Crystal Structure of a Dimethylene Sulfone-Linked Ribodinucleotide Analog

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Received February 15, 1995

Although the pairing of complementary natural oligonucleotides to form a double helix is a well-studied phenomenon, relatively little is known about the impact on the conformation of the double helix induced by small modifications of the backbone structure. This impact is especially interesting when the backbone modification involves the loss of charge. Nonionic oligonucleotide analogs are believed to stand a better chance of entering cells than their polyanionic counterparts, and they are being sought as gene-targeted and antisense reagents in many laboratories.[1]

The dimethylene sulfone unit offers a largely isosteric replacement of the phosphodiester group in natural nucleic acids,[2,3] removing the charge associated with the phosphodiester without introducing a stereogenic center or undesired reactivity (Figure 1). We report here the crystal structure of a Watson−Crick base-paired miniduplex of the guanylyl-(3′,5′)-cytidine ribodinucleotide analog containing this linker.

The structure is remarkable first for its similarities to the structure of the natural RNA dimer duplex, $[r(G_pC)]_2$.[4] In the crystal, two dimethylene sulfone-linked $r(G_{SO_2}C)$ dimers form a fragment of a right-handed double helix with antiparallel orientation of strands that are related via a crystallographic twofold rotation axis, as in $r[(G_pC)]_2$ (Figure 2). Conformations around the glycosidic bonds (*anti*), ribose puckers (C3′-*endo*-type), and all backbone torsion angles (Table 1, Figure 3) fall into the same ranges as those in natural RNA duplexes. Further, the overall dimensions of the two duplexes (e.g., relative S(P)···S(P) and C1′···C1′ distances, Table 2) are the same to within 0.3 Å.

Closer examination reveals several noteworthy differences between the two structures, however. The glycosidic torsion angles differ by 21° for G residues and by 18° for C residues, and torsion angles α, δ, and ε each differ by at least 10° in the two duplexes (Table 1). Although torsion angles δ and χ in $[r(G_{SO_2}C)]_2$ are within the ranges observed in natural RNA oligonucleotide duplexes (Figure 3), they are at the extremes of these ranges.

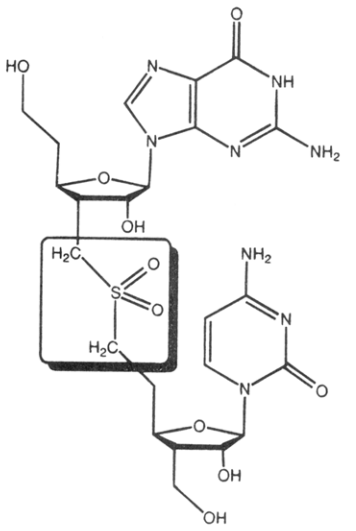

Figure 1. Structure of $r(G_{SO_2}C)$.

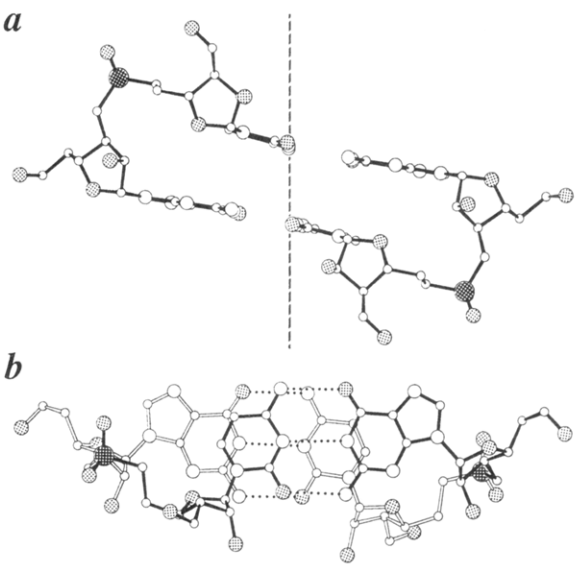

Figure 2. $[r(G_{SO_2}C)]_2$ viewed normal to the helix axis (dashed line, a) and roughly normal to the top base pair (solid bonds, b). Oxygen stippled in gray, sulfur stippled in black, carbon drawn with smallest radii, and hydrogen bonds dotted. Only the major site (occupancy 54%) of the disordered 3′-terminal oxygen of cytidine is depicted. Single crystals were grown at room temperature by slow evaporation of a ~5 mM (single strand) 1:1 H_2O/MeOH solution. A crystal (~0.15 × 0.05 × 0.02 mm) was mounted with mother liquor in a glass capillary. Space group is orthorhombic $P2_12_12$; cell constants a = 9.965(8) Å, b = 24.527(4) Å, c = 14.647(2) Å, V =3580(3) Å^3, and Z = 4. Data were collected at room temperature on a four-circle diffractometer (Enraf-Nonius CAD4, Cu Kα, ω-scan, 3024 unique reflections, 755 with $F_o \geq 3\sigma(F_o)$). Nominal data resolution is ~1.35 Å. Intensities were corrected for Lorentz and polarization effects, but not for absorption. The structure was solved by direct methods (SHELXS-86[5]). Missing fragments were built into the initial structure using graphics. The dimer was first refined as the phosphodiester compound. Limited data resolution necessitated restraints (X-PLOR,[6] standard RNA dictionary). Electron density maps were generated in the CCP4 suite[7] and visualized with CHAIN.[8] Solvent molecules were added gradually, and the structure was further refined as the dimethylene sulfone-linked dimer (SHELXL-93[9]). Hydrogen positions were calculated and included in the refinement. The final asymmetric unit contained 51 heavy atoms, including eight water molecules and one methanol molecule, resulting in an R factor of 11.8%. Coordinates and displacement parameters will be deposited with the Cambridge Crystallographic Data Centre.

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(2) The O3′−PO_2^-−O5′ linkage of natural oligonucleotides is replaced by C3″H_2−SO_2−C6′H_2. Preparation followed a general strategy[3a] similar to one previously reported for dimethylene sulfone-linked *deoxy*ribonucleotide analogs.[3b,c] Details of the synthesis of RNA analogs will be reported separately.

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Table 1. Comparison of Backbone[a] and Glycosyl (χ) Torsion Angles as Well as Pseudorotation Phase Angles (P) (in deg) for $r(G_{SO_2}C)$ and $r(G_pC)$[4]

	α	β	γ	δ	ϵ	ζ	χ	P
G(1)			176	93	−140	−66	−178	12
C(2)	−58	−179	44	86			−170	29
G(1)[4]			49	82	−150	−66	−157	7
C(2)[4]	−75	−176	51	75			−152	17

[a] The angular notations for backbone torsions are as follows: O5′-(C6′)$^{\beta}$-C5′$^{\nu}$-C4′$^{\delta}$-C3′$^{\varepsilon}$-O3′(C3″)$^{\zeta}$-P(S)$^{\alpha}$-O5′(C6′).

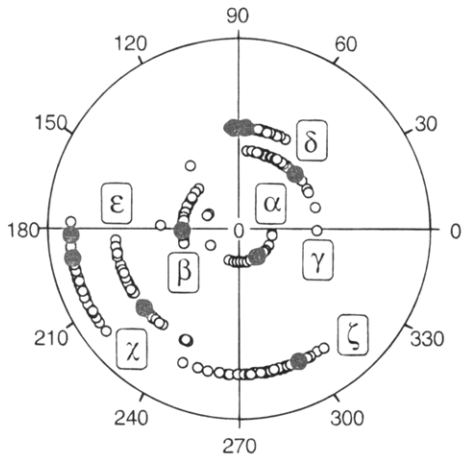

Figure 3. Backbone and glycosidic torsion angles of $[r(G_{SO_2}C)]_2$ (●), compared with those of $[r(U(UA)_6A)]_2$,[10] $[r(CGCGAAUUAGCG)]_2$,[11] and $[r(CCCCGGGG)]_2$[12] (○).

Table 2. Comparison of Helical Parameters[a,13] for $[r(G_{SO_2}C)]_2$ and $[r(G_pC)]_2$[4] (angles in deg, distances in Å)

	rise	twist	incl	slide	roll	S···S or P···P	C1′···C1′	buckle	prop twist
$G_{SO_2}C$	2.92	20.8	9.4	−3.2	0.7	18.0	10.6	6.0	0.8
G_pC	2.59	34.7	28.0	−1.3	7.9	17.7	10.7	5.8	2.7

[a] Atoms selected to determine the helical operator were C1′, N1(9), C2′, and O4′.[14]

The helical parameters of the two duplexes also differ modestly (Table 2). Bases are stacked essentially parallel in the sulfone duplex, with a spacing of 3.40 Å, instead of the 3.68 Å in $[r(G_pC)]_2$. Base pairs in $[r(G_{SO_2}C)]_2$ are offset by 1.9 Å along their long dimensions relative to their positions in $[r(G_pC)]_2$. This increased slide is accompanied by less intrastrand stacking (Figure 2b) and a more pronounced skewing of the dimethylene sulfone backbone with respect to the helical axis (Figure 2a). The sulfone duplex has a low twist (Table 2), and an extended duplex structure built by extrapolation from the geometry of the dimer would therefore have ~17 residues per helical turn rather than the 11 residues in standard A-type RNA.

The differences in the overall structures between the duplexes appear to arise from several factors. First, the S−C6′ bond (1.78 Å) is 0.2 Å longer than the corresponding P−O5′ bond. Since the S−C6′ vector points roughly in the direction of Watson−Crick hydrogen bonds (Figure 2), this elongation moves the cytosines from opposite strands toward each other by a total of ~0.4 Å. The bond length and angle differences incurred at S−C3″−C3′ in comparison to P−O3′−C3′ of $[r(G_pC)]_2$ appear to be mutually exclusive in their effect on the structure.

Second, the C6′ methylene group in the sulfone duplex would interact sterically with the hydrogen atom of C6 in cytosine if C6′ occupied the same position as the corresponding O5′ in the natural RNA duplex. This interaction is avoided by an increased C6′−C5′−C4′ angle (115°) and a slight change in the cytidine ribose conformation (compare P and δ values of residues C(2) of the two duplexes in Table 1). Increasing δ toward 90° (in the upper range of δ values in natural oligonucleotides, see Figure 3) brings about a larger separation of C6′ and C6 by changing the sugar pucker from C3′-*endo* toward C4′-*exo*. The C6′−H_R hydrogen thereby occupies the approximate position assumed by O5′ in the natural duplex. The resulting contribution to sliding from these structural rearrangements in both strands is about 1.2 Å.

Third, to avoid an unfavorable contact between the C6′−H_R hydrogen and the C2′ hydrogen of guanosine, both the cytidine torsion angle α and the guanosine torsion angle ϵ are smaller than the corresponding angles in the natural duplex. The decrease in ϵ is accompanied by an increase in antiperiplanarity of the glycosidic torsion in guanosine and hence further sliding of base pairs. In RNA duplexes, ϵ and χ torsion angles seem to be weakly correlated.[10,11]

The low twist in the $[r(G_{SO_2}C)]_2$ double helix appears to result predominantly from the bond angle of 111° for S−C6′−C5′, instead of the 120° found in RNA for the corresponding P−O5′−C5′ angle. Because the plane defined by atoms S, C6′, and C5′ and the cytosine base plane are roughly parallel (Figure 2a), and because helical twist is defined by the relation between the glycosidic bonds of adjacent bases, the tighter angle in the sulfone RNA backbone has the effect of unwinding the duplex by about 9° per nucleotide. The altered cytidine sugar pucker, as discussed above, may also occasion low twist.

The absence of the negative charge in sulfone RNA has remarkably little effect on the overall duplex solvation. The sulfone crystal carries eight water molecules and one methanol (per strand); the natural duplex carries nine water molecules. However, instead of forming contacts with a sodium ion and three water molecules, as seen with the phosphate, the sulfone group forms contacts with one water molecule, a cytosine NH_2 group from a neighboring duplex, and the CH_3 group of a methanol molecule.

This is the first crystal structure of a nonionic RNA analog. Further, it provides another structure of an oligonucleotide analog involving sugars that, through substitution of the O3′ by carbon, lack some of the ring *gauche* effects displayed by ribosides.[15] The main driving force behind conformational changes in the sulfone RNA duplex compared to the natural RNA duplex is most likely the C6′ methylene group replacing O5′. Otherwise, the overall conformations of the dimethylene sulfone RNA analog and natural RNA duplexes are remarkably similar.

Acknowledgment. We thank Dr. Paolo Lubini for help with helix analysis programs.

Supporting Information Available: PDB-format coordinates and isotropic temperature factors for $[r(G_{SO_2}C)]_2$ (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA950532T

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