X-ray Crystallographic Observation of "In-line" and "Adjacent" Conformations in a Bulged Self-Cleaving RNA/DNA Hybrid

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Abstract

The RNA strand in an RNA/DNA duplex with unpaired ribonucleotides can undergo self-cleavage at bulge sites in the presence of a variety of divalent metal ions. Transesterification proceeds via an *in-line* mechanism, with the 2′-OH of the bulged nucleotide attacking the 3′-adjacent phosphate group. The site-specificity of the reaction is most likely a consequence of the greater local conformational freedom of the RNA backbone in the bulge region. A standard A-form backbone geometry prohibits formation of an *in-line* arrangement between 2′-oxygen and phosphate. However, the backbone in the region of an unpaired nucleotide appears to be conducive to an *in-line* approach. Therefore, the bulge-mediated phosphoryl transfer reaction represents one of the simplest RNA self-cleavage systems. Here we focus on the conformational features of the RNA that underlie site-specific cleavage. The structures of an RNA/DNA duplex with single ribo-adenosyl bulges were analyzed in two crystal forms, permitting observation of 10 individual conformations of the RNA bulge moiety. The bulge geometries cover a range of relative arrangements between the 2′-oxygen of the bulged nucleotide and the P-O5′ bond (including *adjacent* and near *in-line*) and give a detailed picture of the conformational changes necessary to line up 2′-OH nucleophile and scissile bond. Although metal ions are of crucial importance in the catalysis of analogous cleavage reactions by ribozymes, it is clear that local strain or conformational flexibility in the RNA also affects cleavage selectivity and rate. The geometries of the RNA bulges frozen out in the crystals provide snapshots along the reaction pathway prior to the transition state of the phosphoryl transfer reaction.

Methods and Materials

Two crystals forms of the 11mer r(GCG)d(ATAT)r(ACGU) were grown by hanging drop vapor diffusion in the presence of either spermine (SPM-form) or spermidine (SPD-form) within a pH range of 5.5 and 9.0. Various data sets were collected on either in-house x-ray equipment or on the 5-ID beamline of the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) at the Advanced Photon Source (APS), Argonne National Laboratory, Argonne, Illinois. Crystals of the spermine form diffracted to 1.65 Å resolution and contain 3 molecules (6 bulges) per crystallographic asymmetric unit. Crystals of the spermidine form diffract to about 2.4 Å resolution and contain 2 molecules (4 bulges) per asymmetric unit. All data were indexed and merged in the DENZO/SCALEPACK suite. The structures of the two crystal forms were determined by the Molecular Replacement method, using the previously determined structure of a RNA/DNA duplex with single nucleotide bulges as a model. Refinements were carried out with the program CNS using the most recent parameter files.

Acknowledgments

This work was supported by the Petroleum Research Fund, administered by the American Chemical Society (PRF AC-32434 to M.E.) and the National Institutes of Health (R01 GM-55237 to M.E.). Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. The DuPont-Northwestern-Dow Collaborative Access Team Synchrotron Research Center, located at Sector 5 of the Advanced Photon Source, is supported by E. I. DuPont de Nemours & Co., The Dow Chemical Company, the National Science Foundation, and the State of Illinois.

Reference

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