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Structural basis for substrate helix remodeling and cleavage loop activation in the Varkud satellite ribozyme

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Abstract

The Varkud satellite (VS) ribozyme catalyzes site-specific RNA cleavage and ligation reactions. Recognition of the substrate involves a kissing loop interaction between the substrate and the catalytic domain of the ribozyme resulting in a rearrangement of the substrate helix register into a so-called ‘shifted’ conformation that is critical for substrate binding and activation. We report a 3.3 Å crystal structure of the complete ribozyme that reveals the active, shifted conformation of the substrate, docked into the catalytic domain of the ribozyme. Comparison to previous NMR structures of isolated, inactive substrates provides a physical description of substrate remodeling, and implicates roles for tertiary interactions in catalytic activation of the cleavage loop. Similarities to the hairpin ribozyme cleavage loop activation suggest general strategies to enhance fidelity in RNA folding and ribozyme cleavage.

Graphical Abstract

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Supporting Information: Supporting Figures S1-S7, Supporting Table S1. This material is available free of charge via the Internet as <http://pubs.acs.org>.

Web Enhanced Objects: A movie illustrating the structural changes in the substrate upon binding to the catalytic domain *en-route* to catalysis in mpeg format is available.

The authors declare no competing financial interests.

The Varkud satellite ribozyme remodels its substrate

Keywords

Ribozymes; X-Ray Crystallography; RNA folding; Kissing loop; interaction; Register shift

Introduction

RNA folding frequently occurs in a hierarchical manner, in which secondary structure forms before tertiary structure. However, local secondary structures can be altered through the formation of tertiary interactions facilitated by RNA folding or upon complexation with ligands or proteins.¹⁻⁶ For example, metabolite binding to riboswitches can facilitate changes in secondary structure that regulate gene expression at the transcriptional or translational level.⁶ The P4-P6 independently folding domain of the group I intron ribozyme contains a helical element that rearranges upon Mg^{2+} induced formation of its tertiary structure.^{1, 2} Similar rearrangements of RNA secondary structure facilitated by tertiary interactions upon RNA folding organize catalytically competent conformations in Group II intron ribozyme catalysis.^{4, 7} Spliceosomal snRNAs undergo dramatic changes in secondary structure during snRNP maturation, spliceosome assembly, and splicing brought about by protein binding and the action of ATP-dependent helicases.⁸ Although secondary structure rearrangements likely feature commonly in RNA folding and function, in relatively few cases have the pre- and post-rearrangement structures been defined. Herein we focus on the substrate helix of the Varkud satellite (VS) ribozyme, which rearranges upon binding to the catalytic domain of the ribozyme to create the active site.

As the largest member of the class of endonucleolytic ribozymes, the VS RNA self-cleavage motif consists of seven helical segments (helices 1-7) (Figure 1, Figure S1) organized by three three-way junctions. The substrate helix (helix 1), which consists of helix 1a, an internal cleavage loop, helix 1b and the terminal loop, can be separated from the rest of the RNA and undergo reaction *in trans* with helices 2-6. (ref. 9, 10) The substrate helix docks into the catalytic domain (helices 2-6) through tertiary interactions that include a kissing

loop interaction between the closing loops of helices 1 and 5 and interactions between the internal cleavage loop of the substrate and helices 2 and 6 (Figures 1B and 2). Mutations that disrupt the kissing interaction decrease substrate binding and substantially reduce the rate of substrate cleavage.^{11, 12} Biochemical data, *in-vitro* selection,¹³ DMS probing,^{13, 14} and measurements of binding affinity and cleavage¹⁵ show that the kissing loop interaction triggers a rearrangement in the base-pairing in helix 1b that facilitates substrate binding and cleavage. To characterize this rearrangement in substrate secondary structure and to understand how it serves to activate the substrate for catalytic cleavage, we obtained a 3.3 Å X-Ray crystal structure of the wild-type substrate helix docked into the VS catalytic core.

Recent crystal structures of the VS ribozyme revealed a domain-swapped dimer, where the substrate helix from one protomer docks into the catalytic cleft created by helices 2 and 6 of the other protomer, forming two active sites *in trans* through close association of the internal cleavage loop (also referred to as the G638 loop as it contains the putative general base G638) within helix 1, helix 2 and the A730 loop (containing the general acid A756) within helix 6 (Figure 1). (ref. 16) The crystallized constructs contained a mutation that pre-organizes the substrate helix into the shifted secondary structure and thereby eliminates the thermodynamic penalty for shifting incurred upon substrate binding. Here we report the crystal structure of the VS ribozyme dimer (helices 1-7), containing a substrate helix that lacks the pre-organizing mutation (Figure 1, Figure S1). The new structure enables comparison to previous NMR structures of the substrate helix in isolation,^{17, 18} thereby providing a physical description of the extensive remodeling imposed on the substrate helix upon docking into the catalytic core. The structural data suggest how the kissing loop interaction stabilizes a shift in secondary structure, facilitating reorganization of the internal cleavage loop into its catalytically active conformation.

Results

We have solved the crystal structure of the VS ribozyme at 3.3 Å resolution, revealed as a domain swapped dimer (Figure 1). The crystallization construct has the same mutations as the VSx_G638A construct used in our previous work,¹⁶ except that our new construct lacks the C634G mutation present in VSx_G638A construct that forced the substrate helix into a constitutively shifted register of helix 1b.^{15, 16, 19} Therefore, this new construct mimics the natural substrate helix (Figures S1A and S1B). The global structure of this RNA is identical to those we reported earlier (Figure S1C).¹⁶

Interactions involving the docked substrate helix

In the dimer structure, the substrate helix of each protomer forms binding interactions with the other protomer in two regions: the terminal stem loop engages the loop of helix 5 to form a kissing loop interaction, and the internal loop, which harbors the cleavage site and the putative general base, G638, interacts with a cleft formed by helix 6 (containing the general acid, A756) and helix 2 (Figure 2). These interactions rearrange the substrate helix and organize the active site for catalysis. The kissing loop interaction consists of three Watson-Crick (WC) base-pairs, G630-C699, U631-A698 and C632-G697, and a non-canonical base pair C629-A701 (Figure 2, Figure S2A and S7A) arranged in an A-form helical

conformation that renders stacking between helix 5 and the substrate helix contiguous (Figure S3A).

Two unpaired nucleotides in the docked substrate helix, G633 and C634, contribute to this stacking (Figure 3A, Figure S3A). The base-paired region under the hairpin loop, referred to as helix 1b, consists of three WC base pairs: G623-C637, G624-C636, G625-C625 (Figures 3, 4A and B), and one non-canonical C626-C634 pair (Figure S4A).²⁰ The internal cleavage loop contains the nucleotide A638 (catalytic G638 in wild-type), positioned in close proximity to the scissile phosphodiester that links G620 and A621 (Figure S4B).

The G623-C637 base pair at the base of helix 1b (Figure 4B and Figure S5C) and a sheared G620-A639 base pair at the top of helix 1a (Figure 4B and Figure S5C) close the internal loop from above and below, respectively. Within the internal loop immediately following G620, two nucleotides, A621 and A622, flip out of the helix and insert into individual binding pockets involving nucleotides from helices 2 and 6 (Figures 2 and 3) as described in later sections.

NMR structure of the isolated substrate helix—Hues, Dieckmann and co-workers have described the NMR structure of an RNA oligonucleotide corresponding to the natural sequence of the substrate helix (nucleotides U617 to G642) which encompasses a portion of helix 1a, the internal cleavage loop, helix 1b and the terminal loop (PDB ID: 1HWQ).^{17, 18} Their analysis showed that helix 1b adopts the so-called “unshifted” secondary structure, consisting of four Watson-Crick base pairs (G623-C636, G624-C635, G625-C634, C626-G633) capped by a dynamic hexanucleotide loop (Figure 3B); the predicted G627 and C632 nucleotides do not form a stable base-pair.

The internal cleavage loop samples a compact ‘closed’ conformation and a more dynamic ‘open’ conformation in a pH dependant manner.¹⁸ The closed conformation characterized by three non-canonical base pairs: two tandem sheared G-A pairs (G620-A639 and A621-G638) and a sheared A⁺-C pair (A622-C637), in which A622 is protonated at N1, predominates at lower pH (Figures 3B and 4C, Figure S5A).^{17, 18} In this closed conformation, A639 forms a cross-strand stack with nucleotide A621, which likely stabilizes the non-canonical pairs and disfavors the splayed conformation at the cleavage site (Figure 4C, Figure S5A). At higher pH values, A622 deprotonates and the open conformation predominates. In this conformation, A622 disengages from its pairing with C637 and adopts an extra-helical position (Figure 4C, Figure S5C). The internal loop becomes more dynamic but the sheared G-A pairs and base stacking of nucleotides opposite A622 remain intact. Disruption of the A622-C637 pair by deletion of C637 (ΔC637) also shifts the internal cleavage loop to more ‘open’ conformation,²¹ suggestive of the role of the A622-C637 base-pair in the conformational dynamics of the cleavage loop. The ΔC637 substrate helix (PDB ID: 1OW9) preserves the G620-A639 sheared pair, but G638 forms a shared-sheared base pair with both A621 and A622 (Figure S5B).²¹

Rearrangements in the substrate hairpin

Comparison of the NMR structure of the isolated substrate helix (PDB ID: 1HWQ)^{17, 18} with our crystal structure of the full-length ribozyme, which captures the conformation of

the substrate engaged in the kissing interaction, reveals the conformational rearrangements that occur upon docking of the substrate helix into the ribozyme. Beginning with the closing base-pair of the hairpin loop, in the docked structure, the WC edge of C632 faces away from G627, its potential pairing partner in the undocked helix, and instead participates in the kissing interaction, forming a base pair with G697 in stem-loop 5. In this respect, a dynamic or absent G627-C632 base pair in the undocked helix as implied by the NMR data likely facilitates substrate binding relative to a stable base pair;¹⁵ nevertheless, the exocyclic amines of G627 and C632 reside within 3.1 Å of one another (Figure S4C).

The nucleotide immediately downstream of C632, G633, forms a base-pair with C626 in the unbound substrate helix, closing the hexaloop (Figures 3B and 4). (ref. 18) This base-pair was predicted to persist in the bound form of the substrate based on the lack of DMS reactivity at C626.^{13, 14} In addition, C634, a nucleotide that forms a base pair with G625 in the unbound substrate, was predicted to be unpaired and extruded out of the helical stack in the bound substrate, on the basis of its increased reactivity to DMS. In contrast to these expectations, the crystal structure shows that in the docked state, C626 and C634 have disengaged from the Watson-Crick base pairs with G633 and G625 observed in the undocked state, respectively, and form a non-canonical base pair,²⁰ in which the C626N3 accepts a hydrogen bond from the C634 exocyclic amino group (Figure S4A). This rearrangement directs the WC edge of C626 toward the interior of helix 1b (Figure 4B), consistent with its observed protection from DMS modification in the presence of saturating ribozyme.^{13, 14} In contrast, the WC edge of C634 becomes solvent exposed in the docked state, consistent with the observed increase in DMS reactivity caused by the presence of saturating ribozyme or isolated stem-loop 5. (ref. 13, 14) Disengaged from C626, G633 sits between C634 and G697, rendering the stacking between helix 1b and the kissing duplex contiguous (Figures 2 and 3, Figures S3A and S3B).¹³ This stabilizes the kissing-loop interaction, thereby facilitating substrate binding to the catalytic domain of the ribozyme.

The kissing interaction expands the hairpin loop (Figure 3) and causes significant changes in backbone pseudo-torsional angles (η , θ) compared to the undocked state; (Figure 5). Upon docking, the backbone undergoes a sharp turn between C632 and C635 on the 3' side of the substrate helix (Figure 4A) with nucleotides G633 and C634 remaining inside the helical stack (Figures 2, 3A and 5). Concomitant with the disruption of the C626-G633 base pair in this loop expansion, helix 1b undergoes a reorganization of three GC pairs, consistent with the biochemically inferred secondary structure.^{13, 14} In this reorganization, the three GC base pairs in the unbound substrate (G625-C634, G624-C635 and G623-C636) are replaced by three new GC pairs (G625-C635, G624-C636 and G623-C637), which represent a change in register in helix 1b forming the so-called 'shifted' substrate helix (Figures 3 and 4B, Figure S7B).

Rearrangements in the internal cleavage loop

The conformational changes associated with substrate docking extend beyond the terminal loop and helix 1b and into the internal cleavage loop, disrupting two of the sheared pairs (A622-C637 and A621-G638), and adjusting the conformation of the third (G620-A639). A622 disengages from C637; the latter forms the G623-C637 base pair in helix 1b of the

docked substrate as part of the register change. *En route* to the docked state A622 rotates out of the helical stack from the major groove face into the minor groove of helix 2 near junction 2-3-6 (Figures 2 and 4C). With its nucleobase now oriented nearly parallel to the substrate helix, A622 stacks with A657 and forms hydrogen bonds with G654 and the G655-C770 base pair (Figures 2 and 3A, Figure S2B). A621 disengages from its pairing partner in the undocked state, the putative general base G638, rotating out of the helical stack from the minor groove face of the substrate helix into the minor groove of helix 2, where it forms a base triple with the G653-C771 base pair in the docked state (Figures 2 and 3A, Figures S2C and S7C). In a similar manner, A639 rotates out of the helical stack maintaining both its interaction with its pairing partner G620 and coaxial orientation with A621. However, the strand shifting upon docking displaces G620 from the A639 plane and creates a 10.6 Å space between the A639 and A621 nucleobases (Figures 3A and 4C, Figure S5C). Filling this gap are two nucleobases from the A730 loop, C755 and A756, that project from an S-turn to form a contiguous (A621-A756-C755-A639) stack (Figures 2 and 3A). The internal loop restructuring creates a sharp turn in the phosphodiester backbone, reflected by high local (η , θ) values, and splay the nucleotides flanking the scissile phosphate (Figures 4A and 5, Figures S4B). As a result, the docked state brings the reactive groups closer to the in-line geometry, and G638 (A638 in this structure), released by the extrusion of A621 now stacked with G620, and A756, inserted beneath the extruded A621, become poised to mediate general acid/base catalysis (Figure S4B).

Supporting the functional relevance of the new crystal structure, A621 and A622 interactions have significance for VS ribozyme catalysis: an A621G mutation decreases the cleavage rate by 40-fold as do mutations of the interacting nucleotides (G653 and C771); (ref. 22, 23) an A622U mutation decreases the cleavage rate by three orders of magnitude; and mutations of the nucleotides interacting with it (A657U and C771G) decrease cleavage substantially.^{23, 24} These effects underscore the importance of the tertiary interactions between the substrate and the catalytic domain.

Discussion

In this work, we have defined the structural differences between the docked and undocked states of the VS ribozyme substrate helix. We used a complete VS ribozyme crystallization construct containing a substrate helix that adopts an unshifted secondary structure in isolation and a shifted secondary structure upon docking to form the active site (Figures 3-5). The structure enabled us to map the structural changes and tertiary interactions that lead to the creation of an active substrate from an inactive one (Figures 3 and 5). Collectively, these interactions and structural changes create an 'open' configuration of the internal cleavage loop that splay the cleavage-site nucleotides, allowing juxtaposition of the catalytic nucleobases and the scissile phosphate (Figure 3). The creation of this 'open' configuration could occur stochastically in an isolated substrate as suggested by NMR¹⁸ but the available data are not sufficient to suggest stochasticity in the register shift in helix 1b. However, docking of the helix into the catalytic domain accompanies the formation six additional stacking interactions (9 compared to 3), two additional WC base-pairs (6 compared to 4) and five additional non-canonical interactions (7 compared to 2). The structure reveals how the shifted register of helix 1b physically couples the kissing loop and

the internal cleavage loop. The register shift disengages G633 and C634 from their respective base pairs, which allows them to mediate contiguous stacking of the kissing helix and helix 1b. The register shift also draws C637 into helix 1b, resulting in a compression of the internal cleavage loop. The compression extrudes nucleotides at the 5'-side of loop, A621 and A622, towards the cleft created by nucleotides from helices 2 and 6, where they form stacking and tertiary interactions, splay the cleavage site nucleotides to bring the reactant atoms closer to the in-line geometry, and frees G638 to mediate catalysis (Figure 4C, Figure S5C). Consistent with our structural observations, substrates that preorganize helix 1b into the shifted register bind to the ribozyme 100-fold stronger than the wild-type substrate binds,¹⁵ reflecting an energetic penalty for the register shift due to unpairing of two WC base-pairs.

The remodeling of the internal cleavage loop observed in our structure bears some similarity to the remodeling of the internal cleavage loop observed in the hairpin ribozyme which, like the VS ribozyme, contains the cleavage site and guanosine general base. Both VS and hairpin ribozymes feature a sheared pair between the nucleotide upstream of the scissile phosphate (N-1) and the nucleotide directly downstream of the general base (A639 in VS, A9 in hairpin) (Figures S5 and S6A).²⁵ In the unbound substrate of both ribozymes, the nucleotide immediately downstream of the scissile phosphate (N+1) forms a non-canonical pair (N+1A-G638 in VS, N+1G-A9 in hairpin) (Figures S5A and S6B) that gets disrupted upon docking, forcing the N+1 nucleotide to flip out of the helix to create the splayed conformation necessary for nucleolytic cleavage. (Figures S5C, S6A and C).²⁵⁻²⁷ Both ribozymes accommodate the extruded N+1 nucleotide through a stacking interaction with the general acid (A756 in VS, A38 in hairpin).^{26, 27} Thus, analogous reorganization strategies configure the active sites of the hairpin and VS ribozymes for catalysis (Figures S5 and S6). Molecular dynamics studies of the hairpin ribozyme support the inferred structural reorganization at the active site.^{28, 29} Similar cleavage site reorganization accompanied by structuring of the substrate helix upon Mg²⁺ binding in the Twister ribozyme has been demonstrated using 2-aminopurine fluorescence.³⁰ Internal loop rearrangements likely occur frequently in RNA folding and offer a mechanism to enhance folding fidelity.

Secondary structure reorganization activates the branch-point in domain 6 of the Group II intron ribozyme.⁷ This domain toggles between a 1-nucleotide bulge (adenosine branch-point) and a 2-nucleotide bulge that includes the branch-point, utilizing a reshuffling of tandem guanine-pyrimidine base-pairs. The conformation harboring the 1-nucleotide bulge is specific for branching, whereas the conformation with a 2-nucleotide bulge is specific to exon ligation. This restructuring of the branch-point therefore regulates the first and second steps of splicing. However, the role of tertiary interactions in this structural reorganization remains unclear.⁷

Remodeling through a register shift in RNA secondary structure also occurs during the folding of the independently-folding P5abc subdomain derived from the Tetrahymena Group I intron ribozyme.² This subdomain adopts a non-native secondary structure in the absence of Mg²⁺. Upon addition of Mg²⁺, the bulge in P5a and the three-way junction between P5a and P5c undergo extensive secondary structure rearrangement involving the formation of two tandem GA pairs from P5b and a single residue shift in the base pairing register of P5c

as a result of a reorganization of tandem guanine-pyrimidine base-pair reminiscent of the register shift in the VS substrate helix 1b and domain 6 of the group II intron ribozyme.² A recent study combining ¹⁵N relaxation dispersion NMR with chemical probing revealed a transient, 'excited state' intermediate in the folding pathway for the P5abc subdomain. Specifically, P5c rapidly samples a native-like, register shifted state at a low level (~3%) that facilitates acquisition of the native state.³¹ Native state acquisition through transient 'excited states' formed from alternate secondary structures may occur frequently during RNA folding to prohibit misfolding, control native architecture, or enhance the specificity of RNA binding interactions.³¹

The pathway for substrate docking in the VS ribozyme may also involve 'excited state' intermediates. The open conformation sampled transiently by the internal cleavage loop could represent an on-pathway intermediate. This conformation disrupts the A622-C637 pair and positions A622 outside the helical stack as in the docked state, freeing C637 to engage in the helix 1b register shift. Based on NMR observations, Ferre-D'Amare and co-workers speculated that the hairpin ribozyme operates by a similar mechanism of 'conformational capture'.³² However, formation of the loop-loop kissing interaction most likely occurs first *en-route* to the docked complex, as the majority of nucleotides involved are single-stranded and therefore need not undergo any rearrangement of secondary structure before engaging in base-pairing interactions. Indeed, an isolated substrate helix can form a complex with an isolated stem-loop 5, indicating that the kissing interaction can form in the absence of other tertiary interactions. Moreover, a recent NMR study showed that an isolated mutant substrate helix lacking an internal cleavage loop undergoes a register shift upon forming a kissing interaction with an isolated stem-loop 5 (Figures S3B and S3C).¹⁹ Thus, a plausible kinetic pathway for docking could involve initial formation of the kissing interaction leading to an increase in the population of helix 1b with shifted register. The register shift would increase the opening frequency of the internal cleavage loop and allow capture of A621 and A622 in extrahelical positions by tertiary interactions to create the splayed conformation necessary for nucleophilic attack.

Coupling cleavage loop activation to the kissing interaction via a helix register shift provides a mechanism for VS ribozyme motifs to enhance cleavage site specificity and modulate the cleavage-ligation equilibrium. Register shifts involving reorganization of base-pairs might serve as a general strategy to achieve folding specificity and regulate function within structured RNAs.

Experimental Methods

Cloning of crystallization constructs

DNA representing the crystallization construct was synthesized by PCR with overlapping primers (IDT). The resulting PCR fragment was subcloned into EcoRI and XbaI sites of pUC19 (New England Biolabs) under a T7 promoter. The sequence of the DNA representing the crystallization construct VSx_C634wt (Figure S1B) is:

taatagactcactataGGCGCTGTGTCGCAATCTGCGAAGGGCGTCGTCGCCCCAAGCGG
TAGTAAGCAGGGAACCTCCAATGAAACACATTGTCGTAGCAGTTGACTACT

GTTATGTGATTGGTAGAGGCTAAGTGACGGTATTGGCGTAAGCCAATACCGCAGCA
CAGCACAAGCCCGCTTGCGAGATTACAGCGC

The lowercase letters indicate the T7 promoter, italicized nucleotide indicates C634 and bold nucleotides denote the 5' and the 3' ends of the ribozyme.

RNA synthesis and purification

Template for transcription reaction was prepared by PCR amplification of the plasmid containing the relevant DNA sequence. The primer sequences were: 5'-CAG TGA ATT CCG TAA TAC GAC TCA CTA TAG-3' and 5'-mGmCG CTG TAA TCT CGC AAG C-3' for the forward and the reverse primer, respectively. The first two nucleotides of the reverse primer were designed to contain the 2' OMe modification (those preceded by lowercase "m") to reduce transcriptional heterogeneity at the 3' end.³³

RNA was prepared by *in vitro* transcription for 2 hrs at 37°C in buffer containing 40 mM Tris-HCl pH 7.9, 2 mM spermidine, 10 mM NaCl, 25 mM MgCl₂, 10mM DTT, 30 U/mL RNase Inhibitor (NEB), 2.5 U/mL TIPPase (NEB), 4 mM of each NTPs, DNA template 30 pmol/mL, T7 RNA polymerase 40 µg/mL. Transcription reactions were quenched by the addition of 10 U/mL DNase I (Promega) and incubation at 37°C for 30 min.

RNA was purified using a protocol described previously.¹⁶ RNA was P/C/I extracted (pH 4.3) twice and loaded onto a NAP-10 column pre-equilibrated with gel filtration (GF) buffer (10 mM Tris-HCl pH 7.0, 25 mM KCl, 5 mM MgCl₂). RNA was eluted with 1.5 mL of GF buffer and loaded onto HiLoad 16/60 superdex 200pg gel filtration column (GE). All gel filtration runs were carried out at 4°C. Elution peaks containing the VS ribozyme dimer were collected and concentrated to 7.5 mg/mL using an Amicon Ultra-15 column (30 kDa molecular weight cut-off) and stored at -80°C.

Crystallization

RNA was quickly thawed and passed over 0.2 µm centrifugal filter units (Millipore). HT hanging drop vapor diffusion screens (100 nl RNA + 100 nl buffer condition) were setup using Mosquito liquid handling robot (TTP Labtech). HT-crystallization trays were stored at RT (20-25°C). Initial hits were observed in 0.1M bis-Tris pH 5.5, 2.0 M ammonium sulfate. Conditions were optimized in large drops (1 µl RNA+1 µl buffer condition) in standard VDX 24-well-plate (Hampton Research) using 500 µl mother liquor in the reservoir. High-diffracting crystals were obtained in 0.1 M sodium cacodylate (pH 6.8), 1.8-2.0 M ammonium sulfate. Crystals grew to full size in 2-3 days. For cryoprotection, drops bearing suitable crystals were brought to 4.4 M ammonium acetate, keeping all other buffer compositions isotonic.

Data Collection and Processing and Structure Analysis

Screening of hits from high throughput crystallization screens was performed at the Advanced Photon Source (APS) NECAT.

Datasets were integrated and scaled by RAPD (<https://rapd.nec.aps.anl.gov/rapd>). Phases were obtained by molecular replacement using the structure (4R4V) as search model using

Phaser.³⁴ TFZ score and LLG for the molecular replacement were 34.2 and 2645, respectively. Model building was completed with COOT³⁵ with aid of RCrane.³⁶ Refinement was carried out with the Phenix/ERRASER pipeline.^{34, 37} Simulated annealing protocol in Phenix was used to prevent model bias.³⁴ Simulated-annealing omit maps for important regions relevant for this work was generated with Phenix.³³ The final model had an R_{work} of 0.21 and R_{free} of 0.24 (Table S1). The coordinates and structure factors have been deposited to RCSB Protein Data Bank under PDB ID code 5V3I. The numbering in the PDB file offsets the traditional numbering of the ribozyme nucleotides 3' of A675 by 5 nucleotides. For continuity with literature, we use the traditional VS ribozyme numbering for nucleotides 3' of 675. (ref. 16) Pseudo-torsion angles were calculated using Amigos II.³⁸ All figures were made in Pymol (Schrodinger)³⁹ and edited in Illustrator (Adobe).

Video Preparation

The movie depicting the transition from an undocked to a docked VS ribozyme substrate was generated using the Morph function in PyMol.³⁹ The coordinates from the NMR structure of the isolated, undocked substrate (PDB ID: 1HWQ)¹⁸ and the docked substrate from our crystal structure of the full-length RNA were superimposed using the ALIGN function in PyMol.³⁹ For the docked state, helices 2-7 were not shown for clarity. The movie was compiled using the eMovie plugin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structure of the VS ribozyme

(A) Three-dimensional structure of the VS ribozyme dimer in cartoon representation. Color scheme of the top RNA as in Figure S1, bottom RNA is shown in light blue. Scissile phosphates and catalytic nucleobases are represented as red spheres and red sticks, respectively and C634 nucleotides are shown in black. (B) Secondary structure of the VS ribozyme as obtained from the crystal structure in (A). Coloring scheme as in (A). Scissile phosphates and catalytic nucleotides are shown in red, C634 nucleotide in black.

Figure 2. Substrate binding in VS catalysis

The VS substrate (green) docks into the catalytic core of the ribozyme via tertiary interactions between the substrate and nucleotides from helix 2 (magenta), helix 5 (orange) and helix 6 (blue). Catalytic nucleobases are shown in red. Cleavage site is shown in red and in the zoomed structure as a red sphere. H-bond interactions between substrate and catalytic domain are shown as orange dashed lines. For details see Figure S2.

Figure 3. Changes in the secondary structure of the VS substrate on binding

(A) Substrate docked to the catalytic domain of the ribozyme. The docked substrate contains a reorganized stem loop that interacts with the stem loop nucleotides of helix 5, a shifted helix 1b with a new set of WC base-pairs and a more flexible internal cleavage loop. Coloring scheme as in Figure 1. WC H-bonds are shown by black lines, non-canonical interactions are shown by red lines, red double-headed arrows indicate additional stacking interactions. On docking the substrate internal cleavage loop becomes more flexible as A621 and A622 gets extruded and the distance between the planes of A621 and A639 increases to 10.6 Å. (B) Isolated substrate (as obtained from NMR structure PDB ID: 1HWQ). The hairpin nucleotides are disorganized, helix 1b is unshifted and the internal cleavage loop is 'closed' and consists of three non-canonical base-pairs that keep cleavage site nucleotides, G620 and A621 inside the helix.

Figure 4. Overlay of undocked (yellow) and docked (green) substrate

(A) Complete substrate helices superimposed on each other shown in cartoon mode. Helices have been anchored on the U631 nucleotide for best superposition results. Nucleotides are depicted in ladder mode. Nucleotides that undergo the substantial changes on docking are colored in red (See Figure 5). The backbone region that undergoes the largest conformational change is shaded red. (B) Helix 1b superimposed on each other. The docked helix reveals a shift in register, replacing three WC base-pairs in the isolated substrate with three new WC base-pairs. (C) Internal cleavage loops superimposed on each other. The cleavage loop becomes more flexible on substrate docking primarily as a result of the disruption of three non-canonical base-pairs in the cleavage loop of the isolated substrate and the extrusion of two bases, A621 and A622 from the loop. PDB ID of undocked substrate structure: 1HWQ.

Figure 5. Analysis of changes in backbone geometry of the substrate on docking

Major changes in backbone conformations are observed in the internal cleavage loop and stem loop of the substrate, after it docks into the catalytic domain of the ribozyme.

Nucleotides involved in the loop-loop kissing interaction with stem-loop 5 are circled, catalytic nucleobase G is shown in red, C634 is shown in black and the cleavage site is indicated by a red arrow. $(\eta, \theta)^4$ indicates the differences in pseudo-torsion angles between docked and undocked substrate helices; greater its value, greater is the conformational change. Color-coding as follows: Red bars: $(\eta, \theta) > 150$, Brown bars: $150 > (\eta, \theta) > 80$, Purple bars: $80 > (\eta, \theta) > 50$, Blue bars: $(\eta, \theta) < 50$.