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

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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 tools[1,2] as well as in etiological studies of nucleic acid structure.[3,4] Among the chemical modifications of DNA and RNA that lead to structural preorganization are those that target either the sugar moiety alone[1,2,5–7] 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 C3′ and C5′ atoms are connected by an ethylene bridge that is fused to a cyclopropane ring. Oligonucleotides composed of tcdA and tcdT[10] engage in highly stable self-pairing[11,12] 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 ΔT_m/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′-GCGTA-TACGC-3′ and the Dickerson–Drew dodecamer (DDD) DNA 5′-CGCGAATTCGCG-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-ATTCGCG)]$_2$ 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 Ba^{2+} 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

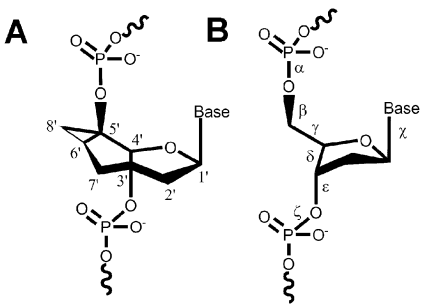

Fig. 1 Configuration and linkage of (A) tricyclo-DNA (tc-DNA) and (B) DNA. Individual atoms in tc-DNA are numbered and backbone and glycosidic torsion angles common to tc-DNA and DNA are labeled in B.

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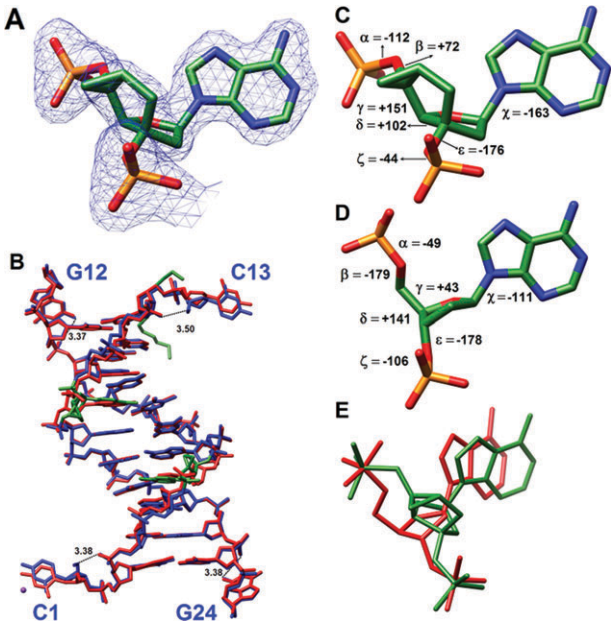

Fig. 2 (A) ($2F_o - F_c$) Fourier sum electron density drawn at the 1σ level around residue tcdA5. (B) Superimposition of the native DDD[15] (red, space group $P3_212$) and the DDD with A5 and A17 replaced (blue, space group $P3_2$) by tcdA (highlighted in green). The view is into the major groove, approximately along the molecular dyad. Residues at the 5′-and 3′-termini are labeled. Thin solid lines indicate hydrogen bonds between 5′-terminal hydroxyl groups of C1 and C13 and phosphates of residues G2 and G14, respectively, as well as between N1 amino groups of G12 and G24 and phosphates of residues C11 and C23, respectively. A Zn^{2+} ion coordinated to N3 of C1 is depicted as a purple sphere, and a spermine molecule $^{+}H_3N(CH_2)_3NH_2^{+}$-$(CH_2)_4NH_2^{+}(CH_2)_3NH_3^{+}$ (highlighted in green) is located near one end of duplex, inside the major groove. (C) Conformation of tcdA17 in the duplex d($C_1G_2C_3G_4$)-tcA_5-($A_6T_7T_8C_9G_{10}C_{11}G_{12}$):d($C_{13}G_{14}C_{15}$-$G_{16}$)-tc$A_{17}$-($A_{18}T_{19}T_{20}C_{21}G_{22}C_{23}G_{24}$). The angle values for residue tcdA5 are −114, +74, +152, +107, −179, −53 and −164° for α, β, γ, ε, ζ and χ, respectively. (D) Conformation of the corresponding adenosine in the crystal structure of the native DDD (PDB code 1ehv).[15] Backbone torsion angles are labelled α to ζ and χ is the glycosidic torsion angle. (E) Comparison of the conformations for PO_3^-–tcA_{17}–PO_3^- (green) and PO_3^-–dA_5–PO_3^- (red; native structure[15]) by superimposing positions of 5′- and 3′-phosphorus atoms.

notable differences concern the sugar pucker and the backbone torsion angles β and γ (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.[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 $sc^-/\underline{ap}/\underline{sc}^+/ap/ap/ac^-$ (α to ζ) conformational ranges and the glycosidic angle has an ac^- conformation (Fig. 2(D)). With tcdA residues backbone torsion angle fall into the $ac^-/\underline{sc}^+/\underline{ap}/ac^+/ap/sc^-$ (α to ζ) conformational ranges and the glycosidic angle has an *ap* conformation (Fig. 2(C)). Thus, the conformations of β and γ torsion angles in tc-DNA (underlined above) are swapped relative to B-DNA. The changes in α ($sc^- \rightarrow ac^-$) and γ ($sc^+ \rightarrow ap$) seen with tc-DNA are somewhat reminiscent of the crankshaft motion around the β angle leading to an extended backbone variant in RNA.[18] However, in RNA all three angles including β are adopting an *ap* conformation and the combination seen in tc-DNA with β in the *sc* range is unique. In fact an *sc* conformation of β 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 *ap* to *sc* for β and from *sc* to *ap* for γ 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 α and ζ in tcdA relative to the corresponding adenosines amount to ~60°. 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 pucker and the glycosidic angle in tcdA residues of −163° 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°; 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.[9,19] In bc-DNA, torsion angle γ widens to an *ap* conformation, but the β angle is in the ac^+ range, apparently prohibiting the compensatory conformational effect seen in tc-DNA. Moreover, bc-DNA residues adopt C2′-*endo* or C1′-*exo* puckers that are associated with B-form DNA. In the crystal structure of the DDD with tcdA 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 nuclease 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[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, ESI†).

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 (3σ) near the nucleobase of extrahelical 5′-terminal cytidines The tetrahedral coordination geometry and distances are consistent with Zn^{2+} (Fig. S2A, ESI†),[21,22] although the crystallization conditions did not include this ion. X-Ray absorption spectra recorded using a fluorescence detector of a [d(CGCG)-tc(A)-d(ATTCGCG)]$_2$ crystal at a synchrotron beamline confirmed that the ion was indeed Zn^{2+} (Fig. S3, ESI†). Zn^{2+} contamination could have resulted from a particular purification procedure or traces of the metal ion contained in certain chemicals used to produce the crystallization solutions. The Zn^{2+} ion coordinates to phosphate groups from 3′-terminal Gs and N3 atoms from 5′-terminal Cs belonging to four symmetry-related duplexes (Fig. S2, ESI†). Our structure

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.[23–26] 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 Co^{2+} or Ni^{2+}, but were of inferior quality compared to Zn^{2+} 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 Zn^{2+} 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 Zn^{2+} 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 tcdA 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 tcdA and the conformation of the nucleoside (the glycosidic angles of tcdA 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-nucleases and leads to the superior resistance against degradation exhibited by tc-DNA. Finally, the fortuitous observation of a Zn^{2+} binding site in the crystal structure of the tcdA-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 Mg^{2+}.

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Notes and references

‡ *Structure determination and refinement*: The structure was determined by the Molecular Replacement technique with the program MOLREP,[27,28] using coordinates for the DDD duplex with PDB ID code[29] 1ehv as the search model. Initially the duplex was refined as an all-DNA model in CNS.[30] At a later stage the 2′-deoxynucleotides A5 and A17 were replaced by tc-DNA nucleotides using the coordinates of tricyclo-DNA in the crystal structure of (3′*S*,5′*R*)-N^6-benzoyl-9-(2′-deoxy-3′,5′-ethano-5′,6′-methano-β-D-ribofuranosyl)adenine[13] as an initial model and for updating the bond lengths and angles dictionary files. Further isotropic as well as anisotropic refinement was carried out with the program Refmac,[31] randomly setting aside 5% of the reflections for calculating the *R*-free.[32] Water molecules were added into regions of superimposed ($2F_o - F_c$) sum and ($F_o - F_o$) difference Fourier electron density and the full model was refined in both space groups $P3_212$ and $P3_2$. Refinement in the latter space group (in which the geometry of the duplex is not restricted by a crystallographic dyad) consistently resulted in lower values for *R*-free and *R*-work (typically around 7% difference). Final refinement parameters and r.m.s. deviations from ideal bond lengths and angles are listed in Table S1, ESI.†

Data Deposition: Final coordinates and structure factors have been deposited in the Protein Data Bank:[29] PDB entry code 2RF3.

1 L. Kværnø and J. Wengel, *Chem. Commun.*, 2001, 1419–1424.
2 E. Lescrinier, M. Froeyen and P. Herdewijn, *Nucleic Acids Res.*, 2003, **31**, 2975–2989.
3 P. S. Pallan, C. J. Wilds, Z. Wawrzak, R. Krishnamurthy, A. Eschenmoser and M. Egli, *Angew. Chem., Int. Ed.*, 2003, **115**, 5893–5895.
4 M. Egli, P. S. Pallan, R. Pattanayek, C. J. Wilds, P. Lubini, G. Minasov, M. Dobler, C. J. Leumann and A. Eschenmoser, *J. Am. Chem. Soc.*, 2006, **128**, 10847–10856.
5 M. Petersen and J. Wengel, *Trends Biotechnol.*, 2003, **21**, 74–81.
6 M. Egli, G. Minasov, V. Tereshko, P. S. Pallan, M. Teplova, G. B. Inamati, E. A. Lesnik, S. R. Owens, B. S. Ross, T. P. Prakash and M. Manoharan, *Biochemistry*, 2005, **44**, 9045–9057.
7 M. Madeira, S. Shenoy, Q. N. Van, V. E. Marquez and J. J. Barchi, Jr., *Nucleic Acids Res.*, 2007, **35**, 1978–1991.
8 M. Egli, *Antisense Nucleic Acid Drug Dev.*, 1998, **8**, 123–128.
9 C. J. Leumann, *Chimia*, 2001, **55**, 1041–1044.
10 R. Steffens and C. J. Leumann, *Helv. Chim. Acta*, 1997, **80**, 2426–2439.
11 R. Steffens and C. J. Leumann, *J. Am. Chem. Soc.*, 1997, **119**, 11548–11549.
12 R. Steffens and C. J. Leumann, *J. Am. Chem. Soc.*, 1999, **121**, 3249–3255.
13 D. Renneberg and C. J. Leumann, *J. Am. Chem. Soc.*, 2002, **124**, 5993–6002.
14 D. Renneberg, E. Bouliong, U. Reber, D. Schümperli and C. J. Leumann, *Nucleic Acids Res.*, 2002, **30**, 2751–2757.
15 E. Johansson, G. Parkinson and S. Neidle, *J. Mol. Biol.*, 2000, **300**, 551–561.
16 S. Neidle, *Principles of Nucleic Acid Structure*, Academic Press Inc., London, UK, 2008.
17 A. Rich, *Nat. Struct. Biol.*, 2003, **10**, 247–249.
18 T. E. Haran, Z. Shakked, A. H.-J. Wang and A. Rich, *J. Biomol. Struct. Dyn.*, 1986, **5**, 199–217.
19 M. Egli, P. Lubini, M. Bolli, M. Dobler and C. J. Leumann, *J. Am. Chem. Soc.*, 1993, **115**, 5855–5856.
20 M. Teplova, S. T. Wallace, G. Minasov, V. Tereshko, A. Symons, P. D. Cook, M. Manoharan and M. Egli, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 14240–14245.
21 C. W. Bock, A. Kaufman Katz, G. D. Markham and J. P. Glusker, *J. Am. Chem. Soc.*, 1999, **121**, 7360–7372.
22 K. A. McCall, C. Huang and C. A. Fierke, *J. Nutr.*, 2000, **130**, 1437S–1446S.
23 N. G. A. Abrescia, L. Malinina, L. G. Fernandez, T. Huynh-Dinh, S. Neidle and J. A. Subirana, *Nucleic Acids Res.*, 1999, **27**, 1593–1599.
24 M. Soler-Lopez, L. Malinina, V. Tereshko, V. Zarytova and J. A. Subirana, *J. Biol. Inorg. Chem.*, 2002, **7**, 533–538.
25 S. L. Labiuk, L. T. J. Delbaere and J. S. Lee, *J. Biol. Inorg. Chem.*, 2003, **8**, 715–720.
26 N. Valls, G. Wright, R. A. Steiner, G. N. Murshudov and J. A. Subirana, *Acta Crystallogr., Sect. D*, 2004, **60**, 680–685.
27 A. Vagin and A. Teplyakov, *J. Appl. Crystallogr.*, 1997, **30**, 1022–1025.
28 Collaborative Computational Project, Number 4, *Acta Crystallogr., Sect. D*, 1994, **50**, 760–763.
29 Web address: http://www.rcsb.org.
30 A. T. Brünger, P. D. Adams, G. M. Clore, W. L. DeLano, P. Gros, R. W. Grosse-Kunstleve, J.-S. Jiang, J. Kuszewski, M. Niges, N. S. Pannu, R. J. Read, L. M. Rice, T. Simonson and K. L. Warren, *Acta Crystallogr., Sect. D*, 1998, **54**, 905–921.
31 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr., Sect. D*, 1997, **53**, 240–255.
32 A. T. Brünger, *Nature*, 1992, **355**, 472–475.