Crystal Structure of a Parallel-Stranded Duplex of a Deoxycytidylyl-(3′–5′)-deoxycytidine Analogue Containing Intranucleosidyl C(3′)–C(5′) Ethylene Bridges

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Received March 17, 1993

The proposal to use synthetic antisense oligonucleotides for therapeutic purposes[1] has led to a great interest in the modification of natural DNA and RNA molecules by chemical methods.[2] To probe the possibility of stabilizing duplex formation entropically by using antisense oligonucleotides with a conformationally more rigid sugar phosphate backbone as the complex partner for a natural DNA (or RNA) sequence, we designed and synthesized a new type of nucleosides (bicyclonucleosides) that differs from the natural deoxynucleosides by an additional ethylene bridge between the centers C(3′) and C(5′).[3] Studies on homodecamers with the nucleobases adenine and thymine thereof essentially confirmed the expected (numerical) reduction of the entropy term upon duplex formation and furthermore revealed a higher propensity for triplex formation of these analogues.[4] To obtain insight into the structural details of a bicyclo-DNA (bcd) single strand, and thus into its preorganization for duplex formation, we synthesized and crystallized the corresponding dinucleotide analogue, bcd(C_2), of deoxycytidylyl-(3′–5′)-deoxycytidine[5] and unexpectedly found it to form a parallel-stranded, right-handed duplex, paired via C-C^+ base pairs with three hydrogen bonds. The cytosine base pairs are stacked at a distance of 3.44 Å with a helical twist of 34°. Beyond the scope of the investigation, this first high-resolution crystal structure of a homocytosine minihelix is reminiscent of the suggested molecular arrangement of double-stranded poly(dC) at neutral pH.[6] It has been shown previously that poly(C) at low pH[7] forms a parallel-oriented, base-paired duplex; however, no structural details are known so far. Parallel orientation of strands with G-G and C-C^+ base pairs was previously observed in crystals of the duplex $[d(CpG)]_2$ grown at low pH, but the average distance of 4.34 Å between base pairs suggested only weak stacking interactions.[8] Self-pairing of cytosine bases via three hydrogen bonds occurs in crystals of cytosine-5-acetic acid[9] and cytosine hemitrichloroacetate.[10]

A single strand of the bcd(C_2) dimer is depicted in Figure 1, and selected torsion angles are given in Table I. It is apparent that the two bcd(C_2) molecules per asymmetric unit have similar geometries with a rms deviation of 0.02 Å. The backbone torsion angles fall into the ranges *-sc, ac, ac, ac, ap, -ac* (from α to ζ in 5′ to 3′ direction). A comparison of the backbone conformations of this dimer and the B-DNA dodecamer with sequence CGC-GAATTCGCG[11] shows that significant deviations occur only for torsion angles β and γ. In the dodecamer, these two torsions have conformations *ap, sc* in most of the nucleotide units. Thus, β is about 80° smaller and γ is about 80° larger in the parallel-stranded duplex. The alteration of γ seems to be a direct consequence of the chemical modification in the sugar framework and is consistent with observations in the monobicyclonucleosides. In the crystal structure of bicyclothymidine, γ was found to be 149°.[3] From an analysis of the torsion angles in backbones of A- and B-type duplexes, it appears that β and γ do not correlate. Nevertheless, the homopyrimidine base pairs of the $[bcd(C_2)]_2$ duplex can be spanned with such a backbone, rendering the resulting distance between stacked bases similar to the value observed for B-DNA.

The furanose moieties in the bcd(C_2) backbone adopt S-type pucker, as observed in DNA duplexes with B-conformation. For residues 1 and 3, the observed values for pseudorotation phase angles *P* (Table I) are associated with a C1′-*exo* conformation of the deoxyriboses, and for residues 2 and 4, with a C2′-*endo*

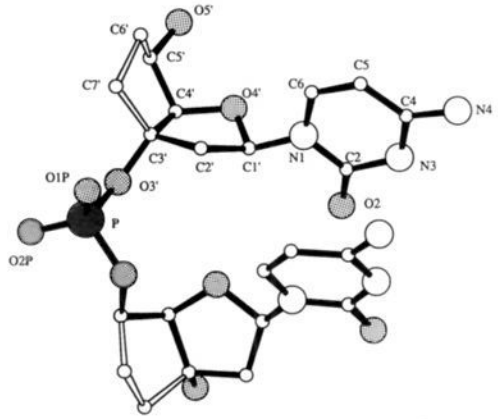

Figure 1. Structure of the bcd(C_2) dimer. Standard DNA backbone and base atoms are connected with solid bonds, and chemical modifications in bcDNA are drawn with open bonds. Oxygen atoms are stippled in grey, the phosphorus atom is stippled in black, and nitrogen atoms are drawn with larger radii than carbon atoms. Single crystals were grown at room temperature by equilibrating sitting drops (10–20 μL) of an aqueous solution of the ammonium salt of bcd(C_2) (20 mM) against a methanol reservoir. The space group is monoclinic $P2_1$ with unit cell dimensions a = 13.37(1) Å, b = 19.107(4) Å, c = 13.37(1) Å, β = 91.45(7)°, and Z = 4. Data were collected at 0 °C on a four-circle diffractometer (Enraf–Nonius CAD4) with graphite-monochromatized Mo Kα radiation (λ = 0.710 69 Å) by the ω scan method. A total of 3304 unique reflections were measured to 1.04 Å resolution, of which 2556 were observed above the $4\sigma(F_{obs})$ level. Intensities were corrected for Lorentz and polarization effects; no absorption correction was applied. The structure was solved with the direct methods program Shelxs-86,[15] and missing atom positions were located in a difference electron density map calculated after several rounds of least-squares refinement with the program Bigshelx-76.[16] The complete duplex was then refined with the program Shelxl-92,[17] including all 2556 reflections. The final asymmetric unit contained 93 heavy atoms, including 15 water and methanol molecules, and refinement was terminated at an R factor of 9.3% ($1/\sigma^2$ weights). Coordinates and displacement parameters will be deposited with the Cambridge Crystallographic Data Centre.

Table I. Backbone[a] and Glycosyl[b] (χ) Torsion Angles and Pseudorotation Phase Angles (P) for bcd(C_2) Strands (in deg)

	C(1)	C(2)	C(3)	C(4)		C(1)	C(2)	C(3)	C(4)
α		−76		−77	ϵ	180		179	
β		99		100	ζ	−103		−103	
γ	154	136	159	133	χ	−107	−143	−108	−146
δ	111	135	115	132	P	114	170	115	166

[a] The standard angular notations are as follows: O5′–β–C5′–γ–C4′–δ–C3′–ϵ–O3′–ζ–P–α–O5′. [b] The glycosyl torsion angle χ is defined as O4′—C1′—N1—C2.

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(1) (a) Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 280. (b) Stephenson, M. L.; Zamecnik, P. C. *Ibid.* **1978**, *75*, 285.
(2) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
(3) Tarköy, M.; Bolli, M.; Schweizer, B.; Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481.
(4) Tarköy, M.; Leumann, C. *Angew. Chem.*, in press.
(5) Details of the synthesis and X-ray analysis will be reported elsewhere.
(6) Inman, R. B. *J. Mol. Biol.* **1964**, *9*, 624.
(7) Langridge, R.; Rich, A. *Nature* **1963**, *198*, 725.
(8) Cruse, W. B. T.; Egert, E.; Kennard, O.; Sala, G. B.; Salisbury, S. A.; Viswamitra, M. A. *Biochemistry* **1983**, *22*, 1833.
(9) Marsh, R. E.; Bierstedt, R.; Eichhorn, E. L. *Acta Crystallogr.* **1962**, *15*, 310.
(10) Gdaniec, M.; Brycki, B.; Szafran, M. *J. Chem. Soc., Perkin Trans. II* **1988**, 1775.
(11) Dickerson, R. E.; Drew, H. R. *J. Mol. Biol.* **1981**, *149*, 761.

0002-7863/93/1515-5855$04.00/0

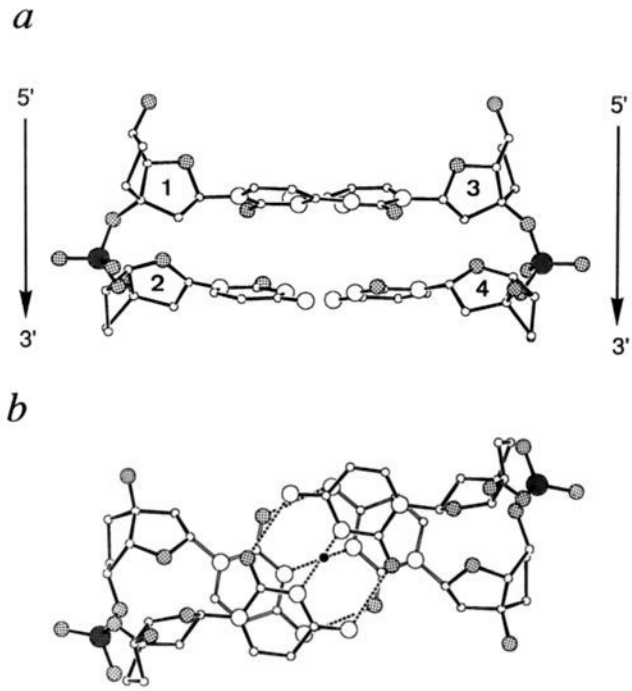

Figure 2. The $[bcd(C_2)]_2$ duplex viewed perpendicular (a) and along the helical axis (b, indicated by a solid dot). Atom types are coded as in Figure 1, arrows indicate parallel orientation of strands, hydrogen bonds are dashed, and res. are numbered.

Table II. Helical Parameters[a] of $[bcd(C_2)]_2$ (angles in deg and distances in Å)

rise	3.25	tilt	0.3
twist	34	P···P	17.0
roll	−0.3	C1′···C1′	9.6

	base pair C(1)·C(3)	base pair C(2)·C(4)		base pair C(1)·C(3)	base pair C(2)·C(4)
buckle	27	−1	N3···N3	2.83	2.74
propeller twist	12	−15	N4···O2	2.86	2.88
O2···N4	2.86	2.83			

[a] Calculated with the program NEWHELIX, version NEWHEL90.[18]

conformation. These two puckering modes are also observed in the free bicyclonucleosides, in which the occurrence of an N-type (C3′-*endo*) conformation can be excluded for steric reasons. Torsion angle δ is thus restricted to the *ac* conformation.[3] The *P* values in the carbocyclic rings are more homogeneous than the ones of the furanose rings and differ by 35° between intrastrand residues. The glycosidic torsion angles χ for all four residues fall into the *anti* range but differ by about 40° for intrastrand residues.

Views of the $[bcd(C_2)]_2$ duplex are depicted in Figure 2, and helical parameters are listed in Table II. A characteristic feature of the parallel-stranded double helix is the lack of local twofold rotation axes between and in the planes of the base pairs, normal to the helix axis, as found in B-DNA. As shown in Figure 2, a pseudo-twofold rotation axis falls together with the helix axis. The different geometries of base pairs C(1)·C(3) and C(2)·C(4), already indicated by different sugar puckers and glycosidic torsion angles, are also apparent from the view into the groove in Figure 2a. Base pair C(1)·C(3) shows a considerable buckle, whereas the buckle in base pair C(2)·C(4) is minimal. However, the hydrogen-bonding distances between bases vary only marginally as a result of such local geometrical perturbations. In both base pairs the cytosine bases are rotated relative to one another around the hydrogen-bonding directions (propeller twisting).

For a homopyrimidine duplex, the resulting stacking interactions between bases both within and across strands (Figure 2b) are believed to be weaker than interactions in duplexes with paired purine and pyrimidine bases. Nevertheless, the arrangement of stacked base pairs in the parallel-stranded duplex is reminiscent of the stacking in B-DNA. The mean distance between bases in the $[bcd(C_2)]_2$ duplex is 3.44 Å, with a helical twist of 34° (B-DNA, 3.4 Å and 36°). Other factors in addition to stacking interactions also contribute to the stability of the duplex in the present case. Figure 2b shows that the relative orientations of keto groups must result in strong dipole–dipole interactions. The large interplanar angle of 17° between both pairs of stacked bases reduces the distance between the keto oxygen of one base and the keto carbon of the base stacked on it (2.88 and 2.91 Å, respectively), leading to a stabilizing electrostatic interaction. The importance of dipole–dipole interactions was noted before with an A-DNA octamer, where correlated changes in the geometry of the duplex resulted in a favorable parallel arrangement of adjacent keto groups.[12] Favorable π–π^* interactions between protonated and neutral intrastrand cytosine bases may account for the relatively close distance between the bases.

There are no intermolecular stacking interactions in the $bcd(C_2)$ crystal lattice. Both N4 amino groups of base pair C(1)·C(3) are engaged in hydrogen bonds to phosphate groups from neighbor duplexes (distances 2.83 and 2.81 Å, respectively). The N4 amino groups of base pair C(2)·C(4) both form hydrogen bonds to hydroxyl groups of methanol molecules. Lattice interactions also include hydrogen bonding between terminal O3′ hydroxyl groups and phosphate oxygens from adjacent duplexes. Each of the phosphorus groups is engaged in four contacts to neighbor molecules, two of which are water molecules.

The lack of structural data for a longer parallel-stranded fragment requires that the topology of such a duplex be derived from helical parameters of short segments such as the $[bcd(C_2)]_2$ duplex.[13] Similarly, crystal structures of double-helical RNA dimers provided insight into topological features of the double helix several years before the structure determination of larger fragments.[14] Although the discrepancy between the calculated positions for atoms in the generated helix and the values observed in the crystal structure is fairly large in the present case, such a model allows a somewhat qualitative analysis of the topological features of this parallel-stranded double helix. The diameter of the double helix is approximately 22 Å, with two identical grooves that are about 6 Å deep and 9 Å wide.

In the case of the $[bcd(C_2)]_2$ duplex, the formation of a zwitterion with positively and negatively charged groups located within the molecule, with no coordinating counterions necessary, may have contributed to the stability and thus facilitated crystallization. However, we were unable to produce diffraction-quality crystals of the native $d(C_2)$ dimer. The knowledge of the detailed geometry of DNA analogues with structural features similar to those of native DNA, such as displayed by the molecule described here, may also contribute to the understanding of DNA structures for which no direct data are yet available.

(12) Shakked, Z.; Rabinovich, D.; Kennard, O.; Cruse, W. B. T.; Salisbury, S. A.; Viswamitra, M. A. *J. Mol. Biol.* **1983**, *166*, 183.
(13) Rosenberg, J. M.; Seeman, N. C.; Day, R. O.; Rich, A. *J. Mol. Biol.* **1976**, *69*, 979.
(14) Rosenberg, J. M.; Seeman, N. C.; Park Kim, J. J.; Suddath, F. L.; Nicholas, H. B.; Rich, A. *Nature* **1973**, *243*, 150.
(15) Sheldrick, G. M. In *Crystallographic Computing 3*; Sheldrick, G. M., Krüger, C., Goddard, T., Eds.; Oxford Univ. Press: Oxford, 1986; pp 175–189.
(16) Maverick, E. *Bigshelx-76*; Univ. of California: Los Angeles, 1985.
(17) Sheldrick, G. M. *Shelxl-92*, Beta version; Univ. of Göttingen: Göttingen, 1992.
(18) Dickerson, R. E., *et al. Nucleic Acids Res.* **1989**, *17*, 1797.