fig. 1) is a second-generation, conformationally constrained DNA analog in which the C30 and C50 atoms are connected by an ethylene bridge that is fused to a cyclopropane ring. Oligonucleotides composed of tcdA and tcdT10 engage in highly stable self-pairing11,12 and homopurine tc-DNAs pair with their complementary strands preferably in the Hoogsteen mode. Heteroduplexes between tc-DNA and DNA are entropically stabilized compared to their native DNA counterparts. By comparison, tc-DNA self-pairing is both enthalpically and entropically favored compared with DNA pairing. Duplexes between all-tcDNA oligonucleotides and complementary DNA and RNA strands exhibited enhanced UV-melting temperatures ΔTm/modification of +1.2 and +2.4 °C, respectively.13 Although hybrids between tc-DNA and RNA do not elicit RNase H, the analog shows promising antisense effects in vitro and in vivo and was found to be completely stable against degradation in heat-deactivated fetal calf serum at 37 °C.14 CD-spectroscopic investigations of tc-DNA indicated that the analog prefers an A-form conformation, consistent with the results from modeling studies.13 However, modeling does not provide a definitive answer as to the preferred conformation of tc-DNA.

To address this issue, we have solved the structure of a DNA duplex with tc-DNA residues.

We chose a DNA decamer of sequence 5'-GCGTACTGCC-3' and the Dickerson–Drew dodecamer (DDD) DNA 5'-CGCGAATTCCGC-3' as templates for incorporation of tcdA or tcdT nucleotides at various locations and subsequent crystallization of the modified oligonucleotides. The best crystals were obtained for a DDD duplex [d(CGCG-tcA-ATTCCG)]2 and its structure was determined by the molecular replacement technique and refined to 1.75 Å resolution.† Selected crystal data and refinement parameters are summarized in Table S1 (see ESI†) and an example of the final electron density is depicted in Fig. 2(A). The unit cell dimensions of the tc-DNA-modified DDD are very similar to those of crystals of the native DDD grown in the presence of Ba2+ and a minor groove binding agent.15 In that crystal, serving as the reference structure here, the DDD is located on a crystallographic dyad and 5'-terminal Cs and 3'-terminal Gs are unpaired and adopt extra-helical conformations. In the structure of the tcdA-modified DDD, terminal base pairs are also disrupted, but the twofold crystallographic symmetry is lost and the duplex is rotated by ca. 120° relative to its position in the unit cell of the unmodified DDD. Despite these differences the conformations of the two duplexes are quite similar (Fig. 2(B)) and the modified dodecamer duplex exhibits non-crystallographic twofold rotational symmetry.

Although the superimposition of the two duplexes reveals only minor changes in their overall geometries, the conformations of tcdA residues and the adjacent phosphate backbone in the modified duplex deviate significantly from the corresponding adenosines in the structure of the native DDD. The most

Fig. 1 Configuration and linkage of (A) tricyclo-DNA (tc-DNA) and (B) DNA. Individual atoms in tc-DNA are numbered and backbone and glycosidic torsion angles common to tc-DNA and DNA are labeled in B.
notable differences concern the sugar puckering and the backbone torsion angles $\beta$ and $\gamma$ (Fig. 2(C), (D)). The 2'-deoxyriboses in both tcdA residues adopt the C2'-exo conformation that is associated with the A-type conformation of DNA and RNA double helices.\textsuperscript{16,17} In the native DNA, the sugars of the corresponding As have a C2'-endo conformation (identical due to the twofold symmetry), commonly associated with a B-form duplex. Their backbone torsion angles fall into the $\text{sc}^-/\text{ap}/\text{sc}^-/\text{ap}/\text{ac}^- (\alpha$ to $\zeta$) conformational ranges and the glycosidic angle has an $\text{ac}^-$ conformation (Fig. 2(D)). With tcdA residues backbone torsion angle fall into the $\text{ac}^-/\text{sc}^+/	ext{ap}/\text{ac}^+/\text{sc}^- (\alpha$ to $\zeta$) conformational ranges and the glycosidic angle has an $\text{ap}^-$ conformation (Fig. 2(C)). Thus, the conformations of $\beta$ and $\gamma$ torsion angles in tc-DNA (underlined above) are swapped relative to B-DNA. The changes in $\alpha$ (sc$^- \rightarrow \text{ac}^-$) and $\gamma$ (sc$^- \rightarrow \text{ap}$) seen with tc-DNA are somewhat reminiscent of the crankshaft motion around the $\beta$ angle leading to an extended backbone variant in RNA.\textsuperscript{18} However, in RNA all three angles including $\beta$ are adopting an $\text{ap}$ conformation and the combination seen in tc-DNA with $\beta$ in the sc range is unique. In fact an $\text{sc}$ conformation of $\beta$ is highly unusual in the contexts of either DNA or RNA and is directly related to the presence of the cyclopropane ring in tc-DNA. Thus, the concerted change in tc-DNA from $\text{ap}$ to $\text{sc}$ for $\beta$ and from $\text{sc}$ to $\text{ap}$ for $\gamma$ appears to have a compensatory effect (Fig. 2(E)) as far as stable self-pairing and pairing with DNA and RNA are concerned. Differences for $\sigma$ and $\zeta$ in tcD relative to the corresponding adenosines amount to $\sim 60\degree$. The distances between the positions of 5'- and 3'-phosphorus atoms with residues tcdA5 and tcdA17 are 6.90 and 6.91 Å, respectively, and therefore similar to the distance between the corresponding atoms in the native DNA (6.71 Å; Fig. 2(E)). In regard to the significantly increased RNA affinity of tc-DNA relative to DNA, the A-type sugar puckers and the glycosidic angle in tcD residues of $-163\degree$ in the crystal structure are particularly noteworthy. This glycosidic angle value is very close to the average value of glycosidic torsion angles in A-form duplexes, whereas the angle of the corresponding adenosine in the native DDD ($-111\degree$; Fig. 2(C), (D)) is typical for B-form duplexes (see supp. info in ref. 4).

The conclusion that the cyclopropane ring in tc-DNA is the cause of the above features is supported by the conformational and pairing properties of bicyclo-DNA (bc-DNA) that lacks cyclopropane.\textsuperscript{9,15} In bc-DNA, torsion angle $\gamma$ widens to an $\text{ap}$ conformation, but the $\beta$ angle is in the $\text{ac}^+$ range, apparently prohibiting the compensatory conformational effect seen in tc-DNA. Moreover, bc-DNA residues adopt C2'-endo or C1'-exo puckerers that are associated with B-form DNA. In the crystal structure of the DDD with tcD residues, the cyclopropane ring is positioned at the edge of the major groove (Fig. 2(B)). The presence of steric bulk in close vicinity of the phosphodiester moiety points to an additional important role of the cyclopropane ring in the superior nucleicase resistance exhibited by tc-DNA. Modeling the tc-DNA backbone at an exonuclease active site based on the crystal structure of the complex between DNA Polymerase I Klenow fragment and a 2'-O-modified RNA\textsuperscript{20} lends support to the notion of a steric origin of the protection against nucleolytic degradation afforded by the tricyclic nucleic acid modification (Fig. S1, ESIF).

Inspection of electron density maps following refinement of the duplex model and placement of water molecules revealed a spermine molecule (Fig. 2(B)) and an intense peak (3-phosphorus atoms with residues tcdA5 and tcdA17; (green) is located near one terminus, and $\text{PO}_3^-$ (red; native structure)\textsuperscript{15} by superimposing positions of 5'- and 3'-phosphorus atoms.

![Fig. 2](image-url)
confirms the previously noted preference, based on crystal structures of DNA, by transition metal ions to coordinate to terminal or extrahelical bases as well as to phosphate groups. However, the particular coordination site involving cytosines observed here is unique and coordination to N7 of G is more common. Crystals of the tc-DNA-modified duplex could subsequently also be grown in the presence of Co2+ or Ni2+, but were of inferior quality compared to Zn2+ crystals and not suitable for high-resolution structure determination. The availability of a crystal structure of the native DDD with extruded terminal C and G residues allows a detailed comparison of the structural changes as a result of Zn2+ coordination. Thus, the aforementioned reorientation of the duplex and the loss of the crystallographic twofold symmetry are the direct result of the coordination of the metal ion. In the absence of Zn2+ 5′-terminal Cs interact directly with phosphate groups of 3′-terminal Gs (Fig. S2B, ESI†).

In summary, the crystal structure of a DNA dodecamer duplex with incorporated tcA nucleotides has provided detailed insight into the conformational properties of tricyclo-DNA, one of the structurally most complex DNA analogs studied so far. Particular features such as the C2′-exo sugar pucker of tcA and the conformation of the nucleoside (the glycosidic angles of tcA residues are consistent with an A-form conformation), and the compensatory effect of the cyclopropane ring on torsion angles β and γ provide a rationalization for the significantly increased RNA affinity of tc-DNA relative to DNA. The cyclopropane ring present on the tc-DNA backbone likely causes an unfavorable steric interaction at the active site of endo- and exo-nuclease and leads to the superior resistance against degradation exhibited by tc-DNA. Finally, the fortuitous observation of a Zn2+ binding site in the crystal structure of the tcA-modified DDD may serve as a guide for the design of active sites in nucleic acid molecules that employ a transition metal ion for catalysis instead of the more commonly found Mg2+.

Financial support from the National Institutes of Health (Grant: GM55237 to M. E.) is gratefully acknowledged. Financial support for NSLS beamlines comes principally from the Offices of Biological and Environmental Research and of Basic Energy Sciences of the US Department of Energy, and from the National Center for Research Resources of the National Institutes of Health. DND-CAT is supported by the E.I. DuPont de Nemours & Co., The Dow Chemical Company, the U.S. National Science Foundation through Grant DMR-9304725 and the State of Illinois through the Department of Commerce and the Board of Higher Education Grant IBHE HECA NWU 96.

Notes and references

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