Crystal Structure of an RNA Duplex Containing Phenyl-Ribonucleotides, Hydrophobic Isosteres of the Natural Pyrimidines

G. Minasov,[1] J. Matulic-Adamic,[3] C. J. Wilds,[2] P. Haeberli,[3] N. Usman,[3] L. Beigelman,[3] M. Egli[2,*]

[1] *Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL, U.S.A.*

[2] *Department of Biological Sciences, Vanderbilt University, Nashville, TN, U.S.A.*

[3] *Ribozyme Pharmaceuticals Inc., Boulder, CO, U.S.A.*

**To whom correspondence should be addressed: martin.egli@ vanderbilt.edu)*

Abstract

Chemically modified nucleotide analogues have gained widespread popularity for probing structure-function relationships. Among the modifications that were incorporated into RNAs for assessing the role of individual functional groups, the phenyl nucleotide has displayed surprising effects in the contexts of both the hammerhead ribozyme and pre-mRNA splicing. To examine the conformational properties of this hydrophobic base analogue, we determined the crystal structure of an RNA double helix with incorporated phenyl ribonucleotides at 1.97 Å resolution. In the structure, phenyl residues are engaged in self-pairing, and their arrangements suggest energetically favorable stacking interactions with 3′-adjacent guanines. The presence of the phenyl rings in the center of the duplex results in only moderate changes of the helical geometry. This finding is in line with those of earlier experiments that showed the phenyl analogue to be a remarkably good mimetic of natural base function. Because the stacking interactions displayed by phenyl residues appear to be similar to those for natural bases, reduced conformational restriction due to the lack of hydrogen bonds with phenyl as well as alterations in its solvent structure may be the main causes of the activity changes with phenyl-modified RNAs.

Methods and Materials

Crystals were stabilized in reservoir buffer supplemented by 25% glycerol, mounted in nylon loops, and frozen and stored in liquid nitrogen. Data were collected on the insertion device (ID) beamline of the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) located at Sector 5 of the Advanced Photon Source. A crystal was transferred into the nitrogen stream and separate high-resolution (200 frames, oscillation angle 0.5°) and low-resolution (60 frames, oscillation angle 1.5°) data sets were collected at a wavelength of 1.0004 Å, using a MARCCD detector. The high-resolution images revealed strong anisotropy of the diffraction pattern, with reflections of up to 1.6 Å resolution along the stacking direction of duplexes (long cell dimension in the z-direction). Data were integrated and scaled in the DENZO/ SCALEPACK suite with a cutoff limit at 1.97 Å.

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Reference

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