Structural Basis of Cleavage by RNase H of Hybrids between Arabinonucleic Acids and RNA

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Abstract

The origins of the substrate specificity of *E. coli* RNase H1 (referred to as RNase H here), an enzyme that hydrolyzes the RNA strand of DNA-RNA hybrids, are presently not understood. Although the enzyme binds double-stranded RNA, no cleavage occurs with such duplexes. Therefore, the hybrid substrates may not adopt a canonical A-form geometry. Furthermore, RNase H is exquisitely sensitive to chemical modification of the DNA strands in hybrid duplexes. This is of particular relevance to the RNase H-dependent pathway of antisense action. Thus, only very few of the modifications currently being evaluated as antisense therapeutics are tolerated by the enzyme, among them phosphorothioate DNA (PS-DNA). Recently, hybrids between RNA and arabinonucleic acid (ANA) as well as the 2′F-ANA analogue were shown to be substrates of RNase H. Using x-ray crystallography, we demonstrate here that ANA analogues, such as 2′F-ANA and [3.3.0]bicyclo-ANA (bc-ANA), may not be able to adopt sugar puckers that are compatible with pure A- or a B-form duplex geometries, but rather prefer the intermediate O4′-*endo* conformation. Based on the observed conformations of these ANA analogues in a DNA dodecamer duplex, we have modeled a duplex between an all-C3′-*endo* RNA strand and an all-O4′-*endo* 2′F-ANA strand. This duplex exhibits a minor groove width that is intermediate between that of A-form RNA and B-form DNA, a feature that may be exploited by the enzyme to differentiate between RNA duplexes and DNA-RNA hybrids. Therefore, the combination of the established structural and functional properties of ANA analogues help settle existing controversies concerning the discrimination of substrates by RNase H. Knowledge of the structure of an analogue that shows enhanced RNA affinity while not interfering with RNase H activity may prove helpful in the design of future antisense modifications.

Methods and Materials

A crystal of a bc-ANA/DNA dodecamer duplex was mounted in a nylon loop and flash-frozen in a nitrogen stream (100 K). High- and low-resolution data sets were collected on the 5-ID beamline (λ = 1.0000 Å) of the DND-CAT at the Advanced Photon Source, using a MARCCD detector. Data were integrated and merged in the DENZO/SCALEPACK suite (R_{merge} = 6.5%) and are 100% complete to 1.43 Å.

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Reference

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