greater than that of normal leukocytes suggests that this may be the case. It further suggests that a patient suddenly deprived of his peroxidase-mediated antimicrobial systems may be less able to combat infection than a patient with a genetic absence of myeloperoxidase.

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Cyclic Cytidine 2′,3′-Phosphate: Molecular Structure

Abstract. *Monoclinic crystals of the sodium salt of cytidine 2′,3′-phosphate contain two anions in the asymmetric unit. Both bases are in the* syn *conformation, and the nucleotides are stacked together into an antiparallel stranded ribbon with the bases 3.3 angstroms apart. One ribose ring is planar, and the other has oxygen-1′ puckered toward carbon-5′. The phosphorus atoms in the five-membered ester rings are puckered toward the sugars. The conformations about the carbon-4′—carbon-5′ bonds are* gauche-trans *and* gauche-gauche.

Pyrimidine ribonucleotides containing a cyclic 2′,3′-phosphate ester linkage are intermediates in the hydrolysis of ribonucleic acid, a reaction catalyzed by ribonuclease (*1, 2*). The cyclic phosphate ester is then further hydrolyzed to give pyrimidine 3′-phosphate and purine oligo nucleotides. The crystal structures of ribonuclease A (*3*) and ribonuclease S (*4*) are being studied at high resolution, and these structures taken in conjunction with the biochemical studies (*1*) should permit the proposal of precise mechanisms for ribonuclease action. No detailed molecular structures have been reported for the cyclic phosphate esters involved in this process; therefore the results of this study of the crystal structure of the sodium salt of cyclic cytidine 2′,3′-phosphate (2′,3′-CMP) will be useful in evaluating hydrolysis mechanisms.

Crystals of the sodium salt of 2′,3′-CMP (Schwarz BioResearch; lot No. 6802) were prepared by diffusing ethanol into an aqueous solution of the salt over a period of several weeks. The crystals were clusters of clear, thin, strongly birefringent plates which tended to stack together. The crystals are monoclinic, space group $P2_1$, with cell dimensions: a, 6.736 ± .005 Å; b, 11.01 ± .01 Å; c, 19.54 ± .01 Å; and β, 95° ± 0.1°. The measured flotation density (1.66 g/cm^3) suggests four molecules of sodium 2′,3′-CMP and eight molecules of water per cell ($d_{calc} = 1.67$ g/cm^3); there are thus two anions per asymmetric unit. The x-ray diffraction data were collected on a General Electric goniostat with a single crystal orienter with copper radiation. The refinement was based on 1964 nonzero intensities with 2θ less than 125°. These data were collected when the indoor relative humidity was 10 percent or greater; at lower humidities reversible intensity changes were evident, presumably reflecting the loss of some loosely bound water molecules.

One phosphate group and the second unique phosphorus were located in the cell by inspection of a sharpened three-dimensional Patterson synthesis. The phase angles calculated with the use of these atomic positions were refined using the tangent formula (*5*), and a Fourier map based on the refined phases and observed amplitudes revealed the main features of the structure. The trial structure was refined by the method of least squares, with each of the 46 atoms assigned an isotropic temperature parameter. Three cycles of refinement reduced the agreement index (R) to 8.5 percent. The structure described here is that reflected by the coordinates from the isotropic refinement. The estimated errors in bond lengths are ±.02 Å and ±1° in bond angles. We are continuing refinement but most of the unusual features of the structure are clear now. A difference Fourier synthesis is being examined for hydrogen atom peaks; no unexplainable features are evident in this map, and there are no indications of disorder.

From a diagram (Fig. 1) of the 2′,3′-CMP anions looking approximately down the b axis, two features of the structure are apparent. First, the bases are aligned parallel to one another, roughly 3.3 Å apart, and the anions form an anti-parallel stranded base-stacked ribbon. This suggests stabilization of the crystal structure through hydrophobic interactions of the bases; such interactions contribute significantly to the stability of nucleic acid helices (*6*) and are often found in crystal structures of purine derivatives. This stabilization is not as common with pyrimidines; for example, the pyrimidine nucleosides 5-chlorouridine (*7*) and 5-bromouridine (*8*) form

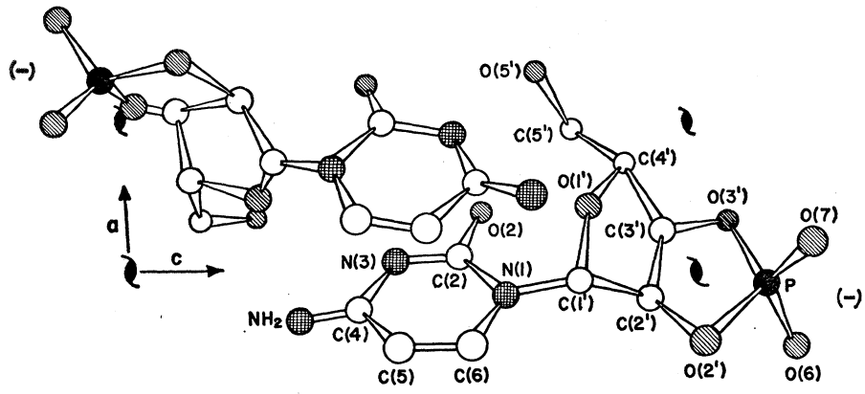

Fig. 1. A schematic view of the 2',3'-CMP anions down the *b* axis. Sodium ions and water molecules are not shown.

parallel stranded ribbons with base-linked hydrogen bond stabilization. The second feature evident from the figure is that the bases are in the *syn* conformation (*9*) about the β-glycosidic C(1')–N(1) bond, with carbonyl oxygen O(2) over the ribose ring. The torsion angles are 253° and 242° in Sundaralingam's notation (*10*) for the two anions, making both in the (−) anticlinal orientation (*10, 11*). Such base conformations are not unusual in purines; similar torsion angles were recently reported for 8-bromoadenosine and 8-bromoguanosine (*12*). These conformations are stabilized by intramolecular O(5')–H . . . N(3) hydrogen bonds. In pyrimidines such conformations are very unusual because of the close contacts between oxygen O(2) and the sugar atoms (*13*); the only other known case is 4-thiouridine (*14*) where the torsion angle was 277°. The intramolecular contact distances for the two 2',3'-CMP anions are 2.81 and 2.91 Å for O(2)–C(2') and 3.02 and 2.88 Å for O(2)–O(1'). There is no indication of disorder in the base orientation; the bases may be held in the *syn* conformation by the O(2) contacts and the rather rigid sugar and ester rings. Some suggested modes of binding of 2',3'-CMP to ribonuclease (*2*) involve a hydrogen bond between a histidine and carbonyl oxygen O(2). This arrangement would be sterically very different if the base were *syn* to the sugar, and it seems possible that either the O(2) oxygen is required to keep the base in the *syn* conformation or there are two distinct isomers of 2',3'-CMP, one *syn* and one *anti*, with a significant energy barrier between them. The latter possibility is now being investigated. Meadows *et al.* (*15*) have suggested, on the basis of a nuclear magnetic resonance study, that 2'-CMP is in the *syn* conformation when bound to ribonuclease, and they showed that this substrate orientation was compatible with the structure of the active site revealed by the x-ray analyses of the enzyme (*3, 4*). Further studies are needed to determine whether ribonuclease selectively binds *syn* 2',3'-CMP.

The allowed values for torsion angles correlate directly with the sugar conformation (*10, 13, 16*). The sugar and phosphate ester rings in the two anions we studied are different and, in the case of the sugars, unusual. In the first anion, the sugar atoms all lie in a plane ±0.001 Å; the other sugar has O(1') out of the plane (±.02 Å) of C(1'), C(2'), C(3'), and C(4') by 0.49 Å on the same side as C(5'). The ester rings have O(2'), C(2'), C(3'), and O(3') in a plane (±.01 and ±.03 Å) and the phosphrous atoms puckered 0.47 and 0.41 Å toward the sugars. The dihedral angles between the five-membered sugar and ester rings are 118° and 117°. Adjacent anions along the *a* and *c* axes are linked through sodium ions and the water molecules. Planar or even nearly planar ribose rings are not generally observed; C(2') or C(3') is usually puckered. These ring conformations suggest that 2',3'-CMP is a strained molecule. The O(2')–P–O(3') angles reflect this, because they are 96.2° and 96.1° rather than the 103° found in less strained esters (*17*), and the C(2')–C(3')–C(4') angles are both 107° rather than the 100° value usually observed (*18*). The C(3')–O(3') distances are also significantly longer (1.48 and 1.46 Å) than the expected 1.40 Å (*18*). The other distances and angles within the anions do not differ significantly from those expected (*18, 19*), and the cytosine base dimensions are, within error, identical to those suggested by Donohue (*19*). The C(1') atoms are 0.21 and 0.09 Å out of the least-squares planes (±.03 and ±.01 Å) through the bases.

Another conformational feature is the orientation of O(5') about the C(5')–C(4') bond. The dihedral angles with O(1') are 43.5° and 61° and with C(3') 161° and 54°; the projected O(5') position falls inside the sugar ring, between O(1') and C(3'), in the second case and outside the ring in the first anion. These correspond to the *gauche-trans* and *gauche-gauche* conformations, both of which are allowed (*10, 16*).

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