Structure of a Dinucleotide: Thymidylyl-(5′→3′)-Thymidylate-5′ (pTpT)

Abstract. *The crystal and molecular structure of the naturally occurring deoxyribose dinucleotide sodium thymidylyl-(5′→3′)-thymidylate-5′ has been determined by x-ray diffraction. There are four molecules of dinucleotide and 52 water molecules in an orthorhombic unit cell of dimensions (in angstroms)* a = *16.06*, b = *15.13*, c = *15.65, space group* $P2_12_12$. *There is a very high degree of conformational consistency between the two halves of the molecule when the dinucleotide is viewed as the combination of two 5′-mononucleotides. The planes of the two thymines are not parallel, but are tilted 38° with respect to each other. An extensive system of hydrogen bonding exists involving the bases, waters, phosphates, and sodiums; no base-base hydrogen bonding occurs. The dinucleotide structural parameters should be of assistance in interpreting DNA fiber diagrams in terms of possible structures.*

Ever since the model building of Watson and Crick which resulted in the proposal of the double-helical structure for DNA, much research has been performed with the intent of obtaining experimental data on the structures of nucleic acids. The crystal and molecular structures of a sizable number of mononucleosides and mononucleotides have been determined, and information so obtained has been used as a basis for the refinement of diffraction data from oriented nucleic acid fibers to postulate atomic coordinates for both DNA and RNA double helices (*1*). However, the structures of monomeric units do not yield information on the conformational features of the 3′,5′-phosphodiester internucleoside linkages—that is, on the favored arrangements of the monomers with respect to each other. The structures of the dinucleoside phosphates uridylyl-3′,5′-adenosine (UpA) (*2*), guanylyl-3′,5′-cytidine (GpC) (*3*), and adenylyl-3′,5′-uridine (ApU) (*4*) were elucidated, yielding data on the mutual arrangement of ribonucleosides about the 3′,5′-phosphodiester bond. We now present the crystal and molecular structure of a naturally occurring dinucleotide, sodium thymidylyl-(5′→3′)-thymidylate-5′ (sodium pTpT). This is the first three-dimensional structural determination of a dinucleotide, and provides the first direct information on the conformational arrangement of deoxynucleosides about the intermonomer linkage, and on comparative conformational features of two similar mononucleotides in the same molecule.

Crystals of the dinucleotide sodium pTpT obtained from a mixture of ethanol and water (1 : 1) (pH ~ 6.3) were colorless plates elongated along a with (001) faces developed (*5*). The crystals were orthorhombic with cell dimensions $a = 16.06$ Å, $b = 15.13$ Å, $c = 15.65$ Å, space group $P2_12_12$, and density (measured in chloroform and bromoform) = 1.587 g cm^{-3}. The density measurement indicated the presence of four molecules of the dinucleotide and 48 to 52 molecules of water per unit cell; thus the asymmetric unit consists of one molecule of sodium pTpT and 12 to 13 water molecules. X-ray reflection intensities were collected on a manual diffractometer with the use of CuKα radiation; the intensities diminished rapidly with diffraction order, and few were observable at spacings smaller than 1.2 Å. A total of 966 reflections had intensities above background, and these were used in the structure refinement.

The structure was solved by direct methods. Positions of the phosphate groups were located on a three-dimensional E map computed from the best set of phases generated by the multiple-phase tangent formula program TANFOR. (The probability criteria for acceptance of phases as correct had to be lowered considerably in order to obtain any solutions at all.) The rest of the atoms, including 2 sodium ions and 13 water molecules per dinucleotide molecule, were found from a number of cycles of Fourier and difference Fourier calculations. Refinement was by least squares with anisotropic thermal parameters for the phosphate groups and isotropic for the other atoms. Positions for 22 of the hydrogen atoms on the dinucleotide were assigned from peaks on a difference Fourier map, and with these included in the structure factor calculations the current discrepancy index $R = 0.116$.

Figure 1 is a stereoscopic drawing showing the molecular conformation of pTpT. The thymine rings are planar within experimental error (maximum deviations from planarity are 0.05 and 0.02 Å in the two rings). The two thymines in the same molecule are not coplanar but are tilted with respect to each other, the acute angle between normals to the rings being 38°. Both bases are in the *anti* conformation with respect to the glycosidic bond, as has been found in the structures of a large majority of the nucleic acid derivatives so far determined. Both deoxyribose rings are in the C2′-*endo* C3′-*exo* conformation; this contrasts with the C3′-*endo* C2′-*exo* conformation adopted by the ribose rings in all three dinucleoside phosphates quoted above. The phosphate groups are approximately tetrahedral in configuration, and the intramolecular P–P distance is 6.64 Å.

A feature of the molecular structure of pTpT is the great similarity of conformational characteristics between the two halves of the molecule. Figure 2 shows pTpT viewed as two 5′-mononucleotides; the molecule labeled A is the 3′-OH end of the dinucleotide. A high degree of conformational consistency has been noted in the nucleoside units of all three ribose-containing dinucleoside phosphates thus far structurally elucidated (*3*); Fig. 2 illustrates that a remarkable conformational consistency exists in the deoxyribose dinucleotide pTpT not only between the two nucleoside components, but including the phosphates as well. Thus pTpT consists of two conformationally virtually identical 5′-mononucleotides with orientation variability provided by

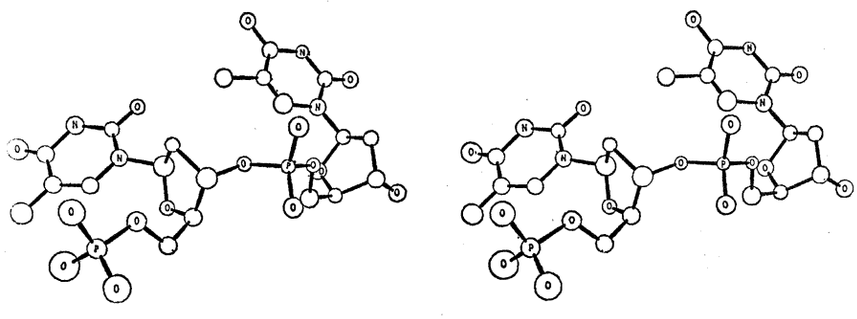

Fig. 1. Stereoscopic drawing of thymidylyl-(5′→3′)-thymidylate-5′.

Table 1. Torsion angles in pTpT.

Angle	Size (degrees)	Angle	Size (degrees)
ω	− 72 (− 83)	χ	34
ϕ	− 173 (173)	χ'	27
Ψ_{0c}	41	ρ	158
Ψ'_{0c}	46	ρ'	155
Ψ_{00}	− 78	ϕ'	− 108
Ψ'_{00}	− 68	ω'	163

possible rotation about the linkage between 5′-mononucleotides.

The similarity in conformation between the two nucleotide halves of the molecule is further seen in the torsion angles listed in Table 1. The angle lettering scheme (Fig. 3) is that previously adopted for dinucleoside phosphates (*6*); the numbers in parentheses refer to corresponding angles in the 5′-terminal phosphate. Comparison of the two values for ω and ϕ and those for ψ and ψ', χ and χ', and ρ and ρ' shows that the largest difference between corresponding torsion angles in the two nucleotide units is only 14°, with the average difference being 8.3°.

Seven basic conformations for nucleic acid structural units have been postulated (*6*) with the use of information from dinucleoside phosphate structures. In terms of the parameters used to define the seven conformations, pTpT fits in the category designated P_2. However, the conformation of pTpT differs considerably from that illustrated for P_2 (*6*); this is due to the large difference (~80° compared to ~150°) between the angles ρ, ρ' in C3′-*endo* nucleotides (used as the basis for the seven conformational postulations) and in C2′-*endo* nucleotides (pTpT), and suggests that new models taking into account the value of ρ and ρ' must be constructed for C2′-*endo* polynucleotides.

There is no thymine-thymine hydrogen bonding in the crystal structure of sodium pTpT. The imino hydrogens of the bases hydrogen bond to phosphate oxygens in other molecules, the O(4) carbonyl oxygens have contacts with waters and with the free 3′-OH group, and the O(2) carbonyl oxygens of the bases form intermolecular attractions only with sodium ions. All of the "free" oxygens of the phosphate groups take part in interactions with water molecules, sodium ions, or imino hydrogens. There is no hydrogen bonding involving carbon-bound phosphate oxygens or the sugar ring oxygens. There is also no close contact between C(6) of the bases and O(5′) of the sugars of the kind observed in UpA (*2*) [C(6) . . . O(5′) distance in pTpT = 3.4 Å].

There appears to be some base stacking in the pTpT crystal. Nucleotide A thymines (Fig. 2) related by the twofold axes are 3.5 Å apart, and their planes are tilted 8° away from being parallel; each is then 3.85 Å from a thymine of nucleotide B whose plane roughly overlaps that of thymine A and is 15° from parallel. Nucleotide B thymines related by the twofold axes have only C(2)–O(2) bonds overlapping: these are 3.5 Å apart and the rings are 10° away from being parallel planes.

Although it has been routinely considered (*7*) that at near-neutral *p*H pTpT is triply ionized, we find only two sodium ions per dinucleotide in the crystal, indicating only a single dissociation for the terminal phosphate group. One of the sodiums is octahedrally coordinated to a bridging-phosphate oxygen, two carbonyl O(2)'s, and three water molecules; the other sodium ion appears to be disordered among two sites, and is coordinated to the terminal phosphate only through water molecules. There is also some disorder in the positioning of at least one of the water molecules.

The structure of pTpT provides the first direct information on the mutual arrangement of deoxyribose nucleotides about an internucleotide linkage. The remarkable degree of conformational similarity in the two 5′-mononucleotide halves of the molecule indicates a high degree of stability for this conformation, and the large amount of hydration in the crystal suggests that the situation in the crystal may be similar to that in a biological milieu. The structural parameters for pTpT should contribute to the understanding of preferred conformations in nucleic acids and to the interpretation of nucleic acid fiber diagrams in terms of model structures for polynucleotides.

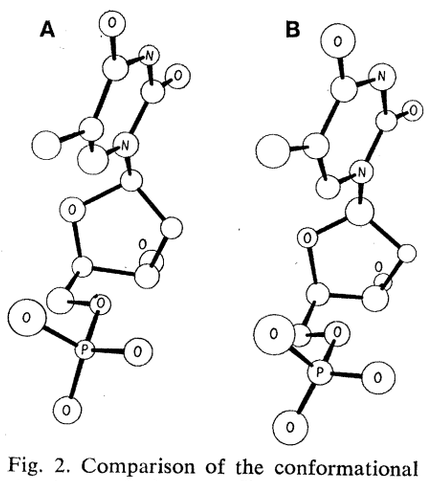

Fig. 2. Comparison of the conformational structures of the two 5′-mononucleotides making up pTpT when viewed in similar orientations. Nucleotide A is the free 3′-hydroxyl end of the dinucleotide and nucleotide B is the free 5′-phosphate end.

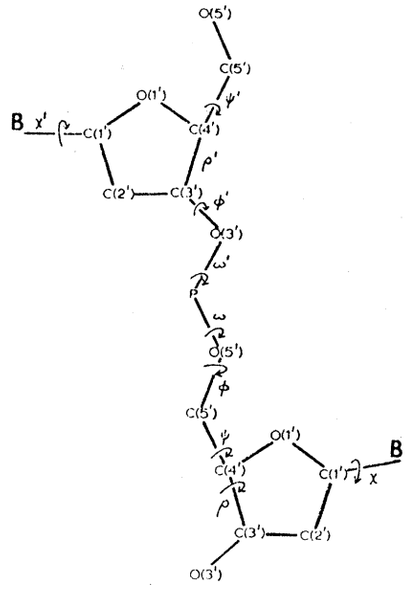

Fig. 3. Nomenclature for torsion angles; *B*, base.

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7. See, for example, P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.* **80**, 6212 (1958).

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