DNAzymes

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Understanding How DNA Enzymes Work

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For RNAs, a wide variety of different structures have been characterized. Non-Watson–Crick base-pairing constellations, but also additional interactions, for example, involving the 2′OH groups, contribute to a variety of structures and subsequent functions, including ligand binding, regulation, and catalysis. By contrast, the 3D structure of the DNA double helix, unraveled by Watson and Crick, remains to provide the fundamental basis for our understanding of how DNA works and functions. Alternative structural motifs in DNA, such as G-quadruplexes found in G-rich DNA strands and i-motifs in C-rich strands, although rare, highlight the rich structural diversity not only of RNA, but also of DNA.

There is considerable interest in utilizing DNA in aptamers, biosensors, antisense oligonucleotides, DNA nanotechnology, information storage, and last but not least, DNAzymes (or DNA enzymes or deoxyribozymes).[1] This interest is based on the chemical stability of DNA, its relative ease of chemical synthesis compared to RNA, and the powerful biochemical methodology, including amplification, ligation, site-directed mutagenesis, available for its production.

The first DNAzyme was developed in 1994[2] by Breaker and Joyce by using the SELEX technology.[3] This DNAzyme was able to cleave a phosphodiester bond of an RNA substrate strand in a Pb^{2+}-dependent manner. Since then, a large number and variety of DNAzymes have been developed for a range of chemical reactions, including bond cleavage in RNA, DNA, and other molecules, C–C bond formation, ligation of biomolecules, and other reactions.[4]

One drawback for using DNAzymes is the high concentration of ionic cofactors required for their catalytic activity and their relatively low in vivo catalytic efficiency compared to enzymes. Therefore, there is a need to make DNAzymes more efficient, in particular in light of the fact that DNA is intrinsically more stable than RNA. Up to now, such studies have relied on an analysis of the effect of mutations as monitored by biological assays

The work by A. Ponce-Salvatierra et al.[5] highlighted herein presents the very first structure of an active DNAzyme (9DB1) in its post-catalytic state. The conclusions drawn from this first structure of a DNAzyme impressively highlight how important structural data are for understanding the function of DNAzymes.

9DB1 is a Mg^{2+}- and Mn^{2+}-dependent RNA-ligating DNAzyme that regioselectively catalyzes the formation of a native phosphodiester bond between the 3′OH and 5′PPP of two RNA strands. The sequence of the DNAzyme was originally obtained through in vitro selection (SELEX).[6] Combinatorial functional mutation interference studies were previously applied by the group of Höbartner[7] to identify the minimally required construct of 9DB1 for catalysis: it comprises 31 nucleotides and is therefore shorter than the original DNAzyme obtained by SELEX in 2004 (see Figure 1 a).

The mutational studies revealed important nucleotides for catalysis (depicted in red in Figure 1). While the structure of the stem is clear, further insight could not be obtained from the earlier studies, and the catalytic mechanism has also remained unclear. Approaches to improve catalytic efficiency have therefore remained at the level of trial and error.

The groups of Höbartner and Pena have now successfully crystalized the DNAzyme in complex with an RNA.[5] They used a 44-nucleotide DNA strand containing the minimal 9DB1 sequence flanked by nucleotides matching a 15-nucleo-

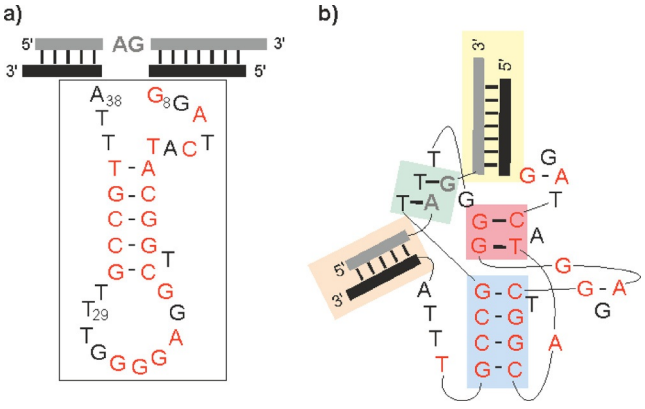

Figure 1. a) The 9DB1/RNA construct used for crystallization. Secondary-structure prediction was based on mutational studies. RNA in shown in gray, DNA in black/red. The section in the box shows the minimal construct for the 9DB1 DNAzyme.[7] The nucleotides highlighted in red are required for catalytic activity for the ligation of two RNAs, one with a 3′adenine and one with 5′GTP. Mutation of the nucleotides shown in black does not significantly abolish catalytic efficiency. b) The secondary structure of DNAzyme/RNA substrate complex, which features a double pseudoknot as determined by the 3D structure. Color coding of the stems: P1 yellow, P2 red, P3 blue, P4 orange, P5 green. The nucleotides are colored as in part (a).

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tide RNA strand, with the ligated nucleosides (AG) left unpaired (see Figure 1a).

The DNA/RNA complex adopts a double pseudoknot fold (see Figure 1b) with an extensive network of tertiary interactions and non-Watson–Crick base pairs not predicted from previous mutational data. A tight structure is formed, which came as a surprise given that the DNA lacks a 2′OH group, which is often considered as a promotor for the formation of a complex structure in RNA. In the DNAzyme, the missing OH group is compensated by greater variety in the puckering of the sugar phosphate backbone.

The authors of the paper point out that the overall shape of the structure (see Figure 2a) is reminiscent of an alpha sign. In total, five stems comprising between two to seven base pairs and denoted P1 to P5 are found. P1 and P4 are the stems formed by hybrid base pairs between DNA and RNA. They span an angle of approximately 120°. P2 (two base pairs,–not predicted previously) and P3 (four base pairs) stack with P1, thereby extending this branch of the alpha sign. The entire structure is stabilized by a number of base multiples comprised of nucleotides from P2, P3, and junctions J1/2 and J2/3. The ligated nucleotides A−1 and G1 are stacked between dA15 (J1/2) and dG27 (J2/3) and are surprisingly base-paired with nucleotides dT29 and dT30, which make up P5 located in the catalytic core (Figure 2b). Both, dT29 and dT30 were not sensitive to mutations in the previous studies, so this interaction had not been predicted.[7] Furthermore, not only A−1 and G1 but a number of nucleotides are accepted for ligation by the DNAzyme (position −1: A,G, U; position 1: A or G).

On the basis of the structural data, the authors were able to manipulate the DNAzyme by mutating a single nucleotide (dT29) to accommodate RNAs previously not ligated, that is, those bearing a C or U at position 1. They were also able to improve the ligation rate of the original RNA by using the mutant dT29dC.

Further mechanistic insight could also be gained from the structure. Hydrogen bonds between the 2′OH of G1 and the dC12 and dG26 base pair in P2 are important for positioning of the phosphate group of G1. Regioselectivity of the DNAzyme is achieved through a hydrogen bond between the 2′OH of A−1 and O4′ of G1, which keeps O2′ away from the ligation site. This was confirmed through the use of modified nucleotides (Figure 2c).

As far as the reaction mechanism is concerned, the distances observed in the structure suggest that the phosphate group of dA13 plays a role in the catalytic reaction. This hypothesis was tested by using phosphate derivatives. The importance of the pro-Sp phosphate oxygen of dA13 for catalysis was revealed by the fact that replacing the non-bridging oxygen with a methyl group abolished catalytic activity.

The role of metal ions for catalysis, however, could not be clarified. Mg^{2+} ions are present in the structure, and high concentrations of divalent ions are needed for catalysis, but no electron density for a Mg^{2+} ion could be observed in the catalytic center. Furthermore, thiophilic-metal rescue experiments, in which the non-bridging oxygen of dA13 was replaced with sulfur and soft metal ions such as Mn^{2+} were used in an attempt to regain full catalytic activity, were not successful.

The results highlight how DNA can achieve a compact structure by exploiting non-Watson–Crick base pairing, base multiples, and a wide range of sugar phosphate angles, thereby compensating for the loss of the 2′OH compared to RNA. The structure provides key insight into the mechanism of catalysis and enables modification of the DNAzyme to accommodate other substrates as presented by the authors. Altogether, the paper nicely highlights how structural data can open the door to provide a rational basis for further design and improvements in the field of catalytic DNAs.

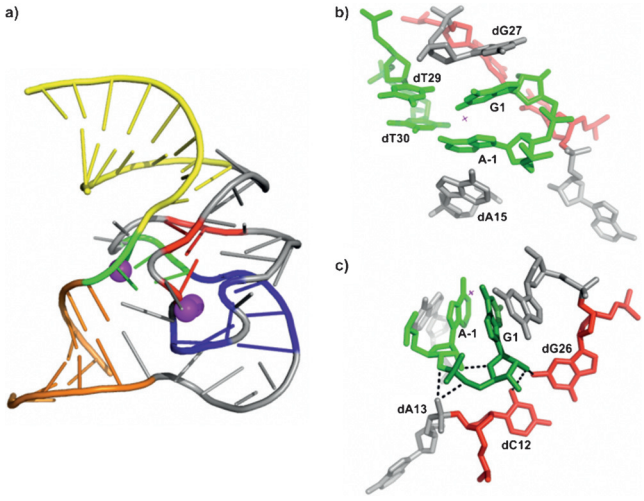

Figure 2. a) Overall fold of the DNAzyme/RNA substrate complex (PDB ID: 5cck).[5] Color coding: P1 yellow, P2 red, P3 blue, P4 orange, P5 green, Mg^{2+} magenta. b, c) Different views of the active site of the DNAzyme/RNA substrate complex.

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